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HPLC-UV Analysis of Chrysophanol in *Senna occidentalis* Extract Obtained by Using the RSM-Optimized Ultrasonic Extraction Process

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Abstract: In this experiment, chrysophanol analysis in *Senna occidentalis* (aerial parts) extract obtained by optimizing ultrasound-assisted extraction (UAE) variables (temperature, time, and liquid-to-solid ratio) using response surface methodology (RSM) was performed by employing the HPLC-UV method. For UAE process optimization, a highly significant quadratic model ($p < 0.001$) was projected to attain maximum chrysophanol yield. The extraction temperature, time, and liquid-to-solid ratio for the best UAE method were determined to be 49.3 °C, 57.7 min, and 18.7 mL/g, respectively. The optimized extract was subjected to a chrysophanol analysis utilizing HPLC-UV (fitted with a Pinnacle C18 column), and a gradient mobile phase composed of 0.5% formic acid (solvent A), acetonitrile (solvent B), methanol (solvent C), at a flow rate of 1.0 mL/min, and an optimum wavelength of 279 nm, respectively. It furnished a compact and intense peak of chrysophanol at Rt = 23.809 min. The experimental value (20.47 mg/g) of chrysophanol obtained was close to the predicted value (19.32 mg/g), indicating that they agreed under the optimized extraction condition. UAE also displayed remarkable improvement in chrysophanol extraction compared with the conventional solvent extraction (CSE) method. Hence, our improved ultrasonic extraction process showed a potential use for effective chrysophanol extraction from commercial herbal supplements comprising the *Senna* species.

Keywords: *S. occidentalis*; Caesalpiniaceae; chrysophanol; HPLC-UV; Box–Behnken design (BBD); response surface methodology (RSM)

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1. Introduction

Senna occidentalis L., a member of the Caesalpiniaceae family, is an important medicinal plant used in Ayurveda. Many names have been ascribed to it, including Coffee *Senna* and Ferid *Cassia* in English, Kaasaari in Ayurveda, Bari Kasaundi in Hindi, and Ka-sondi in Unani, among others. In the United States of America, Africa, Asia, and Australia, it is extensively distributed across tropical and subtropical regions. It is an upright, branching, glabrous, 0.8–1.5 m high plant which can range from a semi-woody to a woody annual shrub in temperate climates. Although its seeds and leaves can be harmful, both people and animals commonly consume this plant, which the locals use as a substitute for coffee [1]. *S. occidentalis* is a key component of Liv. 52 (a hepatoprotective herbal preparation) [2]. In a 1993 study by Humphry et al. on two Hausa communities, this was referred to as "edible weeds of agriculture" or "famine food" [3]. It is utilized in Mali in a conventional recipe-based antimalarial medication. In filarial illness, its roots were found to be useful with black pepper as a decoction. As per "Bhavaprakasam", it is useful for constipation, and the leaves, roots, and seeds are considered purgative in "Wealth of India" [4]. Chrysophanol, aloe-emodin, emodin, essential oils, kaempferol, physcion, and quercetin are some of the identified phytochemical constituents [1] in *S. occidentalis*. Cassiaoccidentalins A, B, and C,

three novel C-glycosidic flavonoids, were obtained from the aerial portions of this plant [5]. Chrysophanol is said to be present in both its leaves and roots, which together make up 4.5% of the plant's total anthraquinones, comprising emodin and chrysophanol [6]. As per climate, the type and quantity of a plant's secondary metabolites fluctuate, as shown by the study on many of its phytoconstituents. It has a number of important biological effects, including antibacterial, antifungal, laxative, analgesic, and diuretic ones [7].

Chrysophanol (Figure 1) is an anthraquinone found in several medicinal plants such as Rhubarb root, *Cassia tora*, *Aloe vera*, and *Senna racemosa*. It has been extensively investigated for its different pharmacological properties such as anticancer, antiviral, antioxidant, and neuroprotective agents during the previous ten years. These pharmacological properties designate that it can be applied in treating and preventing various ailments, including cancer, asthma, diabetic complications, and osteoarthritis. Furthermore, it is proven to be effective against damaged liver (caused by heavy alcohol consumption) and retinal degeneration [8]. It has been analyzed by using high pressure liquid chromatography (HPLC) method in Ganweiqitong tablets [9], *Polygoni multiflora* roots [10], *Rhamnus alpinus* bark [11], and *Cassiae semen* [12]. Ultra-high pressure liquid chromatography–Triple quadrupole mass spectrometer (UHPLC-TQMS) [13], and gas chromatography–mass spectroscopy (GC-MS) [14] techniques were used to analyze it in rat plasma. The extensive literature revealed its high pharmaceutical importance. According to Lo et al., 2022 [15], it is difficult to employ the traditional extraction process to obtain the most chrysophanol possible from plant sources.

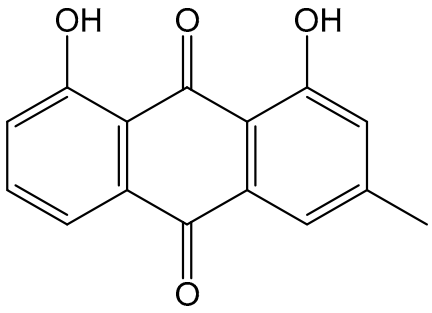

Figure 1. Chemical structure of chrysophanol.

Ultrasonic-assisted extraction (UAE) is a very effective way of extracting natural compounds. The UAE produces cavitation in plant materials due to its thermal and mechanical effect, which ruptures the cell wall structure leading to intermolecular diffusion and thorough discharge of the intracellular constituents [16]. UAE has many benefits compared with the conventional extraction method, such as reduced extraction time, less solvent consumption, and increased percentage of extraction [17]. Several extraction factors including temperature, duration, and the liquid-to-solid ratio are the major factors that impact the UAE process's effectiveness. Henceforth, optimizing the UAE factors to achieve maximum extraction of active constituents from the plant samples is important. In order to obtain the optimum extraction capacity, response surface methodology (RSM) was used to identify the impacts of several UAE factors. RSM permits simultaneous optimization of all factors and forecasts the most productive extraction circumstances with the fewest possible experimental samples [18].

Hence, the study's aim was to assess *S. occidentalis* (aerial parts) extract using the HPLC-UV technique by optimizing UAE factors (temperature, time, and liquid-to-solid ratio) using the Box–Behnken design (BBD) of RSM. This is the first report of chrysophanol analysis in *S. occidantalis* aerial parts using the HPLC-UV technique.

2. Materials and Methods

2.1. Plant Material

Dr. Md. Yusuf, a taxonomist, obtained the aerial parts of *S. occidantalis* (specimen number. 16388) from Fayfa, Saudi Arabia, in 2014. The plant sample was then stored at the herbarium of the Pharmacognosy Department at King Saud University in Saudi Arabia. Very few portions of the obtained plant material were broken down, rinsed with water, dried, and packed firmly in a glass container. Just before the extraction process began, the dried plant pieces were coarsely pulverized.

2.2. Apparatus and Reagents

A standard substance, chrysophanol (≥98.0%), was bought from Sigma-Aldrich (St. Louis, MO, USA). Methanol and acetonitrile, two analytical grade solvents employed in the analysis, were purchased from WINLAB (Market Harborough, UK). An assembly made by Milli-pore Milli-Q® (Bedford, MA, USA) produced high-quality, pure water. The sample was produced using a 0.22 m syringe filter, and the solvent was filtered using a Milli-pore-Millex-HV® filter unit with a 0.45 m pore size membrane filter. An alliance 2695 separation module (Waters Instruments, Inc., Milford, MA, USA) outfitted with a dual wavelength absorbance detector was utilized for the quantitative analysis. Weighing of the crude plant part was performed with Mettler Toledo MS-TS Precision Balances (Fisher scientific, Hampton, NH, USA).

2.3. Extraction Process

2.3.1. Ultrasound-Assisted Extraction of *S. occidentalis* Aerial Parts

The air-dried *S. occidantalis* aerial parts were powdered coarsely and placed in a conical flask (1 g/25 mL). The extraction of plant material was carried out by UAE (Model VCX-750; Sonics, Newtown, CT, USA) using methanol as a solvent. Once the extraction was completed, the final extract was cooled, filtered, and dried out by Rota vapor (R-300, Buchi, Switzerland) to obtain *S. occidantalis* dried extract, and the percentage yield of dried extract was calculated. While optimizing the various extraction parameters for the UAE, the same extraction method was applied. The obtained dried extract was used to analyze the concentration of chrysophanol by the HPLC-UV method.

2.3.2. Conventional Solvent Extraction (CSE)

One gram of the coarsely ground aerial parts of *S. occidantalis* was mixed with 25 mL of methanol, placed in a water bath (Grant W14, Cambridge, UK), and the extraction was carried out at 60 °C for 60 min while being vigorously shaken. The final extract was collected once the extraction process was complete, and it was cooled and dried using Rota vapor to produce the final dry mass. With the use of the HPLC-UV technique, the final dried mass was examined for its chrysophanol content.

2.4. BBD Experimental Design

2.4.1. Single Factor Experimental Design

To examine the impact of different UAE parameters (temperature, time and liquid-to-solid ratio (independent variables)) for the maximum extraction of chrysophanol (dependent variable) from *S. occidantalis* aerial parts, a range of these parameters were fixed with the help of the findings of single-factor impacts on chrysophanol content. The analysis of the single factor effect on chrysophanol content in *S. occidentalis* extract was performed by engaging a range of one extraction parameter, and concurrently two other extraction parameters were kept constant.

2.4.2. Optimization of UAE Parameters Using BBD Method and Method Validity Testing

The optimization was performed according to the Alam et al., 2022 [19] with slight modification. The three extraction variables of UAE (temperature: T_1; duration: T_2; liquid-to-solid ratio: T_3) were optimized at three distinct ranges (low (1), medium (0), and high

(+1)) by using a 3-factorial (3^3) BBD (version 14, Design-Expert Software, Stat-Ease Inc., Minneapolis, MN, USA) (Table 1). The BBD model produced seventeen (17) trials, five of which were fitted with central points to a second-order polynomial equation to optimize the chrysophanol extraction. Using 3D response surface plots and 2D contour plots, it was determined how each of the three extraction factors affected the chrysophanol yields. For each extraction variable, the "biggest-is-best" maxim was applied in order to achieve the most effective results (p-values ≤ 0.05 are regarded as significant). Using adjusted extraction settings, a confirming experiment ($n = 3$) was conducted, and the experimental value obtained was compared with the predicted value to validate the suggested model.

Table 1. Extraction variables selected for BBD optimization.

Independent Variable	Factor Level			Dependent Variables	Goal
	−1	0	+1		
Extraction temperature (°C) (T_1)	35	45	55	Chrysophanol yield (mg/g) (R_1)	Maximized
Extraction time (min) (T_2)	45	55	65		
Liquid-to-solid ratio (mL/g) (T_3)	12	18	24		

2.5. HPLC-UV Analysis of Chrysophanol in the Optimized Extract of S. occidantalis

Chrysophanol content in the seventeen (17) BBD run samples and the optimized extract was evaluated by using HPLC-UV (Waters Instruments, Inc., Milford, MA, USA) equipped with a 2487 dual wavelength absorbance detector. The chrysophanol was separated using Pinnacle C18 column with a size of 250 × 4.6 mm and a thickness of 5 µm at a 25 °C column temperature. The mobile phase was made up of three solvents (A (0.5% formic acid in ultra-pure water), B (acetonitrile), and C (methanol)) and elution was performed in a gradient manner as: 0–4 min (80% A, 10% B, 10% C), 4–6 min (75% A, 15% B, 10% C), 6–11 min (65% A, 20% B, 15% C), 11–16 min (55% A, 25% B, 20% C), 16–21 min (35% A, 35% B, 30% C), 21–25 min (80% A, 15% B, 5% C), and 25–30 min (85% A, 15% B, 0% C) at a 1.0 mL/min of flow rate while injecting 5 µL of application volume. Every sample and reference solution underwent a triple analysis. The peaks were detected by UV at a wavelength of 279 nm, consistent with the scanning mode of the UV detector. The chrysophanol in the *S. occidantalis* extracts was identified by matching its retention times with the standard. Using calibration curves, the amount of chrysophanol in the *S. occidantalis* extracts was measured using an external standard technique, and its concentrations were provided in mg/g. The proposed technique was assessed for accuracy, precision, linearity, limit of detection (LOD), and limit of quantification (LOQ) in accordance with ICH guidelines for the assessment of analytical procedures Q2 (R1).

2.6. Statistical Analysis

The values were provided as mean ± SEM. Data were statistically analyzed using the Student's t-test by Design Expert Software to compare the means, applying a significance level of $p < 0.05$.

3. Results

3.1. Single Extraction Factor Effect on Chrysophanol Content

In order to enhance the extraction of chrysophanol in *S. occidantalis* extracts, a set of all the extraction process variables was fixed. BBD was then used to optimize these fixed variables. To determine their impact on chrysophanol extraction, the following single factor ranges were used: 20–80 min, 30–60 °C, and 8–32 mL/g (Figure 2). The effects of one extraction variable on the chrysophanol extraction was assessed whereas the other two extraction variables remained constant, and the same procedure was performed when investigating the effect of the other two variables. The extraction temperature, time, and liquid-to-solid ratio were all held constant at 45 °C, 50 min, and 20 mL/g, respectively.

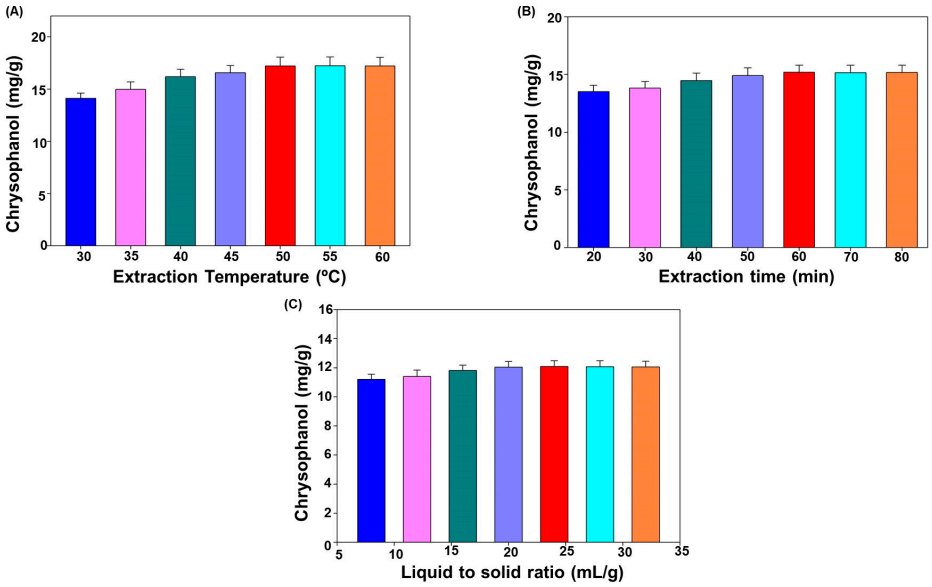

Figure 2. The effects of single factors on chrysophanol yield (mg/g of dry extract). (**A**) Effect of extraction temperature (°C) on chrysophanol yield, (**B**) effect of extraction time (min) on chrysophanol yield, (**C**) effect of liquid-to-solid ratio (mL/g) on chrysophanol yield. Each value represents a mean ± SD (n = 3).

3.2. BBD Optimization of Extraction Conditions

3.2.1. Model Fitting

BBD was used to examine the impacts of UAE parameters ((extraction temperature (T_1; °C), extraction time (T_2; min), and liquid-to-solid ratio (T_3; mL/g)) on the chrysophanol (R) extraction in the linear, quadratic, and interacting modes. Table 1 displays the 3-factorial BBD experimental design and the accompanying response (R). Chrysophanol experimental values varied from 11.5 to 19.1 mg/g of dry extract (Table 2). Results of the ANOVA showed that the quadratic polynomial model was very significant ($p < 0.0001$) compared with other models (Table 3). The values for each response's analysis of variance (ANOVA) are displayed in Table 4.

Table 2. Experimental parameters of BBD and results of chrysophanol yield.

Run	Factor (Coded)			Actual Variables			Chrysophanol Yield (R)		
	(T_1) (°C)	(T_2) (min)	(T_3) (mL/g)	(T_1) (°C)	(T_2) (min)	(T_3) (mL/g)	Experimental Value (mg/g)	Predicted Value (mg/g)	Residual
1	0	−1	−1	45	45	12	15.7 ± 0.61	15.70	0.00
2	0	0	0	45	55	18	19.1 ± 0.81	18.74	0.36
3	0	0	0	45	55	18	18.7 ± 0.83	18.74	−0.04
4	−1	1	0	35	65	18	15.3 ± 0.73	15.30	0.00
5	0	0	0	45	55	18	18.8 ± 0.92	18.74	0.06
6	−1	0	1	35	55	24	13.9 ± 0.43	13.90	0.00
7	1	0	−1	55	55	12	17.1 ± 0.87	17.10	0.00
8	1	−1	0	55	45	18	17.6 ± 0.94	17.60	0.00
9	0	−1	1	45	45	24	16.8 ± 0.95	16.75	0.05
10	−1	0	−1	35	55	12	13.9 ± 0.78	13.85	0.05
11	1	1	0	55	65	18	18.3 ± 0.97	18.25	0.05
12	1	0	1	55	55	24	17.9 ± 0.95	17.95	−0.05
13	0	0	0	45	55	18	18.6 ± 0.86	18.74	−0.14
14	0	1	−1	45	65	12	17.6 ± 0.93	17.65	−0.05
15	0	0	0	45	55	18	18.5 ± 0.94	18.74	−0.24
16	0	1	1	45	65	24	17.5 ± 0.99	17.50	0.00
17	−1	−1	0	35	45	18	11.5 ± 0.61	11.91	−0.47

Table 3. Regression analysis and response regression equation results for the final proposed model.

Dependent Variables	Source	R^2	Adjusted R^2	Predicted R^2	SD	Sequential *p*-Value	Lack of Fit *p*-Value	
R_1	Linear	0.5496	0.4456	0.2919	1.59	0.0133	0.0005	
	2FI	0.5898	0.3437	−0.1314	1.73	0.8060	0.0003	
	Quadratic	0.9846	0.9648	0.7955	0.40	<0.0001	0.0628	Suggested
	Cubic	0.9971	0.9883		0.23	0.0628		

Table 4. Analysis of variance (ANOVA) of the predicted second-order polynomial modes for chrysophanol.

Analysis of Variance (ANOVA)	
F-value (model)	49.69
p-value (model)	<0.0001 [s]
F-value (lack of fit)	5.71
p-value (lack of fit)	0.0628 [ns]
CV(%)	2.37
Adeq. Precision	22.05
Residual	1.12
Pure error	0.21

[s] significant; [ns] not significant.

3.2.2. Influence of Extraction Parameters on Chrysophanol Extraction

The chrysophanol yield in the methanol extract of *S. occidantalis* aerial parts varied from 11.5 to 19.1 mg/g of dried extract (Table 2). The lowest and highest chrysophanol yield for 1 g sample (at constant T_3 = 18 mL/g) was obtained at T_1 of 35 °C and T_2 of 45 min, and at T_1 of 45 °C and T_2 of 55 min, respectively. Table 5 showed that linear effects of all the extraction parameters (T_1, T_2, T_3,) had highly significant ($p < 0.001$) positive effect on chrysophanol yield with T_1 has the highest *F*-value of 207.66. This indicated that T_1 exerts more influence on the chrysophanol extraction, followed by T_2 with *F*-value of 39.40 and T_3 has the least impact. The quadratic effects of all extraction parameters ($T_1{}^2$, $T_2{}^2$ and $T_3{}^2$) were found to have a highly significant ($p < 0.05$) positive impact on chrysophanol yield. The quadratic effect of T_1 was found to be more promising on chrysophanol yield with the *F*-value of 119.73, followed by T_2 and T_3 with the *F*-value of 22.89 and 21.68, respectively (Table 5). Among the interaction effects of all the extraction parameters, T_1T_2 was found to exert significant ($p < 0.05$) influence on chrysophanol yield, while another interaction, T_1T_3 and T_2T_3, was found insignificant ($p > 0.05$) (Table 5). The second-order polynomial equation for chrysophanol was expressed as:

$$R_1 = 18.74 + 2.04\,T_1 + 0.89\,T_2 + 0.23\,T_3 - 0.78\,T_1T_2 + 0.2\,T_1T_3 - 0.3\,T_2T_3 - 2.13\,T_1{}^2 - 0.93\,T_2{}^2 - 0.91\,T_3{}^2$$

3.2.3. BBD Method Validation

Comparison of experimental and predicted values of chrysophanol yield was made in order to validate the BBD approach. The resulting polynomial equation and application of the BBD model are validated by a low percentage prediction error. High R^2 values of 0.9846, indicating extraordinary goodness of fit ($p < 0.0001$), were used to confirm the linear connection between the actual and predicted values of chrysophanol as illustrated by Figure 3.

Table 5. The significance of each response variable effect shown by using the *F* ratio and *p*-value in the near second-order model.

Dependent Variables	Independent Variables	SS [a]	DF [b]	MS [c]	*F*-Value	*p*-Value [d]
			Linear effects			
	T_1	33.21	1	33.21	207.66	<0.0001
	T_2	6.30	1	6.30	39.40	0.0004
	T_3	0.4050	1	0.4050	2.53	0.1556 [ns]
			Interaction effects			
R	T_1T_2	2.40	1	2.40	15.02	0.0061
	T_1T_3	0.1600	1	0.1600	1	0.3505 [ns]
	T_2T_3	0.3600	1	0.3600	2.25	0.1772 [ns]
			Quadratic effects			
	T_1^2	19.15	1	19.15	119.73	<0.0001
	T_2^2	3.66	1	3.66	22.89	0.002
	T_3^2	3.47	1	3.47	21.68	0.002

[a] Sum of squares; [b] degree of freedom; [c] mean sum of squares; [d] *p*-values < 0.05 were considered to be significant; [ns] not significant.

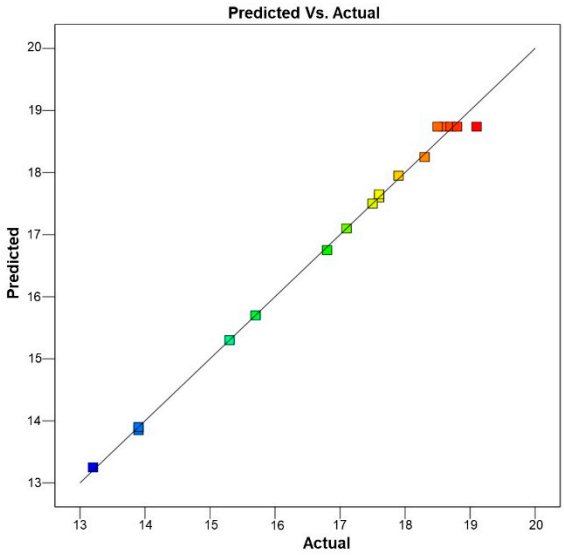

Figure 3. Linear connection plot between the actual and predicted values of chrysophanol.

3.2.4. Optimization of Extraction Conditions and Verification of the Predictive Model

The best extraction conditions for maximum extraction of chrysophanol in methanol extract of aerial parts of *S. occidantalis* were predicted by maximizing the desirability of the response by BBD. Each response has a partial desirability function, where an undesirable or unsatisfactory response is provided the value 0, while an acceptable response has a value between 0 and 1. The range of 0 to 1 represents how closely the response matches its intended value (i.e., minimum to most desirable). The desirability function, therefore, aids in identifying the most advantageous and acceptable location in the design space that satisfies the objectives for dependent variables (response). Design Expert 14 was used in our study to calculate the highest desirability value after the replies were provided their intended aims. The contour plots for the desirability function between T_1 and T_2, T_1 and T_3, and T_2 and T_3 are shown in Figure 4A–C, respectively. The maximum desirability for chrysophanol was found to be 0.920.

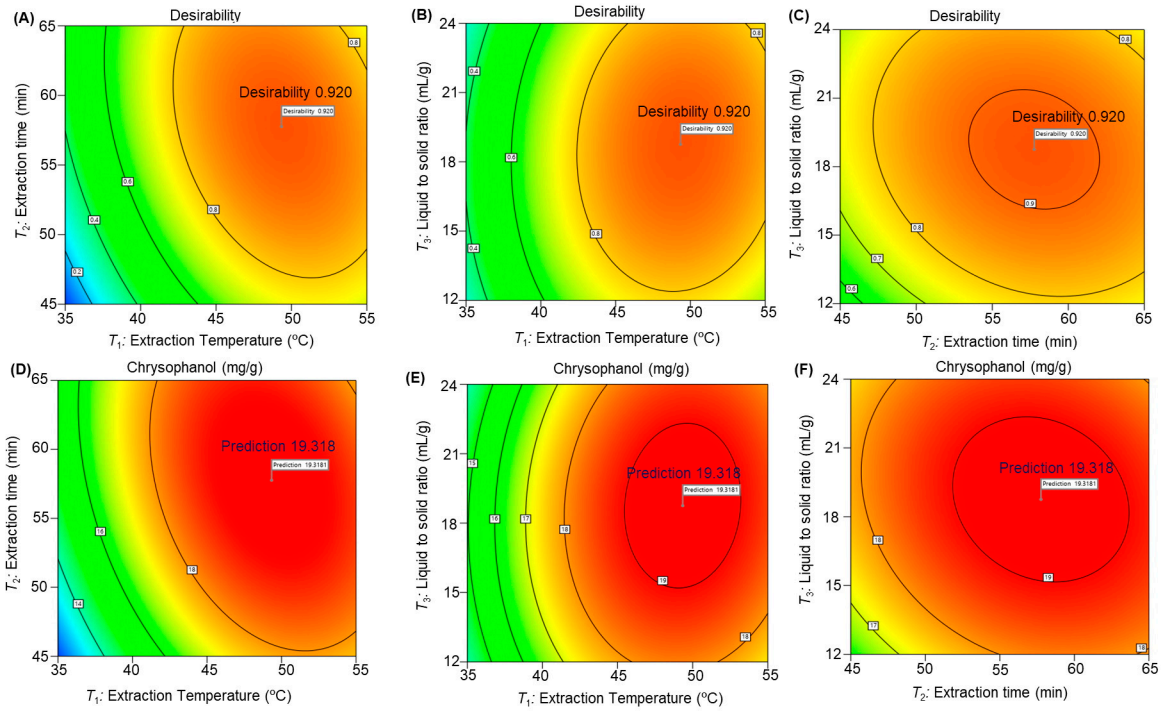

Figure 4. Two-dimensional contour plots showing the interaction effects of the UAE parameters on the desirability and predicted values of chrysophanol (*R*). (**A**,**D**) 2D contour plots shows the effects of T_1 and T_2 interaction on the desirability and predicted values of chrysophanol; (**B**,**E**) 2D contour plots shows the effects of T_1 and T_3 interaction on the desirability and predicted values of chrysophanol; (**C**,**F**) 2D contour plots shows the effects of T_2 and T_3 interaction on the desirability and predicted values of chrysophanol.

The maximum predicted value of chrysophanol (19.31 mg/g of dried extract) is delivered by numeric optimization of the UAE extraction parameters as follows: extraction temperature of 49.5 °C, extraction time of 58.4 min, and liquid-to-solid ratio of 18.9 mL/g (Figure 4D–F). The experiments were performed using the optimized UAE conditions, and the obtained results are enlisted in Table 6. The experimental value of chrysophanol achieved (20.47 ± 0.77 mg/g of dried extract) was found in accordance with the predicted value, which approves the reliability of the BBD model in predicting chrysophanol using UAE.

Table 6. Experimental and predicted values of responses at optimum extraction condition.

Response Variable	Optimum Extraction Condition			Maximum Value	
Chrysophanol yield (mg/g) (*R*)	T_1 (°C) 49.3	T_2 (min) 57.7	T_3 (mL/g) 18.7	Experimental value (mg/g) 20.47 ± 0.77	Predicted value (mg/g) 19.31

3.2.5. HPLC-UV Analysis of Chrysophanol in the Optimized Extract of *S. occidantalis*

The analysis of chrysophanol in methanol extract of *S. occidantalis* aerial parts was performed by RP-HPLC equipped with a Pinnacle DB Aqueous C18 column (Bellefonte, PA, USA; 4.6 × 250 mm, 5 µm) using three solvents (solvent A: 0.5% formic acid in ultra-pure water, solvent B: acetonitrile, and solvent C: methanol) as the mobile phase in a gradient elution system. Figure 5A,B demonstrated the separation of chrysophanol and different constituents present in the optimized extract of *S. occidantalis*, at the UV wavelength of λmax = 279 nm. A gradient system was employed to elute chrysophanol and *S. occidantalis* constituents because it enhances the HPLC column elution strength, sensitivity, and efficiency.

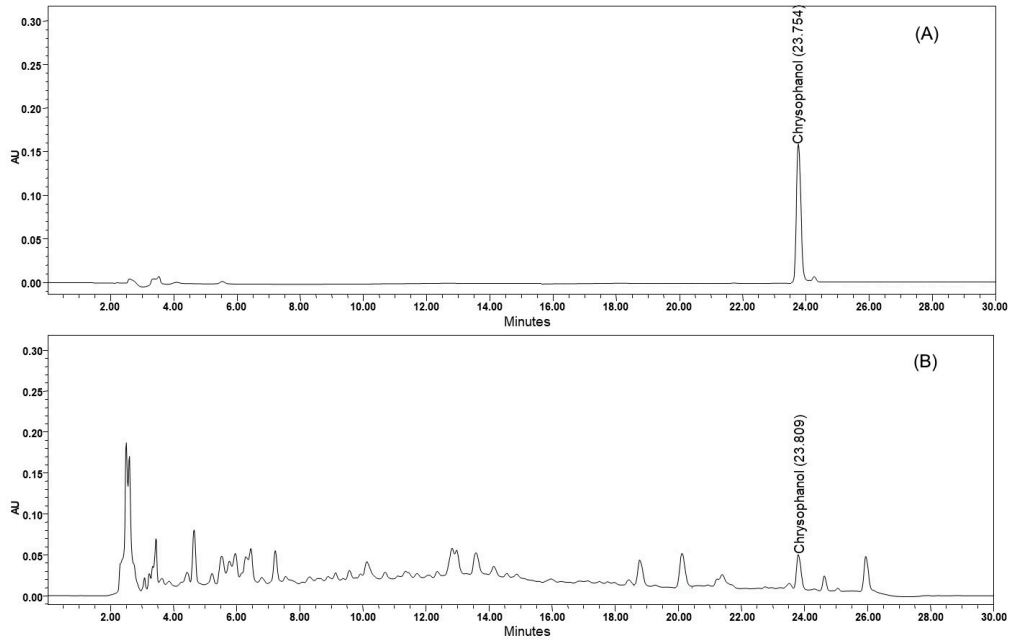

Figure 5. Chromatograms of chrysophanol (1 µg/mL) analysis in methanol extract of *S. occidantalis* aerial parts by HPLC-UV (Conditions: Pinnacle C18 column (4.6 × 250 mm, 5 µm); mobile phase: A (0.5% formic acid in ultra-pure water), B (acetonitrile), and C (methanol) in gradient system; flow rate: 1.0 mL/min; λmax = 279 nm at temperature (25 °C)). (**A**) Representative chromatogram of chrysophanol (Rt = 23.754 min). (**B**) Representative chromatogram of *S. occidantalis* extracts containing chrysophanol.

The gradient system also improves the separation quality and detection limit and decreases analysis time and column degradation due to the strong retention of analytes. Under these conditions, the Rt of chrysophanol was found at 23.754 min comparable to the retention time (Rt = 23.809 min) of the constituent present in the optimized extract of *S. occidantalis*. The developed HPLC method furnished high linearity for chrysophanol with an r^2 value of 0.9996 in the linearity range of 0.5–20 µg/mL. The LOD and LOQ (µg/mL) for chrysophanol were found as 0.017 and 0.053 µg/mL, respectively. The intraday and interday precision (%RSD, relative standard deviation) for chrysophanol were measured at three concentration levels of 5, 10, and 15 µg/mL. The intraday precision (as % RSD) was found in the range of 2.258–2.407%, whereas the interday precision (as % RSD) was found in the range of 4.762–14.766. Such a low precision for the standards indicated that the method is repeatable.

3.2.6. Comparison of UAE with CSE Methods of Extraction

The results of chrysophanol extraction from methanol extract of *S. occidantalis* aerial parts by UAE and CSE are enlisted in Table 7. The UAE method (chrysophanol yield: 20.47 mg/g) increases the chrysophanol extraction significantly ($p < 0.05$) compared with the CSE method of extraction (chrysophanol yield: 14.17 mg/g). Together with the improved chrysophanol extraction ability, the use of solvent and extraction time were also decreased significantly by UAE method in comparison with the CSE method.

Table 7. Comparison of UAE and CSE.

Extraction Method	Extraction Temp. (°C)	Extraction Time (min)	Methanol (mL/g)	Chrysophanol Yield (mg/g)
UAE	49.3	57.7	18.7	20.47 ± 0.77
CSE	60	60	25	14.17 ± 0.46

4. Discussion

In a single extraction parameter experiment, it was observed that during chrysophanol extraction with a range of extraction temperatures, the chrysophanol content increased with the increase in temperature and reached the maximum content (17.24 mg/g of dried extract) at 55 °C, and no further increase was observed upon increasing the temperature (Figure 2A). Similarly, chrysophanol extraction increased with an increase in extraction time and reached a maximum value in 60 min (15.20 mg/g of dried extract), while the maximum extraction of chrysophanol was accomplished at 24 mL/g of liquid-to-solid ratio (12.08 mg/g of dried extract). However, chrysophanol yield did not significantly increase on further increase in time as well as a solvent to drug ratio (Figure 2B,C). These outcomes facilitated the setting of the range for various UAE parameters: extraction temperature of 35–55 °C, extraction time of 45–65 min, and liquid-to-solid ratio of 12–24 mL/g, which were further employed in the optimization of UAE parameters by BBD to achieve the maximum extraction of chrysophanol from *S. occidantalis* aerial parts.

The impact of the three UAE parameters of T_1, T_2, and T_3 on the maximum extraction of chrysophanol were investigated using BBD. Chrysophanol yield values from several BBD runs (Table 2) showed a considerable reliance on various extraction settings, which supports the inevitable need for extraction process adjustment. ANOVA was used to evaluate the fitness and suitability of the suggested model, which was developed as a quadratic polynomial model to improve the extraction technique. ANOVA findings showed that the quadratic polynomial model for chrysophanol extraction was a very significant ($p < 0.0001$) model compared with linear, 2FI, and cubic models (Table 3). The model's *F*-value was discovered to be 49.69, indicating that it was significant ($p < 0.0001$). (Table 4). A model with this high *F*-value may only have a chance of 0.01% due to noise. Compared with the pure error, the lack of fit (*F*-value: 5.71) was found to be non-significant ($p > 0.05$) (Table 4). Noise has a 6.28% possibility of causing a lack of fit *F*-value to occur. A non-significant lack of fit value for the suggested model demonstrated its capacity to predict UAE's extraction of chrysophanol from *S. occidantalis* aerial parts. A reliable and reproducible set of experimental data was available for chrysophanol, for which the coefficient of variation (CV,%) was found to be (2.37) incredibly low [20]. The suggested model's adequate precision was determined to be 22.05. A ratio of greater than 4 is preferred when determining if precision is appropriate. The suggested model's signal-to-noise ratio of 22.05 demonstrated that the signal was sufficient, and the model can be used to explore the design space. The suggested model's residual and pure error were discovered to be 1.12 and 0.21, respectively. The linear, quadratic effects of all three UAE parameters on the yield of chrysophanol are clearly revealed by the data (*F*-value and *p*-value) provided in Table 5. The highest *F*-value (207.66) for the linear effect of extraction temperature compared with other UAE parameters indicated that it has a greater impact on the yield of chrysophanol compared with other factors, which is supported by a low sum of square value (33.21), and highly significant *p*-value (<0.0001). A similar observation was made in the case of the quadratic effect of all the UAE parameters on chrysophanol yield. Here, the quadratic effect of extraction temperature has more effect on the chrysophanol yield compared with the other extraction parameters which are supported by the high *F*-value (119.73), low sum of square (19.15), and highly significant *p*-value (<0.0001) as listed in Table 5. A lower sum of square values indicated a low variability from the mean.

The impact of extraction parameters (T_1, T_2, and T_3) interactions on chrysophanol yield can be seen on the 3D response surface plots (Figure 6A,C,E) and 2D contour plots (Figure 6B,D,F). UAE of chrysophanol from *S. occidantalis* first increased with an increase in temperature and then decreased with a further increase in temperature (Figure 6A,B). Figure 6A,B revealed that the maximum chrysophanol yield was reached (18.9 mg/g) at 49 °C of T_1 after 57 min of T_2 at 19 mL/g of T_3. Apparently, at high temperature, plant tissues become soft, and the cell membranes are affected by weak interactions which leads to a high yield of plant constituents. This is in line with the report published by Zhao et al. [21] and Ruan et al. [22], which indicated that the solubility of chrysophanol

improves with increased temperature. Figure 6C,D displayed the effect of T_1 and T_3 on the chrysophanol yield at constant T_2 (45 min).

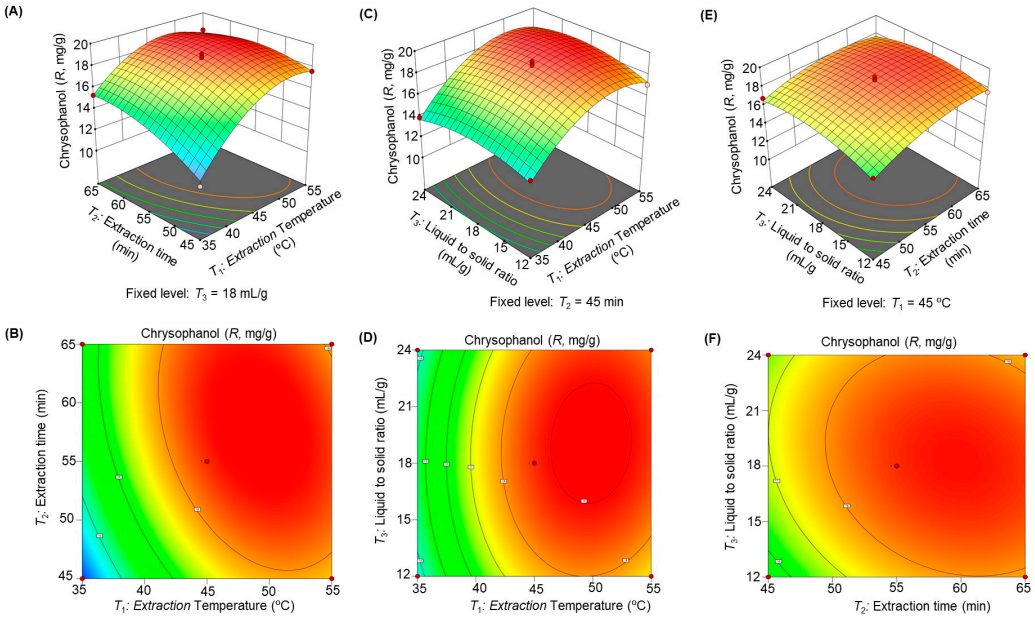

Figure 6. Response surface 3D and 2D contour plots showing the interaction effects of the UAE parameters on the chrysophanol (*R*) yield. (**A**) 3D response surface plots shows the effects of T_1 and T_2 interaction on the chrysophanol yield at constant T_3 (18 mL/g); (**B**) 3D response surface plots show the effects of T_1 and T_3 interaction on the chrysophanol yield at constant T_2 (45 min); (**C**) 3D response surface plots show the effects of T_2 and T_3 interaction on the chrysophanol yield at constant T_1 (45 °C); (**D**) 2D contour plots shows the effects of T_1 and T_2 interaction on chrysophanol yield at constant T_3 (18 mL/g); (**E**) 2D contour plots shows the effects of T_1 and T_3 interaction on chrysophanol yield at constant T_2 (45 min); (**F**) 2D contour plots shows the effects of T_2 and T_3 interaction on chrysophanol yield at constant T_1 (45 °C).

The maximum chrysophanol yield (18.5 mg/g) was found at 48 °C and a liquid-to-solid ratio of 18.5 mL/g. The penetrating power of methanol increased with an increase in extraction temperature causing higher plant material extraction and chrysophanol yield. Further increases in extraction temperature do not significantly change chrysophanol extraction. Figure 6E,F exhibit the influence of T_2 and T_3 on chrysophanol yields. At a fixed T_1 of 45 °C, chrysophanol yield decreases when the T_2 increases from 60 min and T_3 from 18.2 mL/g. The maximum chrysophanol yield was approximately 17.9 mg/g at a T_3 of 18.2 mL/g, and T_2 of 60 min.

Evaluating the results acquired from BBD analysis, the optimum UAE conditions for maximum chrysophanol extraction were found as: extraction temperature of 49.3 °C, extraction time of 57.7 min, and liquid-to-solid ratio of 18.7 mL/g. Experiments were performed using the optimized UAE conditions. The extract of *S. occidantalis* aerial parts obtained using the BBD-optimized UAE extraction parameters was analyzed for chrysophanol content using the developed the HPLC-UV method. The experimental value of chrysophanol in BBD-optimized *S. occidantalis* extract was found to be 20.47 ± 0.77 mg/g, which was in accordance with the predicted value. This proves the consistency of the proposed model in predicting the chrysophanol yield. Along with the improved efficiency of UAE for chrysophanol extraction, it decreased the solvent use and time of extraction compared with the CSE method.

5. Conclusions

In this study, chrysophanol analysis in *S. occidentalis* (aerial parts) extract obtained by optimizing UAE variables (extraction temperature, extraction time, and liquid-to-solid

ratio) using BBD was performed by employing the HPLC-UV method. For the UAE process optimization, a highly significant quadratic model ($p < 0.001$) was projected to attain maximum chrysophanol yield. The results showed that extraction temperature and extraction time had a significant impact on the chrysophanol yield. The optimum extraction condition of UAE for maximum extraction of chrysophanol from *S. occidantalis* was found to be the extraction temperature of 49.3 °C, extraction time of 57.7 min, and liquid-to-solid ratio of 18.7 mL/g. The chrysophanol analysis in the optimized extract was performed by the HPLC-UV method at the flow rate of 1.0 mL/min and λmax = 279 nm using a gradient mobile phase (comprised of A (0.5% formic acid in ultra-pure water), B (acetonitrile), and C (methanol)). This furnished a compact and intense peak of chrysophanol at Rt = 23.809 min. The obtained experimental value of chrysophanol (20.47 ± 0.77 mg/g) was found in agreement with the predicted values (19.31 mg/g) under the optimized UAE conditions. Together with the improved chrysophanol extraction ability, the use of solvent and extraction time were also decreased significantly by UAE method in comparison with the CSE method. UAE exhibited discrete progress upon conventional solvent extraction (CSE) with regard to high extraction efficiency at a lower extraction temperature and extraction solvent. Thus, the improved UAE method and developed the HPLC-UV method can be valuable in high chrysophanol extraction and estimation from *S. occidantalis* aerial parts for industrial purposes.

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