Pharmacognosy Research, 2023; 15(1):145-154.
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Original Article

QbD Based Extraction of Naringin from *Citrus sinensis* L. Peel and its Antioxidant Activity

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ABSTRACT

Background: Naringin, a bioflavanoid possessing multifaceted pharmacological properties present in *Citrus sinensis* peel. Our research demonstrates the process parameter at which high yield of naringin can be extracted from *Citrus sinensis* peel in "one run." **Objectives:** The study compares the efficacy of different modern and conventional methods for naringin extraction. Box-Behnken Design (BBD), was availed for optimization of process parameters for the extraction of naringin from *Citrus sinensis* peels. **Materials and Methods:** Extraction conditions (extraction time, solvent-to-drug ratio and extraction temperature) were optimized by Quality by Design (QbD), specifically BBD. Quantification analysis of naringin in different extracts was done using HPLC. Further, the antioxidant potential of different extracts of *C. sinensis* were assessed with the DPPH method. **Results:** Ultrasound-assisted extraction method gives the highest yield of naringin and ethanol found to be the most effective extractive solvent. Through the use of BBD, the optimal conditions for naringin extraction were established as extraction temperature- 65.508°C, solvent-to-drug ratio- 25.880 mL/g and extraction time- 29.978 min. Under such conditions, naringin was yielded as 2.021 mg/g, which was nearly contiguous to the predicted value of 2.20 mg/g. The ethanolic extract has unveiled significant antioxidant activity with a percentage inhibition of 71.54%. **Conclusion:** The Ultrasound-assisted extraction method stood out to be the best amongst all the other thermal and non-thermal modes of extraction used, and ethanol was proved to be the most efficient extracting solvent. Furthermore, naringin extraction was significantly affected by all three different variables. The present work highlights the use of QbD, a multivariate statistical technique in the extraction field of therapeutically potent phytoconstituents, which makes the optimization method less laborious and time-saving than the traditional optimization method.

Keywords: Naringin, *Citrus sinensis*, Extraction, Optimization, Response surface methodology.

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Received: 26-Aug-2022 ; **Revised:** 28-Sep-2022 ; **Accepted:** 15-Oct-2022

INTRODUCTION

Citrus sinensis (Linn.), commonly kenned as sweet orange, is one of the prime medicinal plants of the family Rutaceae.[1] It is a conventional culinary herb and has been used in different indigenous medicines, especially in Ayurveda traditional Indian medicinal system to treat various diseases.[2,3] The plant is highly valued because of its vast medicinal properties, including antibacterial, antiosteoporotic, relaxant, anxiolytic, sedative, antioxidant, insecticidal, antiproliferative, anti-parasitic, hypocholesterolemic, cardioprotective, analgesic, UV protection, anti-obesity, and anti-fungal activities.[4-20] It is also extensively used in numerous ayurvedic medicines to cure tuberculosis, constipation, obesity, colic, cough, cramps, menstrual disorder, bronchitis, cold, depression, angina, stress, hypertension and anxiety.[3] This plant contains various chemical constituents: peptides, steroids, coumarins, flavonoids, carbamates and alkylamines, hydroxyamides, fattyacids, alkanes, volatile compounds, carotenes and nutritional elements such as magnesium, sodium, calcium and potassium among which naringin is the active constituent.[21-22]

Naringin is a polymethoxylated flavanoid having formula 4,5,7-trihydroxyflavanone-7-rhamnoglucoside, has been copiously explored both *in vivo* and *in vitro*. It possess multifaceted biological profile that includes antiapoptotic, antioxidant, antitumor, anti-asthmatics, anti-inflammatory, anxiolytic, anti-ulcer, neuroprotective, antiosteoporotic, and anticarcinogenic activity.[23] Naringin is mainly isolated and found in plants – *Drynaria fortunei, Citrus aurantium, Citrus medica, Citrus sinensis* and other citrus fruits.[23]

DOI: 10.5530/097484900241

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In this scenario, where phytoconstituents display immense medicinal potential, the Optimization of extraction parameters for extracting the maximum amount of bioactive constituent from plants in a single run has become more significant. Therefore, to solve this problem, RSM is used, a mathematical and statistical technique that uses multivariate methods to optimize complex processes.[34] It is a labor, and time-saving method compared to other optimization methods and requires fewer experiments to assess multiple factors and their interactions.[24] For the Optimization of extraction parameters of various phytoconstituents like flavonoids from *Vitis vinifera*,[25] quercitrin from *Herba Polygoni capitati*,[26] karanjin from *Pongamia pinnata*,[27] embelin from *Emblica ribes*,[28] lupeol from *Ficus racemosa*,[29] carthamin from *Carthamus tinctorious*,[30] glycyrrhizic acid from Glycyrrhiza glabra,[31] atropine from *Atropa balledona*,[32] mangiferin from *Mangifera indica*,[37] piperine from *Piper longum*,[38] and baicalin from Oroxylum indicum,[33,35,36] researchers have exploited this technique.

The present study utilizes RSM to optimize the extraction parameters (extraction time, extraction temperature, solvent to drug ratio) of naringin from *C. sinensis* peel, and quantification analysis is done using HPLC. Several studies have reported the extraction of naringin from *Drynaria fortunei, Citrus aurantium, Citrus medica*.[23] However, none of the researchers have developed the extraction process for the isolation and quantification of naringin from *Citrus sinensis*. Therefore, our study employs different techniques like reflux, soxhlet, UAE, and maceration technique for the extraction of naringin, which has not been reported by any other researcher yet.

MATERIALS AND METHODS

Collection and authentication of the Plant Material

The peels of *Citrus sinensis* L. were procured from the Medicinal plant market, Delhi. Identification and authentication were done from National Institute of Science Communication and Policy Research (NIScPR), New Delhi, with authentication number NIScPR/RHMD/Consult/2021/3883-84.

Chemicals

Standard naringin was acquired from Sigma Aldrich, India. HPLC grade water and acetonitrile were purchased from S.D. Fine Chem Limited, India. All other analytical grade chemicals were obtained from S.D. Fine Chem Limited, India.

Preparation of Plant Material

The peels were properly cleansed to eliminate cling dust and other foreign material and then washed with water. The peels were air-dried, powdered, passed through 14 mesh sieves, and stowed in an air-lock container.

Extraction of Naringin

Four different extraction techniques viz. soxhelation, reflux, ultrasound-assisted extraction, and maceration were employed to extract naringin from *Citrus sinensis* using different solvents- ethanol, acetone, dimethyl sulphoxide (DMSO), and dimethylformamide (DMF) of varying polarity.

Soxhlet Extraction

Extraction was carried out using a soxhlet apparatus (continuous hot solvent extraction) at 50°C for 1 hr using the solvent-to-drug ratio- 10 ml/g. After extraction, the plant residue was filtered, and a rotary evaporator dried the filtrate under a vacuum.

Reflux Extraction

The extraction process was carried out in a reflux apparatus (hot solvent extraction method) using 50 ml solvent at 50°C for 1 hr with solvent-to-drug ratio- 10 ml/g. After extraction, the plant residue was filtered, and a rotary evaporator dried the filtrate under a vacuum.

Ultrasound-Assisted Extraction

Extraction was done using ultrasound-assisted extraction (UAE) method for 1 hr using the solvent-to-drug ratio- 10 ml/g at 50°C in a sonicator (TOSCHON, SW7). After extraction, the plant residue was filtered, and a rotary evaporator dried filtrate under a vacuum.

Extraction by Maceration

Two grams of the powdered drug were taken in a beaker and soaked in 20 ml of solvent for 72 hr at room temperature (solvent-to-drug ratio- 10 ml/g). The menstruum was filtered in a china dish, and the filtrate was allowed to evaporate at room temperature to obtain a brownish-colored sticky mass. The extract was then stored for further analysis.

Comparison of Different Extraction Techniques for Extraction of naringin

Quantification of naringin in different extracts of *Citrus sinensis* was done by using High-Performance Liquid Chromatography (HPLC) on Shimadzu HPLC Quaternary System (Japan) attached with a C18 reverse-phase Lichrospher column (Merck, Germany) of 25x 4.6 mm length and 5 µm particle size. A stock solution of standard naringin and the sample solution of different extracts of *Citrus sinensis* were prepared in HPLC grade methanol, and the dilutions of standard naringin ranged from 20 ug/ml-100ug/ml were also prepared with the same. All the standard and sample solutions were passed through a 0.2 µm membrane filter (Axiva) before injection into the HPLC system. Water and Acetonitrile (85:15) were used as mobile phase at a 1mL/ minute flow rate in isocratic mode,[16] and detection was done at a wavelength of 284 nm. The calibration curve was prepared between concentration

Table 1: Ranges of different parameters assessed in single factorial experiment along with their coded and actual values.

Independent variables	Lower Range	Higher Range	Coded levels		
			-1	1	+1
Extraction time	20	30	20	25	30
Extraction temperature	50	70	50	60	70
Drug-to-solvent ratio	1:10	1:30	10	20	30

for standard naringin and peak area. Naringin content in different extracts of *Citrus sinensis* was then calculated from the linear equation of the calibration curve.

Single Factorial Experiments

After establishing the most effective extraction mode and the best solvent, single factorial trial experiments were conducted on three parameters: extraction time, solvent-to-drug ratio, and extraction temperature. During the experiment, by varying one parameter, and keeping two parameters constant, the effect of that particular parameter on naringin yield was studied. The ranges estimated for different parameters are presented in Table 1. Naringin content in each extract was calculated using HPLC.

Optimization of extraction parameters of naringin

Box-Behnken Design (BBD) was availed to optimize the extraction parameters for naringin using Design-expert software (Version 13), Stat-Ease, Inc. USA. The experimental design consisted of 17 runs consisting of five replicates of the center point and twelve factorial experiments. The three factors selected were named/assigned as Z_1, Z_2, and Z_3 and were designed into three levels encoded/encrypted as -1, 0, +1 for low, intermediate, and high levels respectively, Independent variables were encrypted according to the following equation:

$$z_{i=} \frac{(Z_i - Z_0)}{\Delta Z}$$

Where,

z_i - coded value of an independent variable,

Z_i - actual value of an independent variable,

Z_0 - actual value of an independent variable at the center point, and

ΔZ - step-change value of an independent variable.

The actual and the coded values of three variables are specified in Table 1, and the 17 runs of BBD experiments are specified in Table 2.

Quantification of naringin in Various Extracts by HPLC

Different extracts for BBD experiments were analyzed using HPLC for the quantification of naringin content.

Prefatory phytochemical evaluations

The presence of diverse phytochemicals (glycoside, alkaloids, phenolic compounds, carbohydrate, flavonoids, phytosterols, steroid, triterpenoids, resins and tannin) in different extracts of *C. sinensis* were assessed by prefatory phytochemical investigation.

Antioxidant activity using DPPH

Different extracts of *Citrus sinensis* were assessed for antioxidant potential by employing the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method using UV spectrophotometer at 517 nm. Methanol (5ml) and 1mM DPPH (0.5 ml) were added to the different tubes containing aliquots of 20, 40, 60, 80 and 100 ug/ml as standard. A blank solution consisting of methanol (5ml) and 1mM DPPH (0.5ml) was prepared and further all the solutions were incubated at ambient temperature for 30 min. The antioxidant potential of different extracts were calculated using the equation:

$$\% \text{ scavenging} = \frac{\text{Absorbance of blank solution- Absorbance of test sample}}{\text{Absorbance of blank solution.}} \times 100$$

Data are represented as mean of 4 and linear regression analysis was employed to assess the sample size.

RESULTS AND DISCUSSION

Comparison of different extraction techniques for extraction of naringin

Initial experiments for the extraction of naringin from *Citrus sinensis* using four different modes of extraction techniques employing four different solvents of varying polarity revealed that ethanol was the most effective solvent, and the ultrasound-assisted extraction proved out to be the best mode of extraction technique for extraction of naringin (Figure 1). Quantitative analysis of naringin in each extract was done via HPLC and the ensuing chromatogram of standard naringin and sample naringin in ethanolic extract of *Citrus sinensis* showing naringin peak at

Table 2: Experimental design, BBD.

Run	Extraction time (minutes) Z_1	Extraction temperature Z_2	Drug-to-solvent ratio (g/ml) Z_3	Response (Y) Naringin content (mg/g)		
				Experimental value, Y_e	Predicted Value, Y_i	Y_e-Y_i
1	25	50	30	1.35	1.36	-0.0075
2	25	60	20	2.20	2.20	-0.0040
3	30	50	20	1.24	1.23	0.0063
4	20	50	20	1.18	1.18	0.0013
5	25	60	20	2.20	2.20	0.0040
6	25	60	20	2.20	2.20	-0.0040
7	25	60	20	2.21	2.20	0.0060
8	30	70	20	2.02	2.02	-0.0012
9	30	60	10	1.57	1.58	-0.0062
10	20	60	30	1.79	1.78	0.0062
11	20	60	10	1.17	1.17	-0.0012
12	25	50	10	0.88	0.88	0.0000
13	25	70	10	1.65	1.64	0.0075
14	30	60	30	1.73	1.73	0.0013
15	25	70	30	1.93	1.93	0.0000
16	20	70	20	1.72	1.73	-0.0062
17	25	60	20	2.21	2.20	0.0060

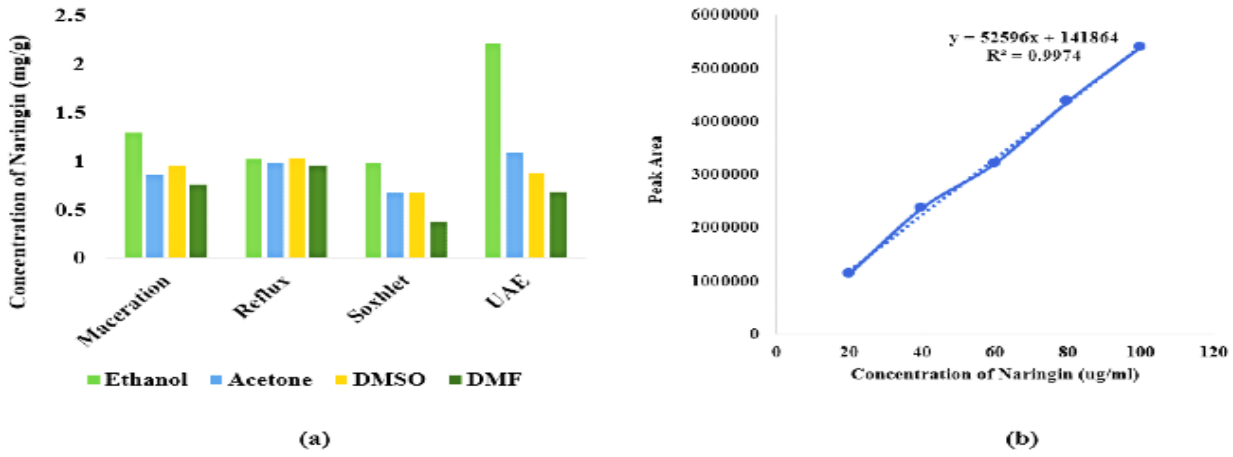

Figure 1: (a) Results of prefatory experiments and (b) Standard Plot of naringin.

retention time 2.831 are shown in (Figure 2 and Figure 3). The retention time, area under the curve and quantification values of naringin in different extracts of *Citrus sinensis* from different extraction methods are shown in Table 3.

Single factorial experiments

The results of single factorial experiments guided determining the ranges of factors for RSM, and the results are shown in Figure 4.

Optimization of Extraction parameters by BBD

BBD was used for further Optimization after determining the ranges of different parameters from single factorial experiments. BBD provided 17 runs with different combinations of three variables. By multiple regression analysis of experimental data, the polynomial equation of second-order was obtained, which established the relationship between the response variable (naringin content) and tested variables as per the equation below:

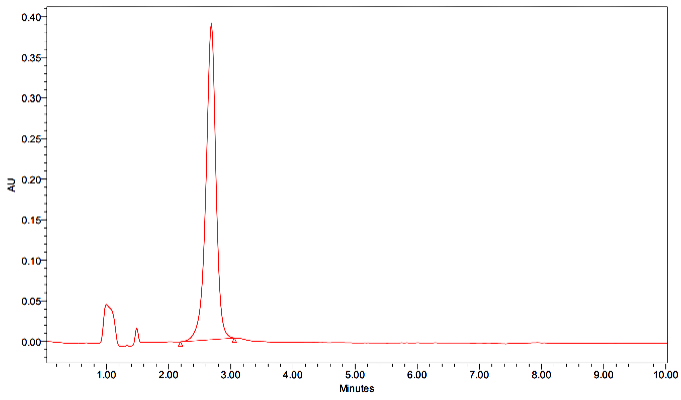

Figure 2: HPLC chromatogram of Standard Naringin.

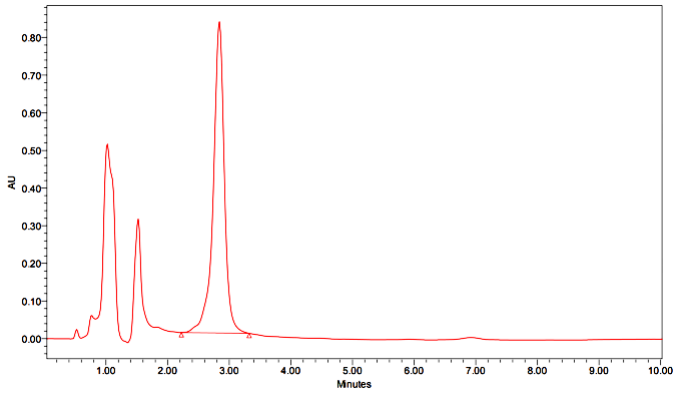

Figure 3: HPLC chromatogram of Ethanolic extract of *Citrus sinensis* peel.

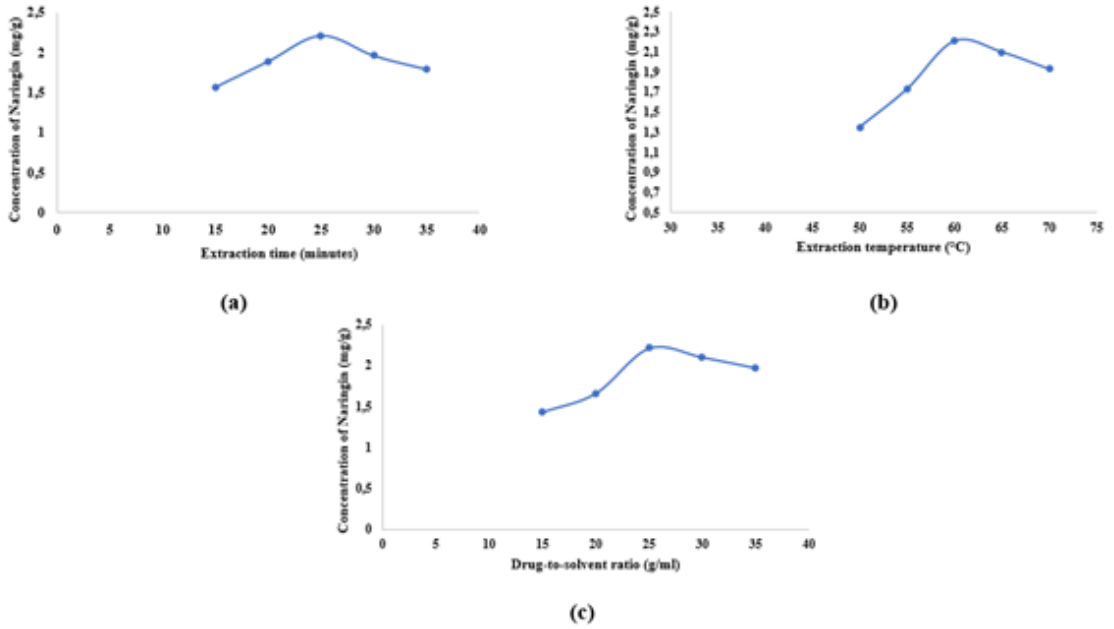

Figure 4: Results of single factorial experiments (a) Effect of extraction time, (b) Effect of extraction temperature, (c) Effect of drug-to-solvent ratio.

Naringin Yield = + 2.20 + 0.0875 A + 0.3338 B + 0.1913 C + 0.0600 AB -0.1150 AC-0.0475 BC-0.2758 A^2- 0.3883 B^2-0.3633 C^2

Where, A- Extraction time (minutes),

B- Extraction temperature (°C),

C = solvent-to-drug ratio (ml/g)

A summary of the variance analysis (ANOVA) was availed for the fitted quadratic polynomial model to determine the model's goodness (Table 4). The coefficient of regression (R^2) was 0.999, which implies the adjacency of the data with fitted regression. A difference of < 0.2 between predicted R^2 and adjusted R^2 signifies fit of the model to be excellent. Concurrently, the coefficient of variance (% CV) of 0.4366, which is a small value, justifies the good dependability of the experimental values. Signal-to-noise ratio, also kenned as Adequate precision, was found to be 229.8068, which shows the desirable ratio, which is more than four and thus shows the goodness of the model. The lack of fit test also determines the goodness of the model. Here, F-value and *p*-value were found to be 3.06 and 0.1544, which implies a lack of fit to be non-significant, making the model suitable. The p-values of each coefficient were checked for the significance of each coefficient, and all the values were found to be less than 0.1 and significant, which implies that the model can be utilized to predict the responses.

Figure 5 shows Contour plots and Three-dimensional response surface plots, which assist in understanding the interactions between the responses and variables more clearly. It is apparent from the 3D graph that naringin yield increases as the extraction time are increased from 25 min to 29.978 min and solvent-to-drug ratio from 20 ml/g to 25.880 ml/g. However, the further increase in both shows a decrease in naringin yield. This implies that both factors are significant for naringin extraction. Similarly,

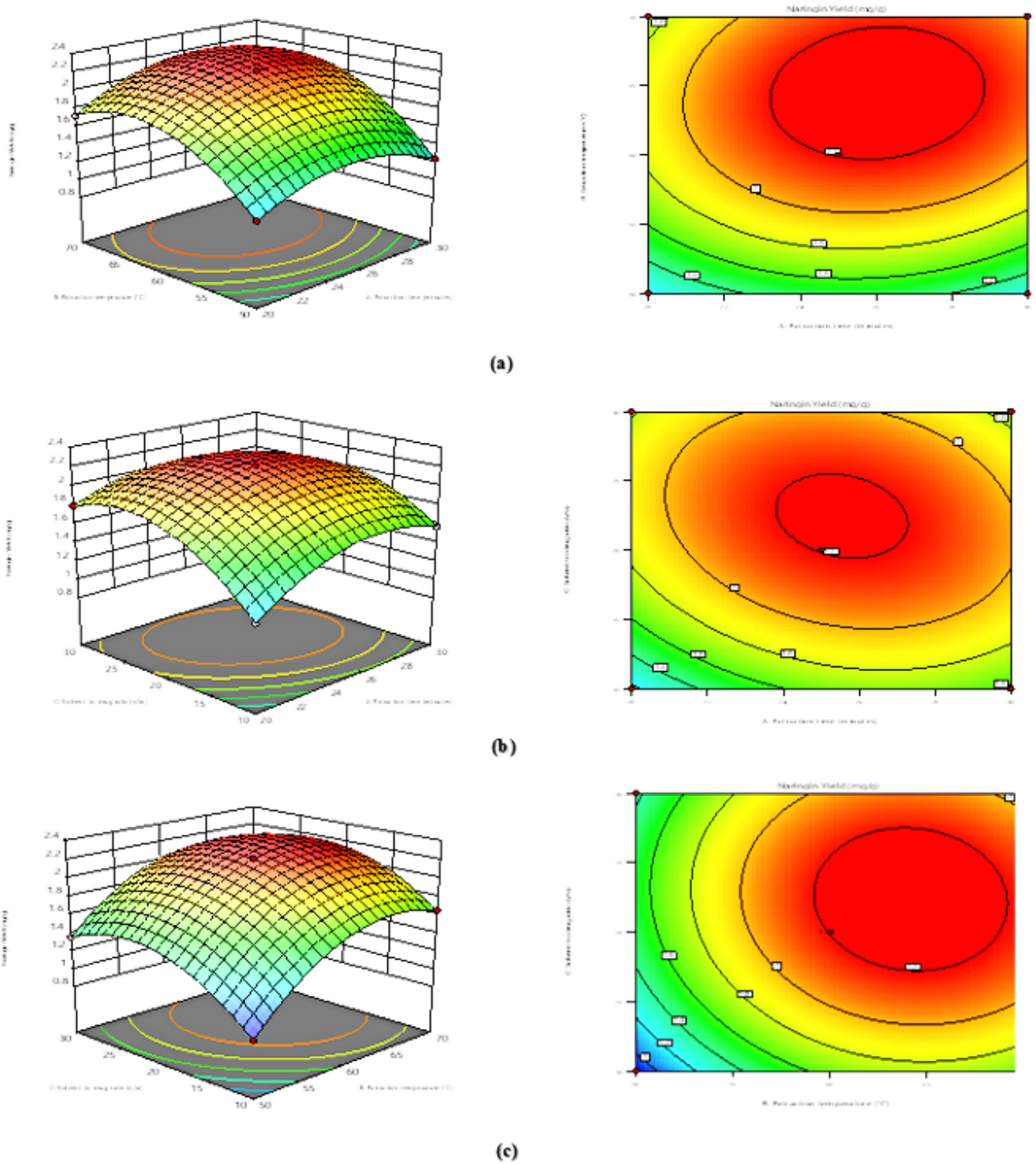

Figure 5: 3D response surface graphs and Contour plots of (a) A and B, (b) A and C and (c) B and C.

the yield of naringin increases as extraction temperature increases from 60°C to 65.508°C and solvent-to-drug ratio from 20 ml/g to 25.880 ml/g and further increase of both factors shows a decrease in naringin yield. Similarly, naringin yield increases as extraction temperature increase from 60°C to 65.508°C and extraction time from 25 min to 29.978 min.

From the point prediction analysis, it was found that the optimal conditions for extraction of naringin from the peels of *Citrus sinensis* are: extraction time- 29.978 min; solvent-to-drug ratio-25.880 ml/g and extraction temperature- 65.508°C. Also, the maximum naringin yield at these optimal conditions was found to be 2.021 mg/g of *Citrus sinensis*.

Table 3: The retention time, area under the curve, and quantification values of naringin in different extracts of *Citrus sinensis* from different extraction methods.

Extraction Method	Solvent Used	Retention Time (min)	Area Under Curve	Naringin content (mg/g)
Maceration	Ethanol	2.821	219,706	1.48
	Acetone	2.831	184,992	0.82
	DMSO	2.839	180,847	0.93
	DMF	2.837	151,834	0.78
Reflux	Ethanol	2.821	198,553	1.02
	Acetone	2.831	190,766	0.98
	DMSO	2.839	196,606	1.01
	DMF	2.837	188,820	0.97
Soxhlet	Ethanol	2.821	196,606	1.01
	Acetone	2.831	136,262	0.70
	DMSO	2.839	136,262	0.70
	DMF	2.837	68,131	0.35
UAE	Ethanol	2.821	436,038	2.24
	Acetone	2.831	233,592	1.2
	DMSO	2.839	198,553	1.02
	DMF	2.837	155,728	0.80

Table 4: ANOVA for response surface quadratic model.

Variables	Sum of squares	Degree of Freedom (DF)	Mean square	*F*-value	*P*-value	Remarks
Model	3.00	9	0.3338	5916.03	< 0.0001	Significant
A	0.0613	1	0.0613	1085.44	< 0.0001	
B	0.8911	1	0.8911	15791.87	< 0.0001	
C	0.2926	1	0.2926	5185.54	< 0.0001	
AB	0.0144	1	0.0144	255.19	< 0.0001	
AC	0.0529	1	0.0529	937.47	< 0.0001	
BC	0.0090	1	0.0090	159.94	< 0.0001	
A^2	0.3202	1	0.3202	5673.73	< 0.0001	
B^2	0.6347	1	0.6347	11247.61	< 0.0001	
C^2	0.5556	1	0.5556	9845.74	< 0.0001	
Residual	0.0004	7	0.0001			
Lack of fit	0.0003	3	0.0001	3.06	0.1544	Non-significant
Pure error	0.0001	4	0.0000			
Cor total	3.00	16				

Model Validation

To validate the model adequacy, the optimal extraction conditions for naringin extraction from *Citrus sinensis* peels were modified, and experiments were done in triplicate to reassess the run. Moreover, the naringin content was found to be 2.20 mg/g of raw material using extraction time- 25 min, solvent-to-drug ratio- 20 ml/g and extraction temperature- 60°C.

However, no significant difference was found between the experimental and predicted yield, which explicit that the response model was satisfactory and adequate for Optimization.

Prefatory Phytochemical Screening

Phytochemical tests of *C. sinensis* extracts in ethanol, Acetone, DMSO and DMF showed the presence of flavanoids and phenolic

Table 5: Prefatory phytochemical screening of the extracts of *Citrus sinensis* stem barks in different solvents.

Plant extract + test reagent	Ethanol	Acetone	DMSO	DMF
Alkaloids	+	-	-	-
Carbohydrate	+	+	-	-
Glycoside	+	+	-	-
Phenolic compounds	++	+	+	+
Flavanoids	++	+	+	+
Phytosterols	+	+	-	-
Triterpenoids	+	-	-	-
Steroid	+	-	-	-
Resins	-	-	+	-
Tannin	+	+	+	+

+ : Present, - : Absent

Table 6: Naringin content determined by the HPLC method in the samples

Sample No.	Sample	HPTLC Naringin (ug/g)
1	E1	1.693 ug
2	E2	0.9203 ug
3	E3	1.096 ug
4	E4	0.637 ug

E1- Ethanolic extract of *Citrus sinensis*, E2- Acetonic extract of *Citrus sinensis*, E3- DMSO extract of *Citrus sinensis*, E4- DMF extract of *Citrus sinensis*

compounds (Table 5). The extractive value of naringin in diverse solvents signifies the extent and character of phytobioactive constituents in each solvent (Table 6). The ethanolic extract of *C. sinensis* revealed the presence of phenolic compounds, reducing sugars, alkaloids, phytosterols, triterpenoids, flavanoids, and glycosides as the major secondary phytochemicals which may be attributed for its therapeutic potential.

Antioxidant activity

The antioxidant potential of *C. sinensis* extracts was investigated using DPPH radical scavenging activity. DPPH free radicals in the form of purple color in test samples were used to estimate test samples. When DPPH solution is exposed to tested samples, it donates a hydrogen atom, resulting in the reduced form, diphenylpicrylhydrazine (yellow color non-radical). The antioxidant capacity of the various extracts were compared to that of Vitamin C (Standard antioxidant compound). For determining the DPPH scavenging activity of different *C. sinensis* extracts, the IC_{50} value was graphically quantified.

The ethanolic extract of *C. sinensis* demonstrated the highest DPPH scavenging activity at a concentration of 100, i.e., 71.54 percent, when compared to *C. sinensis* extracts in acetone, DMSO, and DMF, which were 61.12 percent, 58.79 percent, and

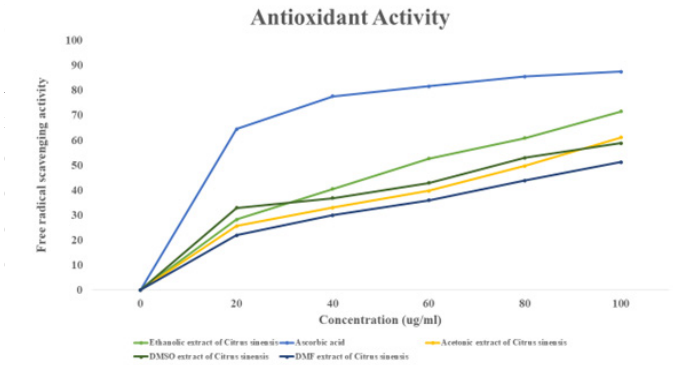

Figure 6: Dose-dependent scavenging of DPPH radicals by the different extracts of *Citrus sinensis* compared with standard drug Ascorbic acid. Each value represent mean ± SD ($n = 3$).

In our present study, both modern and conventional methods were investigated to extract naringin, a flavanoid from *Citrus sinensis* peels. Further, BBD, a modern-day statistical technique, was employed to optimize the extraction parameters of naringin. This optimization method was selected because it is economical, time-saving, less laborious, and has many advantages over other conventional optimization methods. It also helps in studying the interaction between independent variables. In this work, BBD was used as a helpful tool for optimizing the naringin extraction parameters from *Citrus sinensis* peels. Quantitative analysis of naringin was done using HPLC on a C18 reverse-phase column with U.V. detection at 284 nm. An eluting solution consisting of water: acetonitrile (85:15 v/v) was used as a mobile phase at a 1ml/min flow rate in isocratic mode.

Several studies have reported the extraction of naringin from *Drynaria fortunei, Citrus aurantium, Citrus medica*. However, none of the researchers have developed the extraction process for the isolation and quantification of naringin from *Citrus sinensis*. Therefore, our study employs the naringin extraction from *C.*

sinensis using different techniques like reflux, soxhlet, UAE, and maceration technique which has not been reported by any other researcher yet. Further, the antioxidant potential of different extracts of *C. sinensis* were assessed with the DPPH method. The DPPH scavenging activity also reveals that ethanolic extract exhibit highest antioxidant potential and further characterization of this extract can be lucrative for other researchers to discern new therapeutic entities.

CONCLUSION

Single factorial experiments were done before employing BBD, and the results attained from single runs were used in BBD. Our study concluded that ethanol is the most effective extracting solvent for naringin, and the ultrasound-assisted extraction provides a better yield of naringin than other thermal techniques like soxhlet and non-thermal techniques like maceration. In a nutshell, the modern method, which is the ultrasound-assisted extraction technique, stood out to be the best for the extraction of naringin.

By employing multiple regression analysis, the experimental data were being fitted in a polynomial equation of second-order, and optimal conditions for naringin extraction from *Citrus sinensis* peels were estimated using the model equation, which was extraction time- 60 min, solvent-to-drug ratio- 20 ml/g and extraction temp- 60°C. Under these conditions, naringin content was found at 2.20 mg/g, which coincides with the predicted value.

Further, the antioxidant potential of different extracts of *C. sinensis* were assessed with the DPPH method. The DPPH scavenging activity also reveals that ethanolic extract exhibit highest antioxidant potential and further characterization of this extract can be lucrative for other researchers to discern new therapeutic entities.

The outcome of our research will help the upcoming researchers and the pharmaceutical industries who wish to extract naringin in a maximum amount from *Citrus sinensis* peels.

ACKNOWLEDGEMENT

Authors are thankful to the Head, Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Research and Education, Jamia Hamdard, New Delhi, for providing necessary research facilities to carry out this study.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ABBREVIATIONS

3D: Three-dimensional; **°C:** Degree Celsius; **ANOVA:** Analysis of variance; **QbD:** Quality by design; **BBD:** Box–Behnken Design; **G:** Gram; **HPLC:** High performance liquid chromatography; **RSM:** Response surface methodology; **UAE:** Ultrasound-assisted extraction.

SUMMARY

Naringin, a bioflavanoid present in *Citrus sinensis* peel possesses multifaceted pharmacological properties. Our research compares the efficacy of different modern and conventional methods for naringin extraction and demonstrates the process parameter at which high yield of naringin can be extracted from *Citrus sinensis* peel in "one run". In the present study, Box-Behnken Design, was availed for optimizing the process parameters for the extraction of naringin from *Citrus sinensis* peels. Extraction conditions (extraction time, solvent-to-drug ratio and extraction temperature) were optimized by Quality by design (QbD), specifically BBD. Quantification analysis of naringin in different extracts was done using HPLC. The experimental results revealed that Ultrasound-assisted extraction method stood out to be the best amongst all the other thermal and non-thermal modes of extraction used, and ethanol was proved to be the most efficient extracting solvent. Through the use of BBD, the optimal conditions for naringin extraction were established as a solvent-to-drug ratio- 25.880 ml/g, extraction temperature- 65.508°C and extraction time- 29.978 min. Under such conditions, naringin was yielded as 2.021 mg/g, which was nearly close to the predicted value of 2.20 mg/g. Furthermore, naringin extraction was significantly affected by all three different variables. The present work highlights the use of QbD, a multivariate statistical technique in the extraction field of therapeutically potent phytoconstituents, which makes the optimization method less laborious and time-saving than the traditional optimization method. The DPPH scavenging activity also reveals that ethanolic extract exhibit highest antioxidant potential and further characterization of this extract can be lucrative for other researchers to discern new therapeutic entities.

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