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Quality by Design approach for the investigation of critical characteristics of *Phyllanthus emblica* from different vicinities

Grishm Rohilla[a], Priya Masand[b], Pooja Dhama[a], Anurag[b], Sunil Gupta[c], Alok Sharma[b*]

a. Department of Pharmacognosy, ISF College of Pharmacy, Moga, Punjab 142001, India

b. Department of Pharmaceutical Technology, Meerut Institute of Engineering & Technology, Meerut, Uttar Pradesh 250005, India

c. School of Pharmacy, Mangalayatan University, Aligarh, Uttar Pradesh 202146, India

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ABSTRACT

Objective To explore the application of Quality by Design (QbD) tools in assessing geographical variations of *Phyllanthus emblica* (*P. emblica*) from five distinct Indian states.

Methods In the current experiment, the Box-Behnken design with a reduced quartic model and 105 runs was employed with the use of the Design Expert software for randomized response surface mapping. Three different extraction methods (Soxhlet, maceration, and sonication) along with three solventst [distilled water, methanol, and water-methanol mixture (50 : 50 v/v)] were considered in the present study. The anti-oxidant activities, total flavonoid content (TFC), and total phenolic content (TPC) in the *P. emblica* were determined and analysed by gas chromatography-mass spectrometry (GC-MS) to identify the major components.

Results The QbD overlay plot showed that the extractive value of the *P. emblica* was no less than 30% w/w, 2,2-diphenyl-1-picrylhydrazyl (DPPH) no less than 60% mcg/mL (micrograms per millilitre), TFC no less than 75 mg QE/g (milligrams of quercetin equivalents per gram), and TPC no less than 80 mg GAE/g (milligrams of gallic acid equivalents per gram). Moreover, the GC-MS data confirmed the presence of variation in the bioactives of *P. emblica* extracts.

Conclusion The model was significant in describing the variation in extractive value, DPPH, TFC, and TPC. The QbD approach may tend to prioritize thoroughness in the extraction process, ultimately resulting in improved quality in the extracted products.

1 Introduction

Numerous pure chemical compounds derived from plants have demonstrated significant therapeutic effects against a wide range of diseases, and a considerable number of them have since found their way into the modern pharmaceuticals [1]. However, such plants should undergo uniform quality assessment before exerting their medicinal roles. The quality assessment is a crucial step in weeding out various bioactive substances and plant species that could pose risks to human health [2]. The quantitative variation of a specific compound within plant material can be attributed to a variety of factors, including inter or intra-species variability, harvesting time, the part of the plant utilized, environmental influences, as well as several post-harvesting factors like storage

*Corresponding author: Alok Sharma, E-mail: alokalok22@gmail.com.

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conditions, processing approaches, and regional variations. These factors are crucial when it comes to determining the effectiveness, safety, and quality of herbal medicines [3, 4]. Therefore, a systematic procedure that is strategically designed seems necessary for the development of herbal products. As diverse as the raw herbal materials can be, the procedure, following the Quality by Design (QbD) principles, could be considered reliable from the extraction to the purification process in the making of herbal medicines [5, 6].

MYKHAILENKO et al. [7] employed the QbD technique to develop a crude anti-cancerous *Crocus sativus* perianth extract. To evaluate the impact of potentially significant parameters on the effectiveness of extracted compounds from raw materials with either ethanol or water, an experimental design known as Design of Experiment (DoE) was utilized. The findings demonstrated that the QbD technique was a useful tool in assisting the development of quality herbal medicine manufacturing processes. Mucoadhesive lecithin/chitosan nanoparticles loaded with Resveratrol were created by SAHA et al. [8] for sustainable ocular administration of medications. A fishbone diagram was used for the initial risk assessments, while a risk assessment matrix (RAM) was used for the final risk assessments. A definitive screening design (DSD) study was carried out to investigate the relationship between critical process parameters (CPPs) and critical quality attributes (CQAs). The optimal desired formulation value within the design space for the central composite design (CCD) was 0.913. The area under the curve (AUC_{0-6}) and mean residence time (MRT) for the optimal formulation value significantly exceeded those of the resveratrol solution, indicating the enhanced efficiency of implementing QbD principles in the development of resveratrol-loaded mucoadhesive lecithin/chitosan nanoparticles (RMLCNs) [8].

QbD is a systematic strategy that starts with established objectives. It places a strong emphasis on comprehensive product and process understanding, alongside rigorous process control. This methodology is grounded in robust scientific principles and quality risk management [9]. QbD principles have been applied in pharmaceutical manufacturing sectors worldwide [10]. In the QbD framework, the correlation between CPPs and critical material attributes (CMAs) is established, subsequently defining the design space within the permissible ranges of CQAs [11]. It becomes imperative for herbal medicines to have quality standards due to the presence of multiple active pharmaceutical ingredients (API), which may exhibit variations during the production process. The quality of raw herbal materials varies greatly in comparison with that of chemical medications. Technically, even herbs harvested in the same location but at different times may vary dramatically in their compositions, and medicinal herbs that are geographically different exhibit considerable variations as well. Raw ingredients were deemed a primary factor that caused such variations in the quality of herbal pharmaceuticals in different batches [12].

For centuries, *Phyllanthus emblica* (*P. emblica*) has been a traditional remedy for treating illnesses in numerous countries, which is derived from Euphorbiaceae, commonly konwn as Indian gooseberry or Alma, has been a household remedy for addressing issues related to eyes, hair, and immunity. It is typically consumed as a fruit or incorporated into Ayurvedic formulations like Chyawanprash [13, 14]. *P. emblica* fruit is known to contain a rich array of phytoconstituents, including polyphenols like emblicanin A and B, tannins, chebulinic acid, gallic acid, and ellagic acid, flavonoids like flavones, alkaloids such as phyllantine, glycosides, terpenoids, carbohydrates, vitamins like nicotinic acid, riboflavin, amino acids such as lysine, methionine, tryptophan, minerals, and phosphorus [15]. The diversity in compositions is the primary reason that the plant is utilized in traditional Chinese medicine (TCM) for its medicinal benefits [16]. According to a study by LI et al. [17] that investigated the anti-oxidant and anti-proliferative characteristics of five main *P. emblica.* cultivars in China, various *P. emblica* were found to have anti-proliferative and cellular anti-oxidant potentials, hence it is strongly suggested to increase the consumption of the *P. emblica* for health reason. Utilizing 20 polymorphic expressed sequence tag-derived simple sequence repeat (EST-SSR) markers, LIU et al. [18] examined genetic diversity, population dynamics, and genetic structure in 10 *P. emblica* populations collected from regions with either humid or dry climate in Guangxi and Yunnan provinces. Their findings indicated that genetic diversity was significantly affected by precipitation and altitude, with precipitation exerting a slightly stronger impact.

Several pharmacopoeias have described its traditional uses, both in its powdered form and as a commercially standardized extract, utilized for analytical and medicinal purposes. The objective of the study is to show the new application of QbD principles in assessing geographical variation of *P. emblica* from five Indian states, including Gujarat, Himachal Pradesh, Madhya Pradesh, Punjab, and Rajasthan.

2 Materials and methods

2.1 Plant materials

The deciduous trees *P. emblica* fruits are edible and mostly found in India, Pakistan, China, Iran, and Southeast Asia. In this study, fresh, young, and disease-free *P. emblica* fruits were gathered from five different locations in India [Himachal Pradesh (Hamirpur), Punjab (Moga), Madhya Pradesh (Indore), Rajasthan (Jaipur), and

Gujarat (Ahmedabad)], and each with its unique environmental characteristics. It was noted that the harvest season of *P. emblica* lasted from November to February in all areas during which the fruit could be collected. Prior to the study, the samples of *P. emblica* were collected from different geographical areas depicted in Table 1.

2.2 Chemicals

All the chemicals and solvents utilized in this study were of analytical grade. Gallic acid (purity level > 95%), quercetin, Folin-Ciocalteau reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol, sodium carbonate, ethyl acetate, toluene, formic acid (HPLC grade), and dichloromethane were all purchased from Merck Ltd., India.

2.3 Physico-chemical analysis

The powdered drug's physicochemical property, including its moisture content, total ash value, extractive value, water-soluble ash value, and acid insoluble ash value, were all investigated. The official procedures described in the World Health Organization (WHO) standards were used to measure all the physicochemical parameters [19].

2.4 QbD approach

DoE is a systematic and structured approach used to uncover the relationship between essential factors impacting a process and its ultimate outcome. In the present experiment, Design Expert software (version 13, Stat-Ease Inc., Minneapolis, MN, USA) for randomized response surface mapping using Box-Behnken design with a reduced quartic model and 105 runs were applied. The five critical factors studied were independent continuous numeric variables [fraction of methanol in extraction solvent (factor A), maceration time (factor B), sonication time (factor C)], independent nominal categoric variables [location (factor D), and with or without using Soxhlet apparatus (factor E)]. The lowest value of continuous variables (studied at three different levels) was coded as – 1, and the highest value was coded as + 1. The samples were collected from five different locations, which were Gujarat, Rajasthan, Madhya Pradesh, Himachal Pradesh, and Punjab. All five critical variables such as experimental design showing units, type, subtype, minimum & maximum values, coded values, mean value, and standard deviation are summarized in Table 2.

The following responses were measured.

(i) Response 1 was extractive value as % w/w.

(ii) Response 2 was DPPH inhibition reported as % microgram per millilitre (mcg/mL).

(iii) Response 3 was total flavonoid content (TFC) reported as milligrams quercetin equivalents (QE) per gram (mg QE/g).

(iv) Response 4 was total phenolic content (TPC) reported as milligrams of gallic acid equivalents (GAE) per gram (mg GAE/g).

2.5 Extract preparation

Prior to the extraction process, the *P. emblica* was meticulously ground into a fine powder using an electric grinder and then air-dried. Three different extraction methods were used.

(i) Soxhlet. In a round-bottom flask, 5 g of powder were mixed with 50 mL of distilled water, methanol, and

Table 1 *P. emblica* from different geographical areas with their respective characteristics and the geographical distribution

Zone	Part	Extract with sample code	Latitude	Altitude (meters above sea level)	Rainfall (mm)	Temperature (°C)	Soil
Himachal Pradesh	Fruit	Aqueous (HP-A) Methanolic (HP-M) Water : Methanol (50 : 50) (HP-HA)	31.68° N, 76.52° E	786	1 225	12.0 - 25.0	Loamy
Punjab	Fruit	Aqueous (PB-A) Methanolic (PB-M) Water : Methanol (50 : 50) (PB-HA)	30.68° N, 75.24° E	217	498	11.6 - 27.0	Sandy loamy to desert
Madhya Pradesh	Fruit	Aqueous (MP-A) Methanolic (MP-M) Water : Methanol (50 : 50) (MP-HA)	22.71° N, 75.85° E	550	959	9.3 - 30.0	Alluvial to medium black
Rajasthan	Fruit	Aqueous (RJ-A) Methanolic (RJ-M) Water : Methanol (50 : 50) (RJ-HA)	26.912 4° N, 75.787 3° E	431	536	11.2 - 23.4	Loamy fine sand to coarse sand
Gujarat	Fruit	Aqueous (GJ-A) Methanolic (GJ-M) Water : Methanol (50 : 50) (GJ-HA)	23.02° N, 72.57° E	53	782	12.7 - 33.8	Sandy, loamy, well-drained

Table 2 The experimental design, units, type, sub-type, minimum & maximum values, coded values, mean values, and standard deviation

Factor	Name	Unit	Type	Sub-type	Minimum	Maximum	Coded low	Coded high	Mean	Standard deviation
A	Solvent	Fraction MeoH	Numerical	Continuous	0.00	1.00	− 1	+ 1	0.50	0.38
B	Maceration	Hour	Numerical	Continuous	0.00	16.00	− 1	+ 1	4.57	5.86
C	Sonication	Minute	Numerical	Continuous	0.00	30.00	− 1	+ 1	8.57	10.98
D	Location	Name	Categorical	Nominal	Gujarat	Rajasthan	NA	NA	Levels	5.00
E	Soxhlet	NA	Categorical	Nominal	0.00	1.00	NA	NA	Levels	2.00

NA: not applicable in case of categoric variables.

water-methanol mixture (50 : 50) v/v for conventional Soxhlet extraction, and the mixture was refluxed for 6 to 8 h at corresponding boiling points of the solvents.

(ii) Maceration. Similarly, in another classic extraction method known as Maceration, 5 g of powder were separately mixed with 50 mL methanol, distilled water, and water-methanol (50 : 50) v/v at room temperature in the maceration container for 8, 16, and 24 h, respectively. The solid remains were separated from the liquid extracts and subsequently dried using a water bath to determine the extractive yield.

(iii) Sonication. 5 g of powder were separately blended in beakers with 50 mL of methanol, distilled water, and water-methanol (50 : 50) v/v for sonication extraction. To extract the samples, the beakers were immersed in an ultrasonic bath (Labman Scientific Instrument) for 15 and 30 min. The water in the ultrasonic bath was continuously circulated at room temperature (25 °C). Subsequently, the resulting supernatant was similarly processed as described in conventional Soxhlet extraction to generate a dry extract of *P. emblica*, and the extractive yield was determined.

2.6 Determination of TPC in the extract

The TPC from the extract was determined using the Folin-Ciocalteau technique [20], and calculated as mg GAE/g.

2.7 Determination of TFC in the extract

TFC was evaluated using the aluminum chloride colorimetric technique [21]. Three replicates were analyzed for each plant within the three samples to determine the TFC in the methanolic extracts of the plant, specifically focusing on quercetin analogues.

2.8 Evaluation of the anti-oxidant activity in the extracts through DPPH free radical scavenging

The investigation of radical scavenging activity began by preparing a 0.2 mmol/L DPPH solution in methanol, followed by the addition of 1.5 mL of this solution to an equal volume of each test sample dissolved in different methanol concentrations. The mixture was vigorously shaken and incubated in darkness for 30 min. Subsequently, the absorbance (UV-VIS Spectrophotometer Shimadzu UV-1700) was measured at 517 nm, with a blank as the reference. Ascorbic acid and butylated hydroxy anisole (BHA) served as standard references. The formula used to calculate the scavenging activity is as follows [22].

$$\text{Scavenging activity}\,(\%) = \frac{\text{Absorbance of blank solution} - \text{Absorbance of test sample}}{\text{Absorbance of blank solution}} \times 100$$

2.9 Gas chromatograph mass spectrometer (GC-MS) analysis

GC-MS (Shimadzu QP 2010 Ultra with MS) was used to analyze the plant extract at the Central Instrumentation Laboratory (CIL), CUPB, Punjab. The GC-MS analysis was carried out at a high resolution with the GC-MS-QP2010 Ultra Shimadzu (GC-MS-TQ8050 NX) equipped with GC-MS solution software, a high-capacity differential vacuum system, advanced scanning speed protocol (ASSP™), and quadrupole mass filter. The analysis was conducted using an Agilent DB-17 column, which was a medium-polarity 50%-phenyl-methyl polysiloxane column (30 m × 0.25 mm × 0.25 mm), with a column flow rate of 1.00 mL/min and pressure control maintained at 61.3 kPa. The oven temperature was set at 70.0 °C for the column and 280.0 °C for the injection.

2.10 Statistical analysis

Box-Behnken statistical experimental design was used to measure the four different response surfaces (extractive value, DPPH inhibition, TFC, and TPC). The variation in the responses was recorded by changing the independent variables. Multiple independent variables were changed at the same time to obtain maximum information in minimum number of experiments. A mathematical model (using coded variable values) was proposed for each response separately. The importance of the model and each term in the model was determined to identify the significant variables ($P < 0.05$) for each response. The overlay plot of all the four variables helped to find the best operating conditions to maximise all the four responses simultaneously.

3 Results

3.1 Physical parameters

Table 3 displays the physical parameters including moisture content, foreign matter analysis, total ash values, acid insoluble ash value, water soluble ash value, and extractive values of different *P. emblica* solvents.

3.2 QbD

The factors and responses of experimental design are shown in Table 4, while Table 5 provides information on the range of extractive value, DPPH inhibition, TFC, and TPC. In Table 5, it is evident that the extractive value ranges from 9.84 to 38.09% w/w, with a mean value of 22.28% w/w and a standard deviation of 7.68% w/w. Based on the Analysis of Variance (ANOVA), it is evident that the model exhibits statistical significance, as indicated by an F value of 65.34. An F number of this magnitude is typically observed due to random noise in only 0.01% of cases. $P < 0.05$ suggests that model terms are significant. Specifically, in this scenario, B and D are important model terms.

Figure 1A represents the design space of extractive value through a contour plot that visualizes the relationship between the fraction of extraction solvent and the duration of maceration. The extractive value demonstrates an upward trend when changing from methanol to distilled water as shown in Figure 2A. Table 5 shows a variation in DPPH inhibition, which spans from 33.46 to 67.07% mcg/mL, featuring a mean value of 42.64% mcg/mL and a standard deviation of 6.66% mcg/mL. Remarkably, the ANOVA analysis confirms the model's significance with an F value of 52.99. In this context, model terms A, B, and D are indeed significant. Additionally, the actual R^2 value was 0.8185.

Table 3 Physical parameters of *P. emblica* from different vicinities

P. emblica	Parameter				
	Total ash value (% w/w)	Acid insoluble (% w/w)	Water soluble (% w/w)	Foreign matter (% w/w)	Loss on drying (% w/w)
Sample-1 MPG-PG/PE/22/03A	3.75	1.97	4.61	0.30	4.58
Sample-2 MPG-PG/PE/22/03B	4.01	2.22	3.66	0.50	6.57
Sample-3 MPG-PG/PE/22/03C	3.88	1.70	2.94	0.60	5.23
Sample-4 MPG-PG/PE/22/03D	4.23	2.15	3.42	0.40	5.16
Sample-5 MPG-PG/PE/22/03E	4.09	1.68	4.35	0.20	6.85

Plant materials form different regions and their authentication code. MPG-PG/PE/22/03A: Rajasthan, MPG-PG/PE/22/03B: Himachal. MPG-PG/PE/22/03C: Madhya Pradesh. MPG-PG/PE/22/03D: Gujarat. MPG-PG/PE/22/03E: Punjab.

Table 4 Factors and responses of experimental design

Run	Factor A	Factor B	Factor C	Factor D	Factor E	Response 1	Response 2	Response 3	Response 4
1	0.5	0	30	HP	0	20.99	42.15	62.09	67.12
2	0	8	0	GJ	0	26.60	44.91	50.38	74.00
3	1	8	0	RJ	0	11.61	34.45	54.23	54.00
4	0.5	16	0	HP	0	16.27	38.16	59.26	71.32
5	0.5	0	0	MP	1	25.52	42.31	62.30	71.37
6	0	0	15	MP	0	22.27	42.39	57.07	60.87
7	1	8	0	HP	0	21.32	38.23	56.84	56.21
8	0.5	16	0	GJ	0	24.29	40.96	71.09	77.21
9	1	0	15	GJ	0	23.67	49.17	73.01	65.10
10	1	0	15	PB	0	35.41	52.65	71.39	79.35
11	0.5	0	0	PB	1	28.31	49.46	75.20	78.23
12	1	8	0	GJ	0	33.92	40.84	66.29	67.19
13	0.5	0	0	RJ	1	16.84	38.61	58.29	64.94
14	0.5	16	0	PB	0	25.29	42.17	78.29	74.20
15	0	0	15	PB	0	36.75	60.39	76.03	74.09
16	0	8	0	PB	0	34.53	48.93	76.29	75.84
17	1	8	0	MP	0	15.17	33.46	57.32	67.24
18	0	0	15	RJ	0	17.38	38.31	58.32	63.59

Table 4 Continued

Run	Factor A	Factor B	Factor C	Factor D	Factor E	Response 1	Response 2	Response 3	Response 4
19	1	0	15	MP	0	13.87	37.71	65.67	75.05
20	0.5	16	0	RJ	0	9.84	35.71	64.29	66.28
21	0	8	0	MP	0	18.27	39.41	58.36	55.17
22	0.5	0	30	MP	0	16.42	39.21	66.29	70.34
23	0.5	0	0	GJ	1	22.43	44.97	76.30	78.37
24	0.5	16	0	MP	0	17.70	39.48	68.50	78.30
25	0	8	0	RJ	0	15.22	38.60	57.68	57.36
26	0	0	15	GJ	0	24.69	48.76	70.17	68.04
27	1	0	15	HP	0	18.42	46.01	54.06	74.09
28	0	0	15	HP	0	23.19	41.47	58.31	59.62
29	0.5	0	30	RJ	0	10.43	35.20	58.26	64.21
30	0.5	0	30	GJ	0	30.52	49.89	46.29	72.21
31	0	8	0	HP	0	16.04	38.6	53.26	65.84
32	1	0	15	RJ	0	10.77	34.95	61.82	72.31
33	0.5	0	0	HP	1	21.23	42.01	64.30	71.30
34	0.5	0	30	PB	0	36.04	52.39	67.30	79.21
35	1	8	0	PB	0	37.67	42.03	75.26	76.00
36	0	0	15	HP	0	21.82	42.05	57.82	60.03
37	0.5	0	0	PB	1	29.88	50.27	71.26	79.81
38	0	0	15	PB	0	35.51	59.25	78.90	75.32
39	0.5	0	0	RJ	1	16.06	38.07	59.71	67.83
40	0.5	0	30	PB	0	34.12	52.91	68.02	78.00
41	0	0	15	RJ	0	16.63	37.59	59.02	62.08
42	0.5	16	0	GJ	0	26.16	42.98	76.02	80.36
43	0.5	16	0	HP	0	16.79	39.78	58.03	68.29
44	0.5	0	30	GJ	0	29.83	50.17	51.20	75.36
45	0	0	15	MP	0	22.87	39.83	58.39	61.31
46	0.5	0	30	RJ	0	11.91	36.84	54.23	62.82
47	1	0	15	RJ	0	9.85	35.02	62.35	73.62
48	1	8	0	GJ	0	32.73	41.82	64.37	67.98
49	1	0	15	MP	0	12.03	36.65	66.85	74.97
50	1	8	0	PB	0	34.54	44.81	68.27	80.00
51	1	8	0	RJ	0	13.14	35.61	56.16	55.00
52	0.5	0	0	GJ	1	23.71	47.21	73.29	76.91
53	0.5	0	30	MP	0	16.89	37.21	61.23	74.20
54	1	8	0	MP	0	17.75	35.06	53.84	68.21
55	1	8	0	HP	0	18.91	37.23	59.69	58.08
56	0.5	16	0	PB	0	26.67	43.52	76.39	72.03
57	0.5	0	30	HP	0	22.96	42.86	59.23	65.20
58	1	0	15	GJ	0	21.92	48.05	74.54	64.37
59	0	8	0	MP	0	17.96	38.21	56.21	55.01
60	0.5	16	0	MP	0	16.66	38.63	69.20	72.20
61	0.5	0	0	HP	1	22.18	4.37	59.07	68.21
62	1	0	15	PB	0	33.94	50.32	69.02	78.79
63	0.5	0	0	MP	1	20.27	38.94	63.08	73.08
64	0.5	16	0	RJ	0	10.58	34.81	67.30	69.20
65	0	8	0	RJ	0	15.86	35.89	52.39	54.21
66	0	8	0	HP	0	17.72	37.41	48.29	62.27
67	0	8	0	GJ	0	26.93	42.09	54.12	71.98

Table 4 Continued

Run	Factor A	Factor B	Factor C	Factor D	Factor E	Response 1	Response 2	Response 3	Response 4
68	0	0	15	GJ	0	23.94	52.42	69.18	69.91
69	0	8	0	PB	0	34.23	49.5	71.02	78.21
70	1	0	15	HP	0	19.93	45.52	55.18	74.58
71	0.5	16	0	RJ	0	11.70	36.19	68.06	64.20
72	0.5	16	0	PB	0	25.94	44.53	76.06	80.91
73	0.5	16	0	GJ	0	28.22	44.39	72.20	79.20
74	0	0	15	GJ	0	25.01	50.59	70.61	68.87
75	0.5	16	0	MP	0	13.37	36.47	70.19	76.02
76	1	8	0	GJ	0	33.49	44.65	65.29	68.29
77	0.5	0	30	PB	0	35.35	53.67	67.29	79.30
78	0.5	0	0	MP	1	22.12	47.21	65.28	73.17
79	0.5	0	30	MP	0	18.03	38.21	64.39	74.35
80	1	0	15	MP	0	14.70	37.15	66.03	75.62
81	1	8	0	HP	0	17.24	33.48	61.28	60.75
82	0	8	0	MP	0	17.71	35.68	58.20	58.48
83	1	8	0	RJ	0	15.20	34.89	59.36	55.21
84	0	8	0	GJ	0	28.69	44.86	51.20	70.21
85	0	0	15	MP	0	21.83	40.97	59.36	61.95
86	0.5	0	0	GJ	1	24.22	46.51	76.20	74.18
87	1	8	0	PB	0	38.09	43.78	71.81	78.10
88	0.5	0	30	HP	0	21.82	41.08	58.69	68.51
89	0.5	0	30	RJ	0	10.87	34.02	58.69	58.10
90	0.5	16	0	HP	0	16.66	39.40	58.09	73.10
91	1	0	15	PB	0	34.72	51.69	70.85	78.95
92	0.5	0	0	HP	1	23.37	46.71	62.28	69.19
93	0	0	15	PB	0	34.83	60.85	77.53	76.05
94	1	0	15	RJ	0	10.14	35.59	62.72	73.95
95	0	8	0	PB	0	32.55	46.95	75.62	78.51
96	0	0	15	RJ	0	16.02	38.65	58.95	63.98
97	1	0	15	GJ	0	22.87	67.07	73.09	6.52
98	0.5	0	0	PB	1	32.68	52.76	73.32	80.27
99	0.5	0	0	RJ	1	15.75	36.21	56.28	65.05
100	1	0	15	HP	0	78.94	45.24	55.78	75.27
101	0	8	0	HP	0	18.05	36.78	51.20	67.26
102	0	8	0	RJ	0	17.16	37.28	56.20	57.61
103	0.5	0	30	GJ	0	29.33	50.11	49.29	74.64
104	0	0	15	HP	0	22.88	40.83	58.76	59.17
105	1	8	0	MP	0	18.47	36.17	60.37	65.49

Factor A: solvent fraction MeOH. Factor B: maceration hours. Factor C: sonication time (min). Factor D: location name. Factor E: Soxhlet. Response 1: Extractive value (% w/w). Response 2: DPPH inhibition (% mcg/mL). Response 3: TFC (mg QE/g). Response 4: TPC (mg GAE/g). HP: Himachal Pradesh. PB: Punjab. MP: Madhya Pradesh. RJ: Rajasthan. GJ: Gujarat.

Table 5 Ranges of extractive value, DPPH, TFC, and TPC in different vicinities

Location	Extractive value (% w/w)	DPPH (% mcg/mL)	TFC (mg QE/g)	TPC (mg GAE/g)
Punjab*	25.29 - 38.09	42.03 - 60.85	67.29 - 78.90	72.03 - 80.91
Gujarat*	21.92 - 33.92	40.84 - 67.07	46.29 - 76.30	64.37 - 80.36
Himachal Pradesh	16.04 - 23.37	33.48 - 46.72	48.29 - 64.30	56.21 - 75.27
Madhya Pradesh	12.03 - 25.52	33.46 - 47.21	53.84 - 70.19	55.01 - 78.30
Rajasthan	9.84 - 17.38	34.02 - 38.65	52.39 - 68.06	54.00 - 73.95

*The variations meet the proposed specifications.

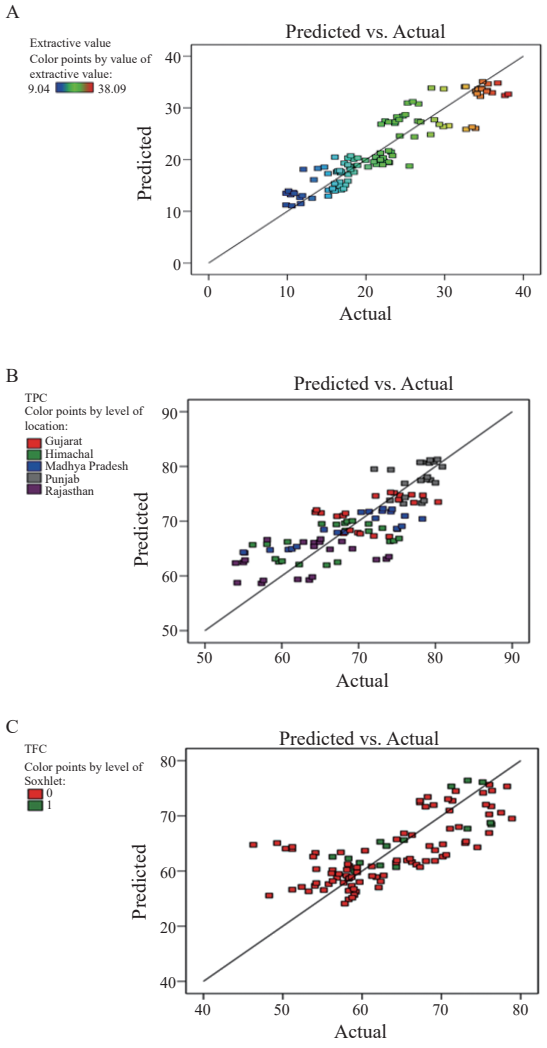

Figure 1 Response surface and contour plot within the design space, revealing the influence of solvent mixture on extractive value, TPC, and TFC

A, predicted vs. actual graph (extractive value). B, predicted vs. actual graph (TPC). C, predicted vs. actual graph (TFC).

Figure 1B depicts the design space of DPPH inhibition values using a contour plot that illustrates the relationship between the fraction of extraction solvent and the duration of maceration. The response in DPPH inhibition value shows an increase as the extraction solvent changes from methanol to distilled water, as demonstrated in Figure 2B. Table 5 demonstrates the TFC value, which ranges from 46.29 to 78.90 mg QE/g, featuring an average value of 63.56 mg QE/g and a standard deviation of 7.97 mg QE/g. According to the ANOVA, the model was statistically significant, as evidenced by an F value of 13.44. In this particular case, model terms A, B, and D are significant. The actual R^2 value is 0.5336.

In contrast, Figure 1C illustrates the design space of TFC by depicting contours that relate to the extraction solvent fraction and maceration duration. The TFC shows a progressive increase as the extraction solvent changes from distilled water to methanol. Table 5 illustrates the TPC value ranging from 54 to 80.91 mg GAE/g, with a mean value of 69.52 mg GAE/g and a standard deviation of 7.35 mg GAE/g (Figure 2C). Based on the ANOVA, the model is highly significant with an F value of 18.20. In this context, model terms A, B, C, D, and E were all significant. The actual R^2 value was 0.6077. In Figure 2D, the design space for TPC is depicted through contour lines that delineate the relationship between extraction solvent fraction and maceration duration. Notably, as the extraction solvent shifts from distilled water to methanol, there is a noticeable increase in TPC.

Finally, the optimal processing conditions were determined through a combination of numerical optimization using the desirability approach and a graphical optimization strategy utilizing overlay plots. Figures 3A and 3B depict the desirability approach graph and overlay graph, respectively.

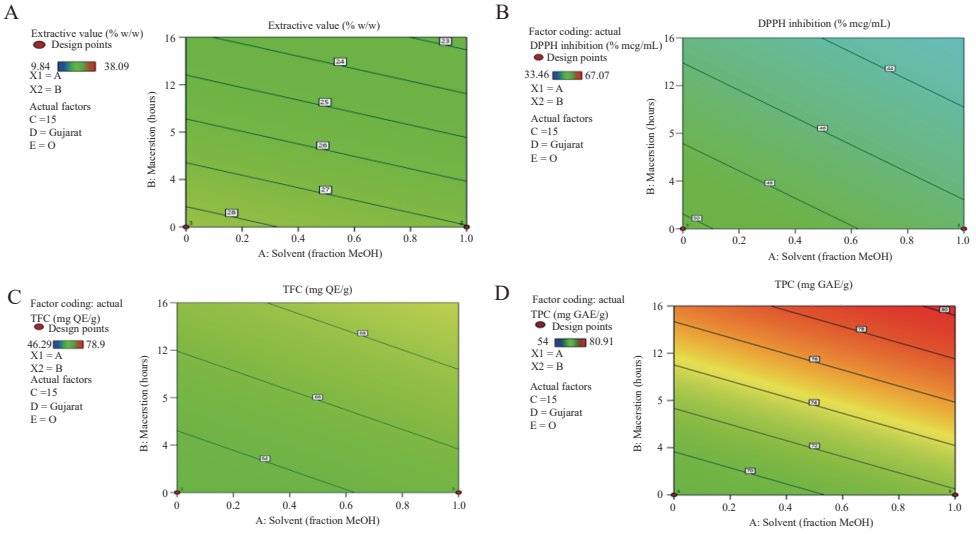

Figure 2 Contour plot for the establishment of the design space

A, extractive value. B, DPPH Inhibition. C, TFC. D, TPC.

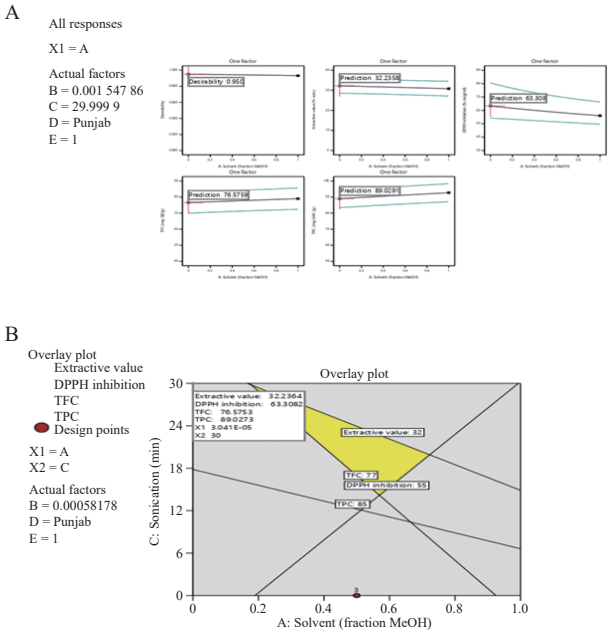

Figure 3 The desirability approach graph and overlay graph

A, desirability approach. B, graphical overlay plot.

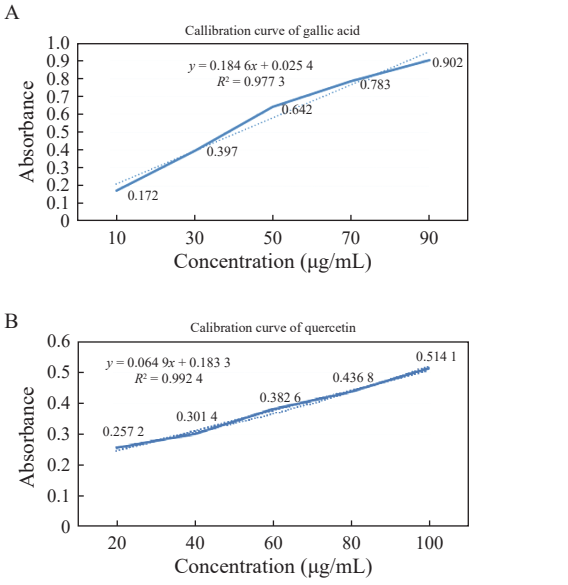

Figure 4 Preparation of calibration curve of gallic acid by spectrophotometric methods for quantitative determination of total phenolic and total flavonoid content

A, calibration curve of gallic acid (TPC). B, calibration curve of quercetin (TFC).

3.3 Estimation of extractive yield

Table 5 provides a comprehensive summary of the extractive yield ranges of *P. emblica*, obtained by refluxing (Soxhlet), maceration, and sonication procedures using water, methanol, and water-methanol. The extractive yield, expressed as the mass of extract/mass of dry matter, was a valuable metric to measure the efficacy of the extraction procedures. Supplementary Table S1 further shows the variations in the extractive value based on different extraction methods employed across various locations.

3.4 TPC estimation

The highest concentration of the TPC, measuring 80.91 mg GAE/g, was detected in the PB-HA when employing the maceration extraction method for 16 h. Figure 4A shows the calibration curve of gallic acid.

3.5 TFC estimation

The highest concentration of the TFC, measuring 80.29 mg QE/g, was identified in the PB-A when using the 30 min sonication extraction method. Figure 4B shows the calibration curve of quercetin.

3.6 Estimation of DPPH free radical scavenging anti-oxidant activity of extracts

The highest DPPH free radical scavenging activity was seen in GJ-A, with detailed results presented in Table 5.

3.7 Estimation of anti-oxidant activity

The most potent anti-oxidant inhibition activity was found in PB-A, extracted using the 30 min sonication extraction method, as indicated in Table 5.

3.8 GC-MS analysis for extracts

The GC-MS data revealed that in the case of GJ-A obtained through Soxhlet extraction, a total of 65 phytochemical compounds were identified within the extract (Supplementary Table S2). The molecules, namely 1,2,3-benzenetriol (65.86%), glycerin (5.99%) 2H-pyran-2,6(3H)-dione (4.75%), and (R)-(-)-2,2-dimethyl-1,3-dioxolane-4 (3.57%), exhibited the most significant peak area percentages, with retention times of 8.469, 6.713, 4.865, and 4.299 min, respectively. In the determination of Himachal Pradesh variation, a total of 41 phytochemical compounds were identified in the extract. The molecules, 1,2,3-benzenetriol (60.14%), 2H-pyran-2,6(3H)-dione (6.84%), and 5-hydroxymethylfurfural (4.13%) demonstrated the most significant peak area percentages, with retention times of 8.364, 4.888, and 7.241 min, respectively. Interestingly, when conducting GC analysis for PB-A (Soxhlet extraction), a total of 45 phytochemical compounds were detected in the extract. The molecules, i.e. 1,2,3-benzenetriol (42.69%), 2H-pyran-2,6(3H)-dione (4.37%), and 1,2,3-propanetriol (2.41%), exhibited the most significant peak area percentages, with retention times of 8.408, 4.864, and 6.387 min, respectively. However, in terms of MP-A variety (Soxhlet extraction), a total of 46 phytochemical compounds were

discovered in the extract. The molecules, namely 1,2,3-benzenetriol (14.15%), tetradecane (10.54%), and phosphoric acid, bis(trimethylsilyl) monomethyl (9.04%), showed the most substantial peak area percentages, with corresponding retention times of 8.371, 8.475 and 8.561 min, respectively. Moreover, in the case of the Rajasthan variety (Soxhlet extraction), the distilled water extract yielded a total of 48 phytochemical compounds. Notably, among these molecules, namely 1,2,3-benzenetriol (15.55%), tetradecane (11.28%), and phosphoric acid, bis(trimethylsilyl) monomethyl (8.54%), exhibited the most substantial peak area percentages, with corresponding retention times of 8.370, 8.477, and 8.564 min, respectively.

When utilizing the 30 min sonication with PB-HA, a total of 38 phytochemical compounds were identified in the extract. The molecules, including 1,2,3-benzenetriol (60.01%), 2H-pyran-2,6(3H)-dione (11.53%), and 5-hydroxymethylfurfura (8.42%), exhibited the most significant peak area percentage, with retention times of 8.827, 4.937, and 7.230, respectively. In contrast, when employing the 30 min sonication method for the GJ-HA variety, a total of 49 phytochemical compounds were identified in the extract (Supplementary Figure S1). The molecules, encompassing 1,2,3-benzenetriol (25.33%), 2H-pyran-2,6(3H)-dione (5.50%), and glycerin (4.81%), exhibited the most significant peak area percentage, with retention times of 8.466, 4.862, and 6.271 min, respectively.

Nearly, all of these compounds have demonstrated pharmacological activities, regardless of the concentration at which they were originally detected. Table 6 and 7 show that the extracts contain eight identical and seven different compounds, each possessing a diverse range of pharmacological activities. The chromatograms of *P. emblica* (Rajasthan) D.W. Soxhlet, *P. emblica* (Punjab) hydro-alcohol sonication 30 min, *P. emblica* (Madhya Pradesh) D.W. Soxhlet, *P. emblica* (Punjab) D.W. Soxhlet, *P. emblica* (Gujarat) hydro-alcohol sonication 30 min, *P. emblica* (Himachal Pradesh) D.W. Soxhlet, *P. emblica* (Gujarat) D.W. Soxhlet, are all revealed in Figure 5.

4 Discussion

The pharmacologically active compounds are often found in minute concentrations within plants. An efficient extraction method yields a high amount of extract with minimal need for alteration to the fundamental extraction processes. The efficacy of a solvent in extracting a substance primarily depends on the solubility of the material in the liquid, the thermodynamics of heat and mass transfer associated with the substance, and the efficiency of the concentration encounter, including constraints related to thermal dispersion frequency. In conventional extraction, energy is dissipated through conduction and convection from the outside. While standard solvent extraction has been extensively utilized to study the separation of phytochemical substances from plants, the current study sought to investigate how different extraction procedures affected the yield and phytochemical features of *P. emblica*.

The model consists of main effects, without observed interactions. The model equation for the extractive value is expressed using the square root function.

Table 6 Common compounds with their biological activity found in the sample extracts of GC-MS

No.	Compound	Biological activity	Reference
1	1,2,3-Benzenetriol	Anti-microbial activity	[23]
2	Tetradecane	Anti-microbial activity	[24]
3	Tetradecanoic acid	Anti-microbial, anti-oxidant, and anti-inflammatory activities	[25]
4	1,2-Benzenedicarboxylic acid	Anti-microbial activity	[26]
5	Hexadecane	Anti-microbial and anti-oxidant activities	[27]
6	Hexadecanoic acid	Anti-bacterial and anti-oxidant activities	[28]
7	Nonane	Anti-oxidant activity	[29]
8	1,2,3-Propanetriol	Anti-microbial activity	[27]

Table 7 Uncommon compounds and their biological activity found in the sample extracts of GC-MS

No.	Compound	Biological activity	Reference
1	Squalene	Antibacterial, anti-microbial, and anti-oxidant activities	[30]
2	Tetracosane	Antibacterial activity	[31]
3	Glutaric acid	Antibacterial activity	[32]
4	Sulfurous acid	Antibacterial activity	[33]
5	Benzoic acid	Anti-inflammatory and anti-oxidant activities	[34]
6	Docosane	Anti-microbial activity	[35]
7	Heneicosane	Anti-microbial activity	[36]

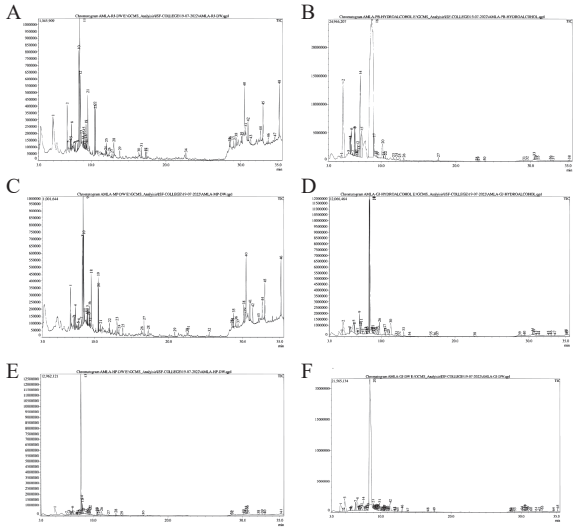

Figure 5 GC-MS chromatograms of *P. emblica*

A, *P. emblica* (Rajasthan) D.W. Soxhlet. B, *P. emblica* (Punjab) hydro-alcohol sonication 30 min. C, *P. emblica* (Madhya Pradesh) D.W. Soxhlet and *P. emblica* (Punjab) D.W. Soxhlet. D, *P. emblica* (Gujarat) hydro-alcohol sonication 30 min. E, *P. emblica* (Himachal Pradesh) D.W. Soxhlet. F, *P. emblica* (Gujarat) D.W. Soxhlet.

The mathematical models for various responses are provided in coded equations.

The extractive value = **− 0.10 * A − 0.23 * B** − 0.13 * C + **0.52 * D1 − 0.20 * D2 − 0.41 * D3 + 1.10 * D4** − 0.054 4 * E **+ 4.45**

The DPPH inhibition value = **2.14E-05 * A + 2.99E-05 * B** − 1.72E-05 * C **− 8.33E-05 * D1 + 1.57E-05 * D2 + 6.50E-05 * D3 − 1.17E-05 * D4** − 1.95E-05 * E **+ 3.67E-04**

The TFC value = 1.23 * A + 2.38 * B + 0.92 * C **+ 1.88 * D1 − 5.86 * D2 − 1.26 * D3 + 9.55 * D4 + 2.77 * E** + 66.95

The TPC value = **1.86 * A + 4.35 * B + 4.97 * C + 2.14 * D1 − 3.07 * D2 − 0.83 * D3 + 8.16 * D4 + 5.02 * E** + 77.10

The coding function employed − 1 for the lower factor value and + 1 for the highest factor value. According to the Ayurvedic Pharmacopeia of India, a standardized extract of *P. emblica* should exhibit certain criteria: an extractive value of > 30% (w/w), DPPH > 60% mcg/mL, TFC > 75 mg QE/g, and TPC > 85 mg GAE/g. However, based on our study, we proposed the specifications of a standardized extract of *P. emblica* as follows: an extractive value of ⩾ 30% (w/w), DPPH ⩾ 60% mcg/mL, TFC ⩾ 75 mg QE/g, and TPC ⩾ 80 mg GAE/g. Table 5 shows the ranges of the extractive value, DPPH, TFC, and TPC of *P. emblica* from different locations.

The model proved highly effective in explaining the variability of extractive value, DPPH, TFC, and TPC with respect to the model terms, which encompassed the composition of the extraction solvent, duration of maceration, duration of sonication, location of sample collection, and the use of Soxhlet method. Notably, statistically significant terms ($P < 0.05$) in the mathematical model are indicated in bold. Specifically, the composition of the extraction solvent and the duration of maceration exhibited a significant impact on extractive value, DPPH activity, and TPC ($P < 0.05$). The Soxhlet extraction process, on the other hand, showed a significant impact on TFC and TPC ($P < 0.05$), whereas the duration of sonication had a significant impact on TPC ($P < 0.05$).

5 Conclusion

Phytochemicals derived from medicinal plants are gaining popularity nowadays. These medicinal herbs are not just consumed individually as single bioactives, but are more commonly employed as components of polyhebral formulations, comprising various bioactives extracted from medicinal herbs in a fixed combination. The QbD approach offers a fresh perspective on extricating potent bioactives for their industrial-scale production. This methodology can be systematically integrated into the optimization of commercial processes for Indian formulations. In addition, the QbD approach helps reduce variations among different batches of raw materials, which may be sourced from different vicinities.

To conclude, this study demonstrates that the QbD approach can markedly enhance the quality of the extraction process of the medicinal herbs. Furthermore, it paves the way for the development and manufacturing process in the herbal industry, ensuring the delivery of high-quality products.

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Competing interests

The authors declare no conflict of interest.

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基于质量源于设计理念研究不同地区余甘子的关键性状

Grishm Rohilla[a], Priya Masand[b], Pooja Dhama[a], Anurag[b], Sunil Gupta[c], Alok Sharma[b*]

a. Department of Pharmacognosy, ISF College of Pharmacy, Moga, Punjab 142001, India
b. Department of Pharmaceutical Technology, Meerut Institute of Engineering & Technology, Meerut, Uttar Pradesh 250005, India
c. School of Pharmacy, Mangalayatan University, Aligarh, Uttar Pradesh 202146, India

【摘要】目的 探讨质量源于设计（QbD）工具在评估印度 5 个不同地区余甘子地理变异中的应用。**方法** 在本实验中，采用简化四次模型的 Box-Behnken 设计，运行 105 次，结合使用 Design Expert 软件进行随机响应面映射。本研究使用了三种不同的浸提方法（索氏浸提、浸渍浸提和超声浸提）以及三种溶剂 [蒸馏水、甲醇和水-甲醇混合物（50 : 50 v/v）]。采用气相色谱-质谱法（GC-MS）测定并分析余甘子的抗氧化活性、总黄酮含量（TFC）和总酚含量（TPC），以鉴定其主要成分。**结果** QbD 叠加图显示余甘子的提取值不低于 30% w/w，2，2-二苯基-1-三硝基苯肼（DPPH）不低于 60% mcg/mL（微克每毫升），TFC 不低于 75 mg QE/g（毫克槲皮素当量每克干原料），TPC 不低于 80 mg GAE/g（毫克没食子酸当量每克干原料）。此外，（GC-MS）数据证实了余甘子的生物活性存在变异。**结论** 本研究模型能较好的描述浸提值、DPPH、TFC 和 TPC 的变化。QbD 方法可能倾向于在浸提过程中优先考虑彻底性，最终提高浸提产品的质量。

【关键词】余甘子；质量源于设计；气相色谱-质谱法；浸提参数；生物活性