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Systematic Extraction Studies for High Recovery of Catechin from *Saraca asoca* (Roxb.) Willd. Seeds Following Analytical Quality by Design (AQbD)

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ABSTRACT

Background: Bioactive constituents from medicinal plants have been used to treat many diseases. Preparation of extract is the critical step for properly isolating active ingredients from the cellular matrix and further utilization in the manufacturing of herbal formulations. The extraction process is affected by many parameters and slight change in the values of these factors could alter the extraction of bioactive. Therefore, it is very crucial to study the effect of parameters properly. Quality by Design is emerging as a new technology to obtain high extractive values with bioactive components. *Saraca asoca* (Roxb.) Willd. (Caesalpiniaceae) has been traditionally used in the management of many diseases. Mainly the stem bark of this plant is used, but other parts also possess many biological activities because of catechin. In the present study, the Quality by Design-enabled extraction process was utilized for maximum extraction of catechin from the seeds of *Saraca asoca* instead of bark as the conservation strategy. The excessive usage of stem bark may affect the plant's physiological process and decrease the plant's life expectancy.

Results: Preliminary studies revealed the use of reflux technique and 75 % ethanol as solvent for catechin extraction. The effect of three variables (60–80 mL/g, 60ºC to 80ºC and 20–40 min) chosen by one factor at a time method was again studied and optimized at three-level by Box-benkhen design. This final optimization by Box-benkhen design have suggested using the reflux technique with 74.71 mL/g of 75% ethanol for 30.65 minutes at 69.70ºC for maximum catechin extraction from the *Saraca asoca* seeds. The statistical analysis of the developed method effectively explain the effect and significance of each selected parameter for catechin content.

Conclusions: This is the first report on optimizing extraction conditions for maximum catechin extraction from *asoca* seeds. The developed extraction method could be helpful for all the extraction processes involved in plant standardization and manufacturing of herbal formulations. Furthermore, this study supports the rational substitution of *Saraca asoca* bark with seeds for the utilization of catechin.

Keywords: *Saraca asoca*, Quality by design, extraction, catechin, design of experiment, Box-Behnken design.

BACKGROUND

Saraca asoca (Roxb.) Willd. (Caesalpiniaceae) popularly known as Sita-Ashoka, is an important indigenous medicinal plant [1]. In the Indian system of medicine, different parts of this sacred evergreen tree, *viz*. Bark, flowers, seeds, pods, and roots were used to treat various disorders. Although the stem bark of this plant is mainly used, other parts like leaves, stems, seeds, and fruits also possess many biological activities on account of polyphenols presence [2–4]. The seeds are traditionally used for cough and ingested in case of kidney stones to avoid urine flow obstruction [5]. Also, the patients are advised to eat powdered seed wrapped in betel leaves empty stomach. Many pharmacological reports are also communicated for the use of seeds. Seeds extracts are very effective against dermatophytes fungi [2]. The aqueous extract suspension of flowers, flower buds, bark, and seeds was used to treat ulcers in rats. And also useful in the treatment of skin diseases like eczema, acne, psoriasis, *etc.* [6, 7]. The seed and flower extract are also used in the treatment of rickets on account of their property to induce osteoid tissue promoter, and reduce calcium deficiency [5, 6]. The recent discovery showed that the isolated lectin 'saracin' from the seed integument induces apoptosis in human T lymphocyte in an in-vitro assay [8]. The bioactive components of different chemical classes results in pharmacological activities [9]. Catechin is an important bio active constituent of *Saraca asoca* present in bark, seed, fruits, *etc.* and is responsible for many biological activities [10,11].

A high quantity of catechin is present in stem bark, and many herbal formulations include *asoca* bark as the main ingredient, the usage of stem bark leads to excessive removal. It puts the *asoca* plant under threat, as stem bark is the outermost layer that protects the plant system from changing environmental conditions and pest attacks. Hence, to protect the plant from physiological damages, it is necessary to find another part of the plant as a source of catechin and a high recovery extraction method, as extraction is the first step towards separating bioactive components from the complex plant matrix. Thus it is very important to identify the best method for extraction [12, 13]. As the extraction process is affected by many parameters like temperature, time, solute-solvent ratio, solvent volume, etc., a little change in the values of these factors could alter the catechin extraction. So, it is crucial to develop and optimize the extraction protocol with optimized values of different parameters to separate major components from plants [14, 15]. QbD is emerging as a new optimization method and could be used in extraction technology to obtain high extractive values with bioactive components [16, 17]. Chromatographic studies, especially HPLC, are suitable for the resolution and identification of marker components in qualitative and quantitative analysis for plant drug standardization [18, 19]. In this view, the present study was carried out to design an extraction process for maximum extraction of catechin from the seeds of *Saraca asoca* following QbD and marker-based HPLC analysis.

METHODS

Chemicals and solvents

All the solvents used in the present analysis were of HPLC grade and procured from different manufacturers. HPLC grade Methanol (Finar), HPLC water (Merck, Mumbai) and Ortho-phosphoric acid (Merck, Mumbai) were used in the current work. The analytical standard catechin purchased from Sigma-Aldrich.

Plant material

Matured seeds of S. *asoca* were collected from the geographical location with GPS data (Latitude: N 22° 34' 35.676"; Longitude: E 88° 26' 4.733") in the months of May; 2022 (Figure 2). The identity of plants was authenticated by botanical and chemical standardization as per the Ayurvedic Pharmacopoeia of India standards. Voucher specimen was also submitted in the museum-cum-herbarium of the Institute for all future references. Seeds were washed, shade dried, and pulverised to get a coarse powder.

Sample and standard preparation

Powdered drug (5 grams) was refluxed with 100 mL of methanol for 30 minutes and filtered (whatmann filter paper, 20 µm pore size). The filtrate was dried up wholly and solvent was recovered in a rotary evaporator under reduced pressure. The residue was reconstituted in HPLC grade methanol to obtain a concentration 1 µg/µL and filtered with nylon syringe filter (0.22µm) prior to HPLC studies. For standard solution preparation, exactly weighed 10 mg of each of catechin dissolved in 10 mL of HPLC grade methanol to obtain a concentration of 1 µg/µL (stock solution).

Preliminary Screening

In the preliminary studies, effect of different parameters were studied to find the effective range of independent variables following one factor at a time studies.

Effect of extraction solvent

The selection of extraction solvent was made by studying the effect of four different solvents of increasing polarity (ethyl acetate, ethanol, methanol and water). 2 grams of plant material was refluxed in 100 mL of different solvents separately for 2hrs at their respective boiling point. The solvent that results in maximum residue weight is used in the next step.

Effect of extraction technique

Four different techniques were also studied namely, reflux (2 hours), soxhlet (8 hours), sonication (45 minutes) and cold maceration (24 hours). 2 grams of plant material was extracted in 100 mL of selected solvent using reflux and soxhlet, at the boiling point of the selected solvent and at room temperature using sonication and cold maceration. The technique with highest residue weight was used in next step.

One-factor-at-a-time (OFAT) studies

The effect of other parameters viz. ethanol percentage (25, 50, 75, 100 %), extraction time (10, 20, 40, 80, 160 minute), extraction cycle (x, 2x, 3x, where x is number of cycle), extraction temperature (50, 60, 70, 80, 90 ºC) and solvent volume (20, 40, 60, 80 and 100 mL/g) were studied by OFAT method. At each step of extraction, 2 g of plant material was used and only one parameters was varied by keeping all other parameters constant at pre-selected conditions and range of independent variables were selected by monitoring the changes in residue weight of extracts for further studies.

High performance liquid chromatography

Instrumentation and chromatographic conditions

Agilent HPLC system (model: 1260 series, Infinity-II) with an auto sampler, quaternary pump and diode-array detector was used in the present analysis. Acquired data from online signal were analyzed by Open Lab software. In the present study agilent HPLC column with dimensions 150 mm length, 4.6 mm internal diameter and particle size 45 µm was used. Chromatographic study was done in gradient mode. The mobile phase consists of A: 0.1% orthophosphoric acid (OPA) in HPLC water and B: methanol. The mobile phase were filtered through a nylon membrane filter (0.22 µm) and degassed by sonication prior to HPLC studies.

Calibration curve of standards

The stock solution of catechin, (1 µg/µl) were appropriately diluted with methanol to prepare five serially diluted working concentration 5.0, 10.0, 20.0, 40.0 and 80.0 µg/ml by transferring 50, 100, 200, 400 and 800 µL of stock solution into 10-mL volumetric flasks and diluting with methanol up to mark, respectively. For each of the working concentration 10 µL was injected in triplicate and area under the curve at wavelength 270 nm was recorded.

Experimental design

After proper inspection of different parameters in preliminary studies, Design expert software version 7 (Stat-Ease Inc., Minneapolis) was utilized for the development and optimization of extraction procedure using Box-Behnken design of RSM. The studies were done at three-level (-1, 0, +1) and the interactive effect of three selected factors on the content of catechin were done. The design of experiments has total 17 runs and optimization was done to maximize the content of catechin. Table 1 depicts the levels of independent variables. Actual and coded values of variables in different combinations are listed in Table 2.

Second-order polynomial equation was used to check the fitness of response variable and to obtain the regression coefficients:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC$$

Where, Y is the response; A, B and C are the variables; β_0 is the regression coefficient for intercept; β_1, β_2 and β_3 represent the regression coefficients for linear terms; β_{11}, β_{22} and β_{33} are regression coefficients for quadratic terms and β_{12}, $\beta_{13}$and β_{23} are regression coefficients for cross product terms. Analysis of variance was used to test the results statistically for the determination of quality assessment parameters of the model.

RESULTS

Preliminary studies

The suitable solvent and extraction technique were selected based on the residue weights of obtained extract, as extracts with high residue weight contain high catechin. Out of the four solvents and techniques, ethanol as an extraction solvent and reflux as an extraction technique results in the highest residue weight of extracts and hence, catechin content. The residue weight obtained by soxhlet techniques is slightly lesser than reflux; results are depicted in Figure 3 (a and b).

Independent variables selection (One-factor-at-a-time)

OFAT protocol was followed to determine the range of independent variables; the results of OFAT studies are presented in Figure 3 (c, d, e, f, and g). The temperature effect was studied at five levels, and the maximum residue was obtained at 70°C but with minimal variation from 60-80°C. The effect of solvent percentage was also studied between 25%-100% ethanol; 75% ethanol results in maximum residue weight followed by 100 % ethanol but with a high difference in residue weight. The variation of residue weight with the solvent volume of 75 % ethanol was also studied at five levels; maximum residue weight was observed for 60 to 80 mL/g of the sample with marginal difference. The extraction time between 20 to 40 minutes results in comparable residue weight; the extraction time was further confirmed by studying the extraction cycle. It was experimentally found that only one cycle was sufficient for extraction as the gain in the residue was found to be less than 10 % for the next cycle. Hence, parameters: time (20-40 min), temperature (60-80°C), and solvent volume/g of the drug (60-80 mL/g); effects the extraction procedure most, and as the residue weight between the mentioned levels are almost the same, and it requires further detail study. At the same time, parameters like extraction cycle (01 cycles), 75 % ethanol, and reflux extraction technique were kept constant.

HPLC method development and validation

For quantitative estimation of catechin in *asoca* seeds (Figure 2), a robust HPLC method was developed, and a well-resolved peak was obtained with mobile phase A: 1 % ortho-phosphoric acid (OPA) and B: Methanol in gradient mode. The separation was achieved by maintaining a gradient flow rate of 0.8 mL/min with 5 %-20% B (0-10 minutes), maintained at 20 % B (10-20 minutes), and 20 %-5% B (20-30 minutes). The injection volume used was 5 µl, and detection was made at wavelength 270 nm. A 5-level calibration curve was constructed, as shown in Figure 4. Figure 5 represents the catechin marker peak and catechin in seed extract.

Design of experiment

Further studies on three selected independent variables- time (A), temperature (B), and solvent volume/g of the drug (C); for catechin content (R1) were carried out by applying second-order polynomial equation; the levels of these parameters are given in Table 1. A total of 17 runs were performed for the optimization of the extraction process in the present Box-Behnken Design (BBD); the design of the extraction method and results of catechin content of different runs are presented in Table 2. The data obtained were used for the prediction of an optimum setting of extraction conditions with high catechin recovery. The ANOVA was used for statistical analysis of significant or non-significant terms. The values of regression coefficients, quality parameters, and lack of fit are given in Table 3. The effects of variables on each catechin content are represented in Figure 6. Figure 7 shows the correlation between predicted and actual values. Figure 8 shows the overlay of different experimental runs for catechin content.

Effect of parameters on Catechin content

The catechin content obtained for different combinations of design matrix is depicted in Table 3, and they ranged from 0.437 to 2.297 mg/g. The highest content was achieved under 70°C, 70 mL/g solvent, and 30 min, while the lowest was noticed at 80°C, 70 mL/g solvent, and 40 min. The model was highly significant ($p<0.0001$) with the experimental results, while analysis of variance (ANOVA) shows significant ($p<0.0001$) linear (B and C) and interactive (AC) effects on the catechin content of extracts. The non-significant value of lack of fit (F-value= 3.93) shows the excellent fit of the model to the experimental data (p-value=0.1098). The coefficient of determination R^2 was 0.9973, predicted- R^2 0.9670, and adjusted-R^2 0.9939. The graph between predicted and actual values is depicted in Figure 7. The fitted second-order polynomial equation derived from software using RSM is given below:

$$Y = +2.27+0.042*A +0.14*B +0.058*C +0.028 * A * B +0.12 * A * C +0.051* B * C -1.08 * A2-0.63* B2-0.022* C2$$

The same results are visualized in 3-D response surface plots given in Figure 6.

Model verification

Further the developed model was validated using numerical optimization method. The parameter as input was entered in a specific range to achieve maximum response i.e. catechin content. The solution with 30.65 min, 69.70°C and 74.71 mL/g with highest desirability (1) was used in the extraction procedure. The results obtained experimentally and software predicted are almost same as given in Table 4.

DISCUSSION

Extraction is the initial step to separate the active constituents from the complex plant matrix. Moreover, involves by many parameters like temperature, time, solute-solvent ratio, solvent volume, etc., that can affects the extraction rate with a slight change in their values. So, it is crucial to develop and optimize the extraction protocol for separating major components from plants. In the present study, instead of stem bark of *asoca*, seeds were utilized for high recovery of catechin.

For preliminary studies residue weight of extracts was monitored as the increase in residue weight tends to increase the catechin content. The preliminary studies revealed that ethanol as an extraction solvent results in the maximum residue weight that might be due to the presence of the −OH group that makes ethanol favorable for the dissolution of polar components (i.e., catechin). Also, many reports support the use of ethanol in the extraction process for the high recovery of catechins [20, 21]. The reflux technique showed a higher residue weight than the heat soxhlet method, with a minor difference. So the reflux technique (2 hours) was selected as it also took lesser time for extraction than the soxhlet technique (8 hours). So it is more convenient to use the reflux technique for further experiments [22, 23]. Temperature is the key parameter that can affect the extraction rate; with an increase in temperature, the residue weight also increases, which might be due to decreased surface tension and solvent viscosity that improves sample wetting and diffusion of solvent in the plant matrix [24-26]. The residue reached the maximum quantity at 80ºC, and after this temperature, it started decreasing, possibly because of the degradation of bioactive at a higher temperature. The residue weights from 60ºC to 80ºC were comparable with a lesser value difference, so a temperature range from 60ºC to 80ºC was selected for further optimization in RSM. The effect of aqueous ethanol concentration at 25%, 50%, and 75% was compared with the results of pure ethanol, as catechin is a polar component, high polarity solvents are selected, and many reports are available on the use of the percentages mentioned above [20, 21]. 75% ethanol resulted in the highest residue weight and was thus used in further experiments. For the proper extraction and immersion of sample, the use of optimal solvent volume is also essential as most of the plant matrix swell during the extraction [25]. 60 mL of solvent per gram of crude drug results in the maximum residue weight followed by 80 mL/g with marginal difference. So, for the other extraction processes, 60–80 mL/g solvent was selected. The duration of extraction is another important influential factor for the extraction of bioactive. In general, the extraction of components increases with an increase in the extraction time. An optimum time is required for the diffusion of solvent into the cells and the desorption of components from the cell to establish equilibration between solvent and matrix (Fick's law of diffusion) [24-26]. The residue weight increases up to 40 min and starts decreasing after 40 min, which might be due to the degradation of components on the extended extraction; difference in residue weight between 20min and 40min is significantly less. So, 20–40min was chosen for the optimization by RSM. Further confirmation of the extraction time was made through extraction cycle; it is essential to finalize the number of extraction cycle for the complete extraction of bioactive [27]. In the present case, no significant increase in the residue weight observed with the repeated extraction cycle. Hence, for the complete extraction of components from marc, only one cycle of extraction was sufficient.

As HPLC is an effective, sensitive, and precise tool for quantitative analysis [28], final optimization was done using the quantitative HPLC method for catechin to monitor the catechin content during extraction. The method was developed to resolve the selected marker compound (catechin) properly in *asoca* seeds following a hit and trial methods by trying a variety of mobile phases in different pH, in isocratic and gradient modes. The final extraction optimization studies were done by the Box Benkhen design of RSM. The experimentally obtained results showed that for all three selected components, maximum AUC was obtained with 70°C, 70mL/g, and 30min. The ANOVA was used for the statistical determination of significant terms in the present model. The values of regression coefficients of linear, quadratic, and interactive terms for a different response and quality assessment parameters are given in Table 3.

For catechin content, the Coefficient of determination (R2) explains the effectiveness of the model and is found close to 1 as desirable. The Predicted- R2 and Adjusted-R2 were in reasonable agreement with the difference of less than 0.2 and showed a good degree of correlation between actual and predicted values (Figure 7). Whereas the adequate precision that measures the signal-to-noise ratio was more significant than 4 (46.269), showing the model was fit for optimization. Further, the Significance of each Coefficient was determined by f-value and p-value. At the same time, the lack of fit that measures the adequacy of the model was not-significant for catechin content with a small f-value and high p-value. On the basis of regression coefficient values, only linear A, B, and C (significant positive effect); interactive terms AB, AC, and BC of the model exhibited positive influence, while all quadratic terms show a negative influence. The positive values represent that with an increase in the values of the parameter, the catechin content also increases, but the negative quadratic terms represent a significant increase in values that tends to decrease the catechin content. The same results are visualized in 3-D response surface plots given in Figure 6. As the solvent volume increases, catechin also increases significantly; the observed results are based on the fact that on increasing the solvent volume, solute-solvent interaction and driving force for mass transfer increases [25]. Also, a significant positive effect of temperature was found on catechin content, which might be due to the reason that increased temperature, decreases the surface tension and solvent viscosity that improves sample wetting and diffusion of solvent in plant matrix to the solubility of the solute [24,25]. So, the content of catechin content increases from 60 to 70 ºC and reach a maximum of 70ºC, beyond 70ºC content starts decreasing, probably due to the degradation of bioactive at higher temperature as flavonoid are temperature sensitive [26]. Also, increase in time, the extraction

increases but only up to some time after that the catechin starts degrading. In the Figure 7, resulted values are evenly distributed by the 45° line that shows a good fit of the optimized method. It also provides a clear idea of good correlation between predicted values and actual values. Furthermore, the model is verified and a high desirability solution was obtained. The experimentally obtained and software-predicted catechin content are in 97.95% agreement. Hence, the software-predicted solutions can be utilized for all future extractions. In the present work, for the first time catechinwas extracted from seeds of *asoca* plant in high amounts that can be utilized in future formulations and products based on catechin.

CONCLUSIONS

For the preparation of extracts with high content of bioactive, careful development, optimization, and validation of the extraction process were required. Response surface methodology successfully achieved the optimization of the extraction method for catechin extraction from *Saraca asoca* seeds. The effect of selected three variables chosen by one factor at a time method was again studied and optimized at three-level by Box-benkhen design. This is the first report on optimizing extraction conditions for maximum catechin extraction from *asoca* seeds. The second-order polynomial model and ANOVA effectively explain the effect and significance of each selected parameter for catechin content. The validated technical information developed during the present study can be used for preparing *asoca* seed extract with a high level of catechin and is expected to be useful for scale-up extraction in industry and manufacturing of *asoca* seed formulations. Furthermore, this study supports the rational substitution of *Saraca asoca* bark for the utilization of catechin.

List of Abbreviations:

1. QbD: Quality by Design
2. HPLC: High Performance Liquid Chromatography
3. ANOVA: Analysis of Variance
4. RSM: Response Surface Methodology
5. BBD: Box-Benkhen Design
6. OFAT: One Factor at a Time
7. OPA: Ortho-phosphoric acid
8. AUC: Area Under the Curve

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have approved the publication of this work.

Availability of data and materials

Will be made available on request.

Competing interests

All authors hereby declare that there is no conflict of interest.

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Authors' contributions

JD and DK designed, carried out all extraction related experimental work and wrote the manuscript. JD and RB carried out statistical analysis. SD authenticated the sample and prepared museum-cum-her-barium sample. SM collected the plant sample. KH and AKM supervised all work. All authors read and approved the final manuscript.

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Plant collection and authentication

Matured seeds of S. *asoca* were collected from the premise of Central Ayurveda Research Institute, CCRAS, Ministry of AYUSH, Kolkata, West Bengal in the months of May; 2022. The identity of plants was authenticated by botanical and chemical standardization as per the Ayurvedic Pharmacopoeia of India standards. Voucher specimen was also submitted in the museum-cum-herbarium of this Institute for all future references.

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Figure legends

Figure 1: Graphical abstract.

Figure 2: *Saraca asoca* (Roxb.) Willd. Seeds.

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Figure 3: Results of preliminary screening.

Figure 4: Calibration curve of catechin.

Figure 5: HPLC chromatograms showing catechin in seed extract of *Saraca asoca.*

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Figure 7. Correlation between predicted and actual values of catechin content.

Figure 8. Overlay of different experimental extracts for estimation of catechin.

FIGURES

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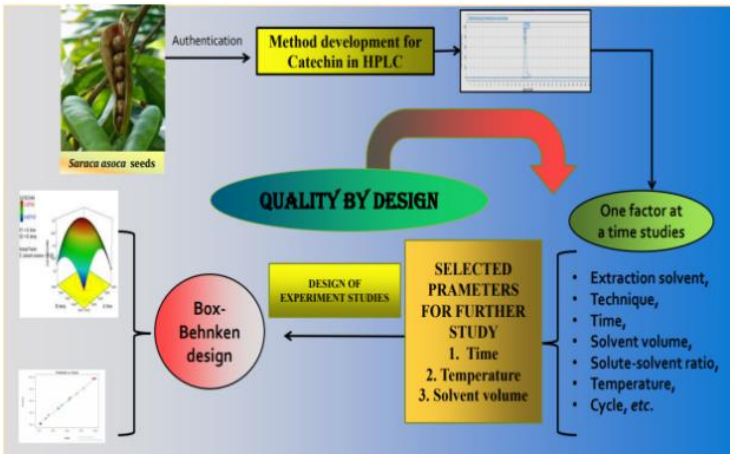
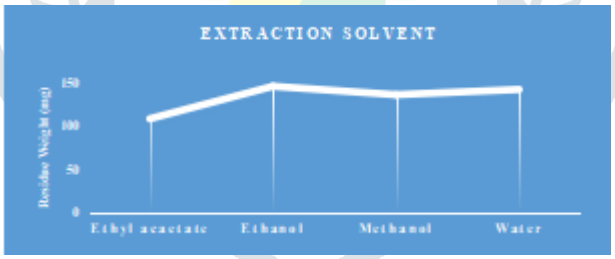


Figure 2: *Saraca asoca* (Roxb.) Willd. Seeds.



Figure 3: Results of preliminary screening.



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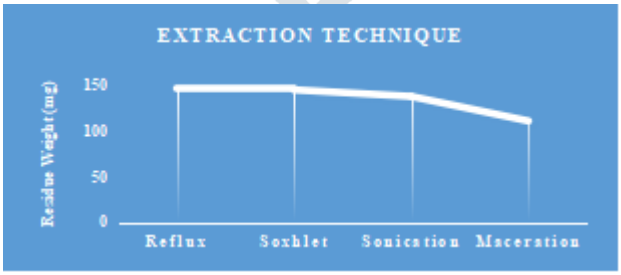

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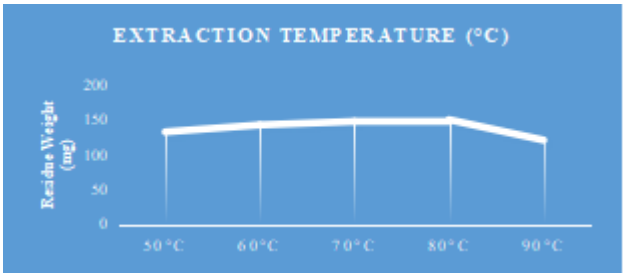

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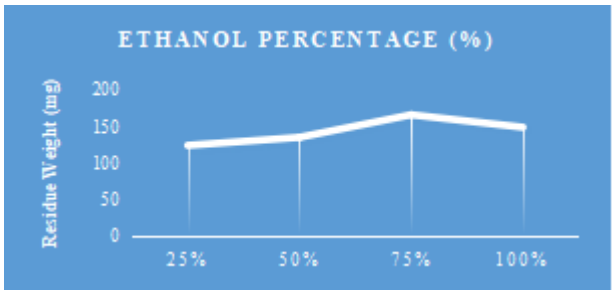

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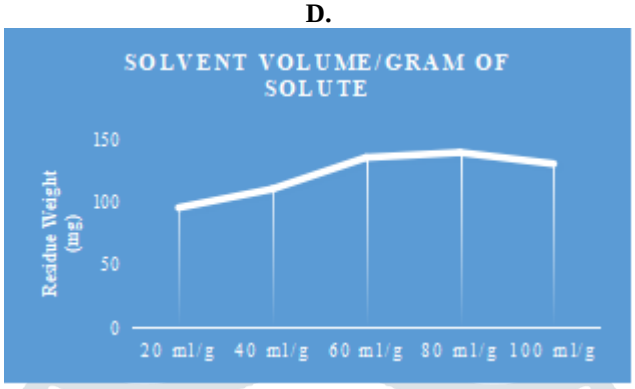

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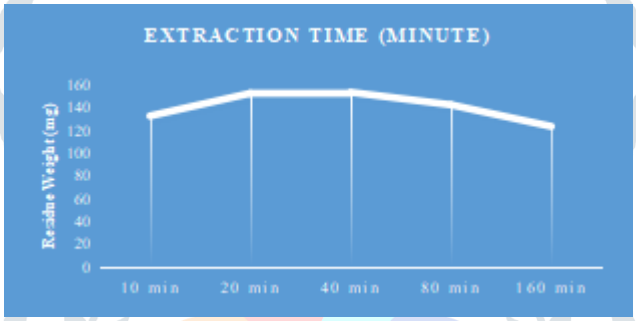

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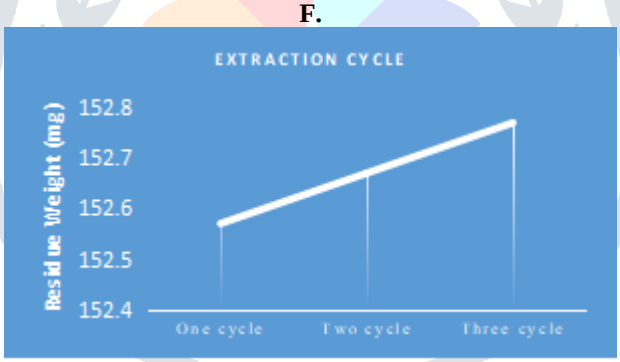


G.

Figure 4: Calibration curve of catechin.

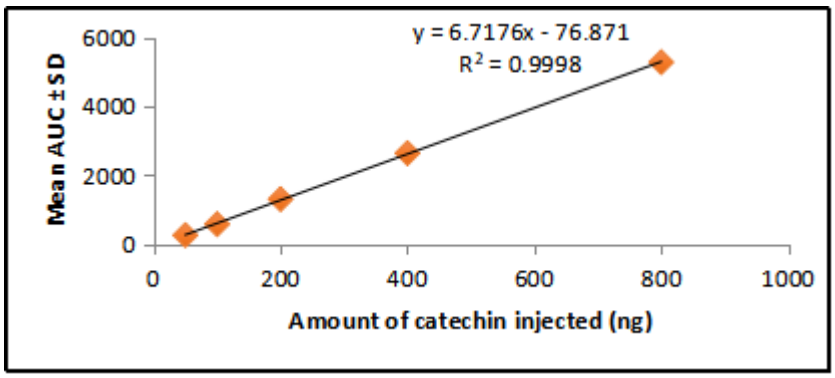
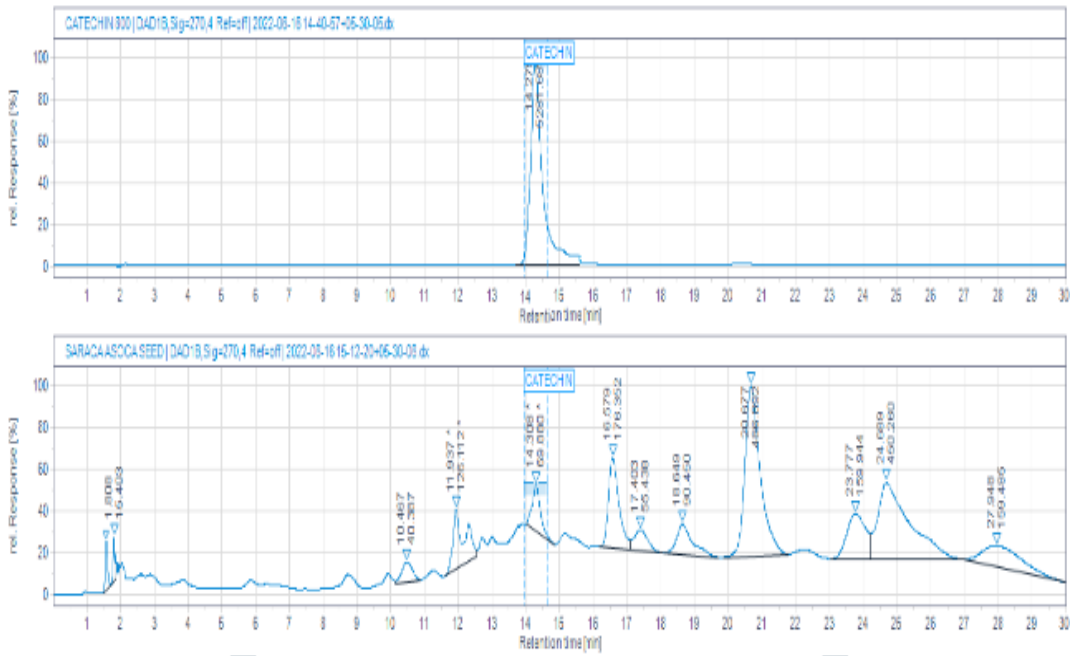


Figure 5: HPLC chromatograms showing catechin in seed extract of *Saraca asoca.*



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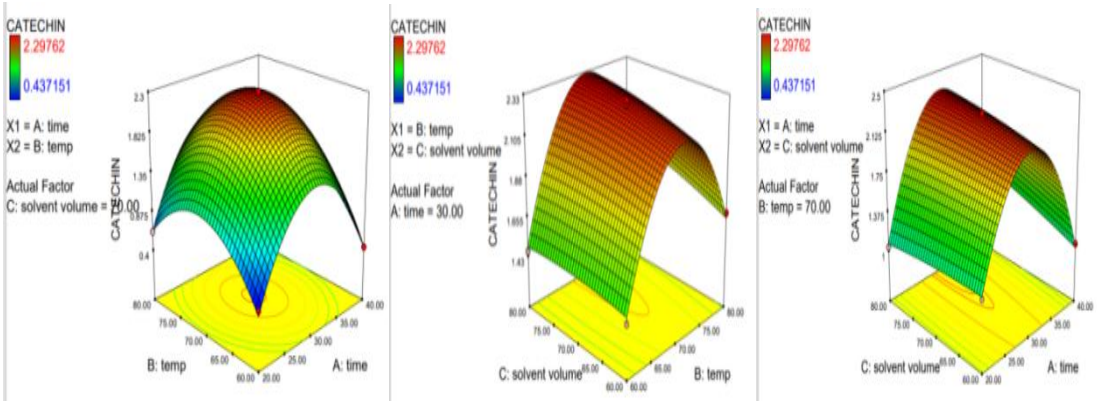
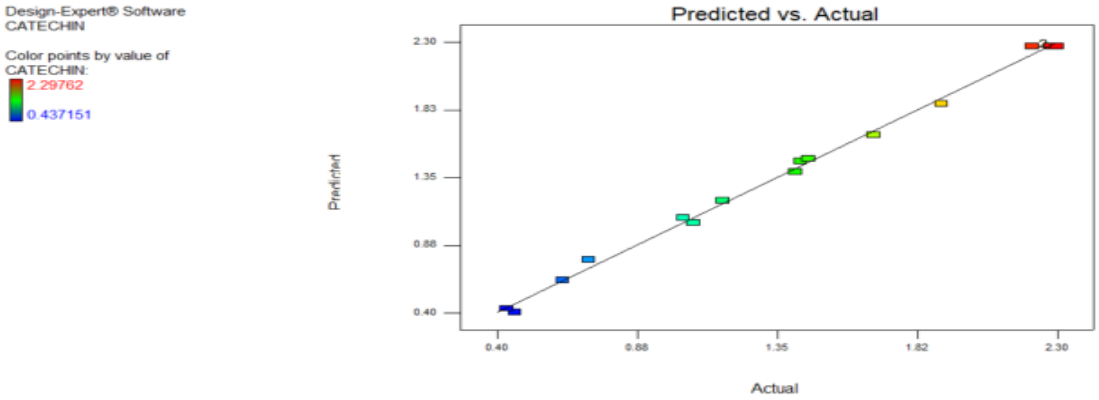


a **b** **c**

Figure 7. Correlation between predicted and actual values of catechin content.



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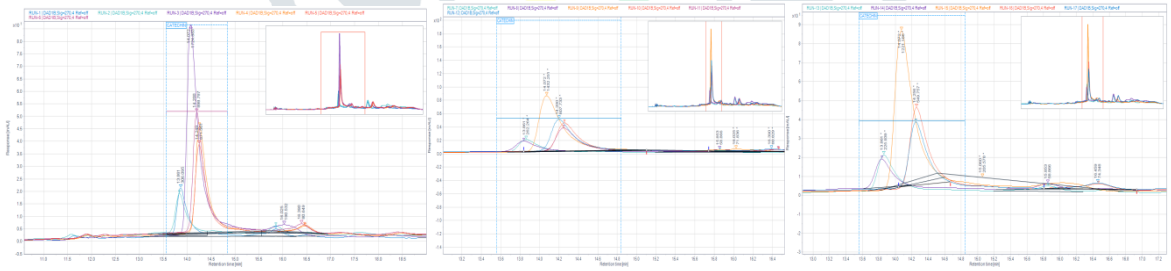

Table 1: Levels of independent variables.

Independent variables	Units	Coded levels		
		-1	0	+1
A: Time	Minute	20	30	40
B: Temperature	Celsius	60	70	80
C: Solvent volume	mL/gram	60	70	80

Table 2: Experimentally obtained results of Box-Behnken design for catechin content.

Run order	A	B	C	R 1
1	30 (-1)	60 (-1)	80 (+1)	1.457
2	20 (-1)	80 (+1)	70 (0)	0.627
3	20 (-1)	70 (0)	60 (-1)	1.166
4	40 (+1)	80 (+1)	70 (0)	0.714
5	20 (-α)	70 (0)	60 (-1)	0.463

6	40 (+1)	70 (0)	60 (-1)	1.430
7	30 (0)	80 (+1)	80 (+1)	1.907
8	30 (0)	80 (0)	60 (-1)	1.677
9**	30 (0)	70 (0)	70 (0)	2.297
10**	30 (0)	70 (0)	70 (0)	2.212
11**	30 (0)	70 (0)	70 (0)	2.275
12	20 (-1)	70 (0)	80 (+1)	1.034
13**	30 (0)	70 (0)	70 (0)	2.297
14**	30 (0)	70 (0)	70 (0)	2.281
15	40 (+1)	70 (0)	80 (+1)	1.412
16	40 (+1)	80 (+1)	70 (0)	0.437
17	40 (+1)	70 (0)	60 (-1)	1.068

*Mean ,**centre points of experimental model, R_1= Catechin content (mg/g)

Table 3. Regression coefficients and quality parameters for investigated responses.

Regression coefficients	**Values**
Intercept	
β_0	+2.27
Linear	
β_1	+0.042
β_2	+0.14
β_3	+0.058
Quadratic	
β_{11}	-1.08
β_{22}	-0.63
β_{33}	-0.022
Cross product	
β_{12}	+0.028
β_{13}	+0.12
β_{23}	+0.051
Quality assessment parameter	
F-value model	290.48
P-value model	<0.0001 (significant)
F-value lack of fit	3.93
P-value lack of fit	0.1098 (non-significant)
R^2	0.9973
Adjusted-R^2	0.9939
Predicted-R^2	0.9670
Adeq. Precision	46.269
C.V.%	3.61

*β_1= time (min.), β_2 = Extraction temperature (°C), β_3=Solvent volume (ml/g), Level of significance *p<0.05, **p<0.0001

Table 4: Predicted and experimental values of response for software predicted solution.

Response variables	**Time (min)**	**Temperature (ºC)**	**Solvent volume (mL/g)**	**Software predicted catechin content**	**Experimental catechin content**
Catechin content	30.65	69.70	74.71	2.299	2.252