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Optimization of process condition to improve percentage purity of aloe emodin from aloe vera by extraction using response surface methodology with the central composite design tool

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***Abstract*---**Extraction is a common separation technique in major chemical and pharmaceutical industries, and it has traditionally been a recommended method for separating active ingredients. The objectives of this research work were to optimize extraction conditions for the separation of derivatives of anthraquinone compounds, especially Aloe Emodin (AE) from Aloe-Vera latex (AVL) using the tool response surface methodology (RSM). This study used three process variables at different levels (20 experimental design runs) proposed by RSM with central composite design (CCD). Multiple regression analysis was used to produce a quadratic polynomial equation to predict extraction condition. The significant effects of the components were investigated using analysis of variance (ANOVA). The first series of single factor studies determined the range of independent variables, including extraction temperature (60-80°C), agitation speed (750-1250 rpm), and solid loading (10-20 gm). Based on the outcomes of single factor trials, the actual values of the independent variables coded were chosen. The optimum conditions for extraction variables for AE were found to be 77.66 °C (±1 °C), 1015 rpm (±10 rpm), and 20.15 gm (±0.01 gm). The maximum experimental purity of AE attained under these optimized settings was 95.36 percent, which was quite near to projected values. As a result, the models developed are suitable, and RSM successfully optimizes the extraction parameters.

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Keywords---aloe emodin, anthequionene, aloe-vera latex, response surface methodology, central composite design.

Introduction

It is possible to extract anthraquinones, sugars, chromones, enzymes, polysaccharides, and other medicinally active components of Aloe Vera using various extractive methods. There are more than 300 species of aloe, most of which are cultivated in Asian continent, Arabia. South Africa and South America[1]. Medicinal and aromatic species with various names: Aloe vera, *A. barbadensis* (*Barbados aloe*), A. ferox (Cape aloe), A.arborescens, A.vulgaris, A. perryi (Socotrine or Zanzibar aloe) [2]. In terms of active component composition and concentrations, each species differs from the others. Its botanical name is Aloe barbadensis miller. It is a plant that belongs to the Liliaceae (Asphodelaceae) botanical family and is arborescent, succulent, perennial, xerophytic, and pea-green in color. It takes around four years for the plant to mature, and it has a lifespan of roughly 12 years.

The aloe plant has thick leaves about 50 cm long and 12 cm broad, with yellow flowers and spikes at the margins. The new parenchymal gel from the leaf's core is transparent. The sticky latex liquid is made from anthraquinones derivatives found in the yellowish-green pericyclic tubules that border the rind of leaf [1]. Degenerated organelles, cell walls, and viscous liquid within the cells make up the structural components of Aloe Vera pulp, which are morphologically and sugar-content-wise separate from one another. Pericyclic cells, which are found just beneath the surface of the aloe vera latex leaf skin and produce bitter yellow latex known as aloe sap or simple latex, are found just beneath the surface of the aloe vera latex leaf skin. Water content in raw Aloe Vera pulp and mucilage or gel is around 98 and 99.5 percent, respectively[3]. Enzymes, organic acids, minerals, phenolic compounds, polysaccharides, water-soluble and fat-soluble vitamins, and other significant therapeutic purpose elements found in the remaining solid material present in 0.5 to 2 percent are among the targeted active components[4] . Aloe-Vera is used in hemorrhoid treatment, uterine stimulant, as laxative and anti-helminthic in Ayurvedic medicine, India's ancient medicine (menstrual regulator)[5] . Several free anthraquinones can be detected in the gel and pulp formed from leaves, including aloin, aloe-emodin, aloetic acid, anthracene, anthranol, chrysophanic acid, emodin, cinnamic acid, ethereal oil, isobarbaloin, and resistannol[6]. The above active ingredients (AI) have therapeutic effects, including anti-cancer, anti-fungal, anti-inflammatory, immunomodulatory, gastroprotective properties, hypoglycemic or antidiabetic effects, and most importantly, wound healing stimulation[7].

Standardization of extraction procedures contributes to the final quality and quantity of herbal drugs. A variety of isolation procedures have been used to separate active components from Aloe vera. Selection of proper isolation methods for AI are based on the degree of separation, the quantity of downstream processing required, and the simplicity with which the solvent may be recovered gives advantages of alcoholic solid-liquid extraction followed by alkaline treatment over other traditional extraction processes[8]. Comprehensive separation

processes, various chemical treatments required for yield augmentation, and a series of unique solvents used for each targeted element identify the most commercially viable technique[6]. Different influencing parameters for the extraction process were discovered due to several investigations undertaken by various researchers to improve extraction parameters utilizing traditional methods for each active component[9]. Aloe Vera extracts are used by many pharmaceutical and cosmetic companies in modern pharmaceuticals and skin-based cosmetics, describing it as a natural source to make cosmetics.

Aloe-emodin is an anthraquinone and isomer of emodin contained in the exudate of the aloe plant, aloe latex. It is a potent stimulant and laxative used in cathartic medicine to induce bowel movement and relieve constipation than any other herb. It has anti-inflammatory properties and is also helpful in cancer treatment[7].

Table 01 Aloe-emodin properties[10]

Sr. No.	Source and Physical Properties	Description
1.	Name	1,8-dihydroxy-3-(hydroxymethyl)anthraquinone
2.	Source	We obtained from the aloe latex as exudate from aloe plant leaves, also known as Aloe sap.
3.	Molecular Formula	$C_{15}H_{10}O_5$
4.	Molecular Weight	270.24 g/mol
5.	Melting Point	224 °C
6.	Solubility	Freely soluble in hot alcohol, benzene with yellow color, ether, ammonia water, and sulphuric acid with crimson color, toluene, and insoluble in water.

Materials and Methods

Aloe Sap as raw material

Aloe sap was collected from Yucca Enterprises, Mumbai. It is separated as a waste by-product from manufacturing commercial aloe juice, gels, powder, jelly, or whole leaf extract and then dried at room temperature to form a tough solid mass of aloe sap waste. This was ground into an ultrafine powder and sieved using mesh sizes of 20µm, 65µm, and 110 µm using a vibrating sieve shaker. During all of the test runs and the extracts condition, it was stored in an airtight plastic container at 4°C, until tested and analysed[11]

Chemicals and reagents

Analytical grade, chemically pure reagents were purchased from Finar Ltd, includes hydrochloric acid, ferric chloride, distilled water, toluene, ethyl acetate, methanol, and potassium hydroxide.

Experimental set-up and procedure

Experimental set-up consisting of a batch extractor, condenser and pump arrangement, hot water bath, and hot plate as represented in Fig. 2 (a). A vacuum

rotatory evaporator (Scientifique make) for making concentrated extract was shown in Fig. 2 (b). Ultrafine powder of Aloe sap was weighted and taken in the batch extractor, dilute HCl (60 ml) and ferric chloride (32 gm) were dissolved in distilled water (130 ml). This mixture was transferred to the extractor. The content was refluxed for 2 hrs to obtain a black residue. This residue was cooled, filtered, and then washed using distilled water till it became a neutral extract. After drying the residue, it was extracted using toluene (60 ml). This extract was concentrated using a vacuum rotatory evaporator. Then the ice cooling was given to extract, which results in the formation of Aloe Emodin crystals. The flow-chart indicates sequential procedures for isolation of Aloe Emodin- aloe anthraquinones were shown in Fig 01.

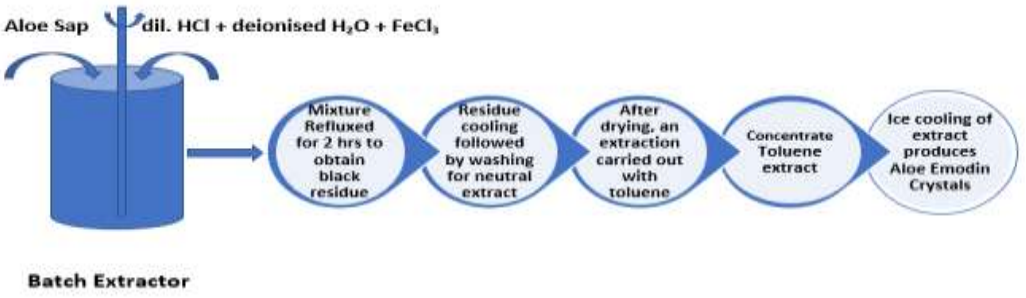

Fig. 01 Flow-chart of AE isolation

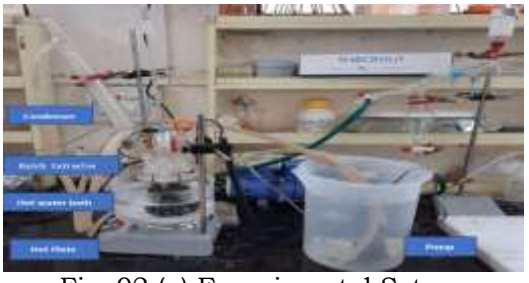

Fig. 02 (a) Experimental Set-up

Fig. 02 (b) Vacuum Rotatory Evaporator

The amount of AE in all separated extracts was measured using the method described before Aloe Emodin standards with a purity of more than 97 percent were utilized for standardization and calibration. The influence of varying temperatures of extraction, speed of agitation, and solid loading at specified extraction conditions, duration of the process pH value of extraction mixture, the

quantity of solvent, and particle size of solute was kept the same for all 20 experimental runs. The method employed for the statistical investigations was RSM and CCD[12]. A software Minitab 20. (Minitab Inc., State College, PA, USA) was used for testing the models, plotting response surface curves in three-dimensional (3D) figuration, and analyzed the results[13].

Table 02 Independent variables in coded level [14]

Independent variables	Symbol	Coded level of independent variables				
		-α -1.681	Low -1	Medium 0	High +1	+ α +1.681
Extraction temperature (^{0}C)	X_1	50	60	70	80	90
Speed of agitation (rpm)	X_2	500	750	1000	1250	1500
Solid loading (gm)	X_3	5	10	15	20	25

Table 03 Independent factors in coded level used in the experiment and Extraction Yield of AE as experimental response [14].

Standard run no.	Run	Coded form of variables X_1	X_2	X_3	Temp. ^{0}C	Speed of Agitation rpm	Solid loading (gm)	Extraction Yield of AE
1	18	-1	-1	-1	60	750	10	89.84
2	15	+1	-1	-1	80	750	10	91.11
3	7	-1	+1	-1	60	1250	10	89.52
4	20	+1	+1	-1	80	1250	10	91.58
5	4	-1	-1	+1	60	750	20	89.19
6	13	+1	-1	+1	80	750	20	94.43
7	6	-1	+1	+1	60	1250	20	88.33
8	12	+1	+1	+1	80	1250	20	95.36
9	17	-α	0	0	50	1000	15	86.69
10	10	+α	0	0	90	1000	15	91.92
11	16	0	-α	0	70	500	15	91.38
12	2	0	+α	0	70	1500	15	92.78
13	5	0	0	-α	70	1000	5	90.86
14	19	0	0	+α	70	1000	25	92.14
15	9	0	0	0	70	1000	15	93.42
16	1	0	0	0	70	1000	15	93.72
17	14	0	0	0	70	1000	15	93.34
18	3	0	0	0	70	1000	15	93.15
19	11	0	0	0	70	1000	15	93.70
20	8	0	0	0	70	1000	15	93.48

Determination of Aloe Emodin (AE) contents

HPLC Method has been developed and validated; prior to this method, Preliminary identification was made by thin-layer chromatography (TLC) and ethyl acetate: Methanol: Water (100:14:10) was used as mobile phase. Detection- 5 % KOH solution in alcohol. HPLC Analysis details were represented in table 04.

Table 04 HPLC Analysis details

Sr. No.	Description and specifications	Value and Ranges
1.	Mobile Phase	Methanol (0.1 % OPA)-Water (65-35)
2.	Detector	UV Visible
3.	Detecting Wavelengths in nm	254, 270, 260, 250
4.	Temperature of Column	25°C
5.	Pump Type	Quaternary pump
6.	Column Specifications	ZODIAC C-18, 250 mm x 4.6 mm, 5 µm
7.	Software	Chromeleon 7
8.	Company Name	Thermo Scientific
9.	Model	Vanquish UHPLC system

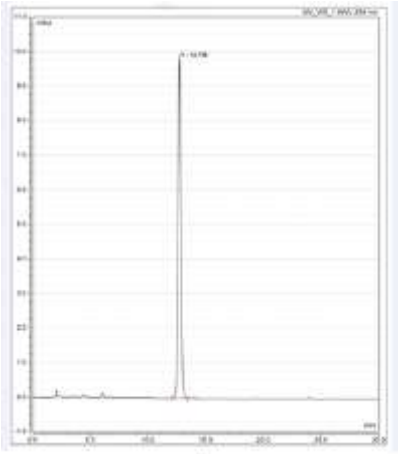

Fig. 03 Chromatogram for Aloe -Emodin by HPLC

Verification of model

The construction of a model explaining the combined influence of X_1, X_2, and X_3 on the percent purity of AE was validated applying the optimal extraction conditions stipulated by the design. The optimal conditions for extracting Aloe Emodin from Aloe sap were determined using RSM's second-order quadratic polynomial model[15]. The model equation's suitability for predicting response values was tested using optimal extraction conditions. In this analysis, a numerical optimization method was employed to find the condition that maximizes the response, and the solution that would be used for verification was chosen based on its desirability and applicability[16]. By comparing experimental and predicted AE response values, the model's validity was verified.

Results and discussion

Optimization of extraction parameter for Aloe-Emodin

The RSM technique is a statistical and mathematical strategy for optimising multivariable systems by determining the actual relationship between the response and a group of independent variables[17]. CCD method optimized AE %

purity by the extraction process, with three components and five levels. Extraction temperature, speed of agitation in extraction, and solid loading were the three variables. The findings revealed the impacts of many parameters on aloe-emodin component yields and purity; for maximum AE purity, three extraction process variables, namely, extraction temperature, speed of agitation, and solid loading, were selected based on previous study's literature, and the ranges of variables were selected based on pilot experiments. In contrast, the extraction time (2hrs), pH value of the extraction mixture (3.5), quantity of solvent (60 ml), and solute particle size (65 µm) were kept the same for all the experimental runs[18]. Ranges of Ethyl acetate: Methanol: Water (100:14:10), solute: solvent ratio (1 gm: 6 ml), extraction time (1 hr to 2 hrs), and solute particle size (20µm to 110µm) were varied, and optimum values of extraction parameters selected.

Single-factor experiments determined three centre levels, whereas an axis distance of α =1.68 determined the remaining two-axis levels. Table 3 has all 20 items run data[19] . Multiple linear regression analysis was used to establish response function (RF) in the form of the empirical quadratic polynomial model, which was calculated using the formula as [20]

$$RF = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j \qquad (1)$$

β_0 and β_i are constant coefficient and linear coefficient of regression respectively, β_{ii} and β_{ij} are quadratic terms and coefficient of interaction respectively, for extraction process X_i is coded value of linear function, X_i^2 coded values of quadratic function and X_iX_j is coded values of interaction effects, respectively; and k represents the number of factors considered in the experiment for optimization, where i< j[21]. The coding of test variables was done according to the equation while developing the regression model.

$$X_i = \frac{(X_i - X_i^*)}{\Delta X_i} \qquad (2)$$

Many reports on the Response Surface Methodology can be found in the past literature [22] [23]. The analysis of variance (ANOVA) based on a 95 percent confidence interval was shown in Table 5. The F statistic and the lack of fit test were used to validate regression models, while R^2 statistics were used to estimate the % variability of optimization parameter [24]. The diffusion and dissolution behaviour of active ingredients from intact plant cell matrix by leaching was a function of extraction temperature, agitation speed, and solid loading. Using RSM, the above three operational factors were adjusted for optimal aloe-emodin (AE) purity. In the estimation of % purity of the extraction of AE and yield (mg/gm), relevant aspects such as solvent quantity, particle size, pH value, and extraction duration were explored independently at first. The AE % purity using RSM ranged from 86.69 To 95.36.

Statistical analysis and model development

The statistical Minitab 20.0 software was used in the investigation to perform regression analysis on experimental data and to create the response surface plot.

ANOVA was also used to estimate the statistical parameters[25]. Tables 4 and 5 indicate the experimental design and results for the extraction experiment, also the requisite coded level of variables and experimental range.

Regression equation in terms of a coded factor represented for percent purity of AE extraction

$$\% \text{ Purity} = 48.4+1.209\ X_1+0.00417X_2-0.823\ X_3-0.01079X_1*X_1-0.000006X_2*X_2 - 0.02120X_3*X_3+0.000129X_1*X_2+0.02235X_1*X_3-0.000008\ X_2*X_3 \quad (3)$$

where the minus sign denotes antagonistic effects, and the plus sign denotes synergistic effects.

Table 05 Variance analysis of experimental results of RSM for Aloe-Emodin extraction

Source	DF	Sum of squares	Mean square	F-Value	P-Value	Remarks
Model	9	89.5588	9.9510	27.27	0.000	Significant
Linear	3	46.8373	15.6124	42.79	0.000	Significant
X_1	1	42.4452	42.4452	116.33	0.000	Significant
X_2	1	0.5700	0.5700	1.56	0.240	
X_3	1	3.8220	3.8220	10.47	0.009	Significant
Square	3	31.8982	10.6327	29.14	0.000	Significant
X_1*X_1	1	29.2649	29.2649	80.20	0.000	Significant
X_2*X_2	1	3.7290	3.7290	10.22	0.010	Significant
X_3*X_3	1	7.0657	7.0657	19.36	0.001	Significant
2-Way Interaction	3	10.8233	3.6078	9.89	0.002	Significant
X_1*X_2	1	0.8320	0.8320	2.28	0.162	
X_1*X_3	1	9.9905	9.9905	27.38	0.000	Significant
X_2*X_3	1	0.0008	0.0008	0.00	0.964	
Error	10	3.6488	0.3649			
Lack-of-Fit	5	3.4115	0.6823	14.38	0.005	Significant
Pure Error	5	0.2373	0.0475			
Total	19	93.2076				

The above results were obtained with MINITAB 20.0 software.

The same software was used to create the model with the response as percent Purity of AE, the regression equation, and the analysis of variance (ANOVA), as shown in Table 05 using equation 03.ANOVA's primary purpose was to combine the results of each experiment run completed in various experimental settings[26]. To evaluate the significant influence of the components and their interaction, a 95 percent confidence interval was employed to analyse the interaction of influencing factors and their effect on the purity of AE. To assess the suitability of the simulated data and regression model, the F statistic test was employed to get the results [27]. A lower P-value (probability value) and a higher

F-value suggest that the related coefficients are more significant. The F-value of 27.27 indicates that the model is both acceptable and significant. Because the value of 'Prob.>F' is less than 0.05, the model terms (individual, square, and two-way interaction) are significant. The 14.38 'lack of fit' F-value indicates that there is only a 0.5 percent probability that the 'lack of fit' F-value arises due to very little noise, which is significant[22].

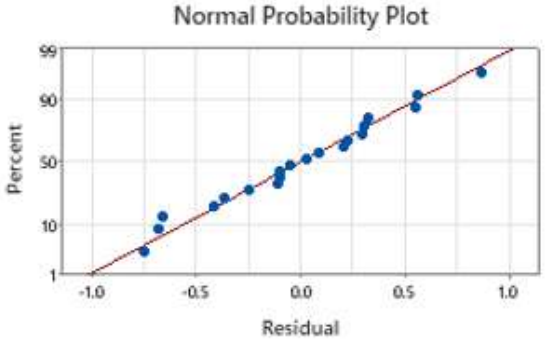

Fig. 05 (a) Nornal Probability Plot

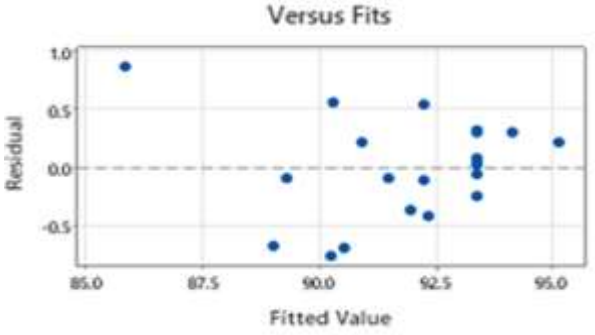

Fig. 05 (b) Fits curve

Figures 05 (a) show normal probability plots of the residuals and 05 (b) represent plots of the residuals vs. fitted values, which were employed to assess the potential of model for improving AE purity. Both figures show no apparent problem with normalcy or response transformation, indicating that all the values of % purity of AE in Fig. 05 (b) are originally constant, implying that the response variable does not need to be transformed. Score in the middle Normal Probability Residual Fig. 5 (a). To test the model and predict its applicability, several studies were conducted, including utilising R^2 statistics to measure the percentage variance of the optimization parameter, displaying probability plots, and comparing residuals and fitted values (see Fig. 05(a). The projected values were compared to the experimentally observed values to assess the model's performance Fig. 05 (c). To validate the model's efficacy, the coefficient of determination was utilised to measure its fit (R^2). To calculate the maximum AE purity, a second-order polynomial equation was developed from the experimental data (Table 3)[27]. Extraction conditions have a significant impact on the extraction of Aloe-emodin components from Aloe Vera leaves. Solvents with lower

polarity can extract higher AE concentrations from plant materials, confirming the essential prediction.

Combined effect of temp, speed of agitation, and solid loading on % purity of AE

To highlight the relationships between response and experimental levels of the independent variables for AE extraction, three-dimensional (3D) surface plots were constructed and displayed in Fig. 6 (b-d), along with the corresponding quadratic polynomial model equation indicated in Eq (3) [28]. The individual and interaction effects of three parameters on percent purity in AE extraction were analyzed employing RSM. The % purity of AE is affected by temperature, agitation speed, and solid loading. The extraction parameters determine the percent purity of AE extracted from aloe vera, according to the RSM analysis method (Table 5). In compared to other variables, extraction temperature (X_1) had the greatest impact, since it affects the solubility of the target active ingredient in each solvent and enhances solvent diffusion and mass transfer rates. However, in AE extraction, speed of agitation and solid loading were found to have a fewer significant influence on percent purity. The interactions between the variables, on the other hand, have little effect on AE extraction; only the temperature has been determined to be more effective in terms of AE purity percent. The quadratic functions of temperature and solid loading had substantially identical effects on the response as the quadratic function of agitation speed, which was the lower-level significance. Table 5 shows the ANOVA for a response surface quadratic model for AE separation from aloe vera. The RSM revealed that the extraction factors were engaged in increasing the purity of AE effectiveness. In three-dimensional response surface plots, Fig. (b-d) show the interactions between the variables, where all axial values are real and indicates the combined effect of temperature (X_1), speed of agitation (X_2), and solid loading (X_3) on % purity of AE from Aloe sap at a constant time (2hrs). The % purity of AE increased due to the more influencing parameters listed were temperature and solid loading at constant extraction time. The highest purity level of AE was confirmed to be 95.36 %. The interaction between solid loading and temperature has proven to be the most efficient parameter for producing high AE purity in the extraction process. As shown in Fig. 6 (d), the percent purity of AE increases as the temperature rises. This could be because increasing the temperature improves the target ingredient's solubility in the solvent, as well as the rate of solvent diffusion and mass transfer. Reduced solvent viscosity and surface tension allow the solvent to penetrate deeper into the cell matrix of the sample plant, increasing extraction efficiency by exposing more surface area of the sample to the solvent. The percent purity of AE increases by 10% when the temperature is elevated from 50 °C to 80 °C. with increased agitation speed. The higher 'F' value of temperature and solid loading, which implies a more considerable contribution to the extraction effect, explains the enhanced percent purity of AE observed in the ANOVA table. Table 5 represents the combined influence of temperature (X_1) and solid loading (X_2) on the percent purity of AE. The effect of solid loading and agitation speed at a constant temperature of 80^0C resulted in a greater purity of AE of 94.43 percent, as shown in Fig. 6. (c). In the same way, the combined influence of temperature (X_1) and agitation speed (X_2) on the percent purity of AE indicated in Fig.(b) ranges from 92 to 93 percent. As shown in the results,

agitation speed and solid loading have less of an impact on the percent purity of AE extracted from aloe sap due to the low value of F statistics [22] .

Contour plots and 3D plots for % purity of AE

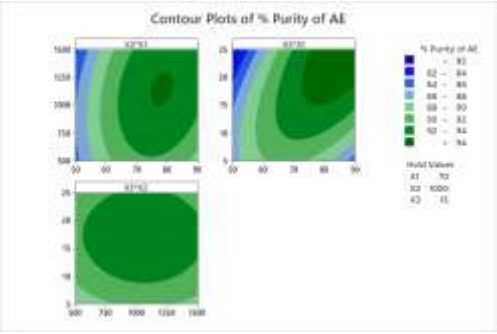

Fig. 06 (a) Contour plot for % purity of AE

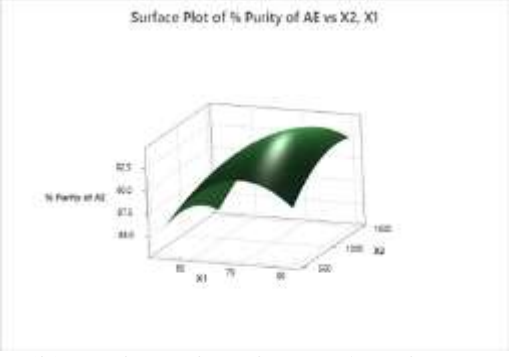

Fig. 06 (b) Surface plot of % Purity of AE vs. X_1 X_2

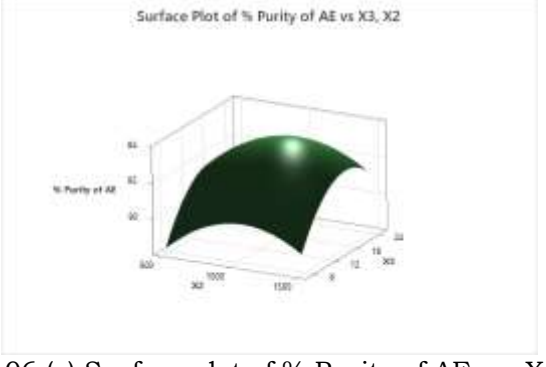

Fig. 06 (c) Surface plot of % Purity of AE vs. X_3 X_2

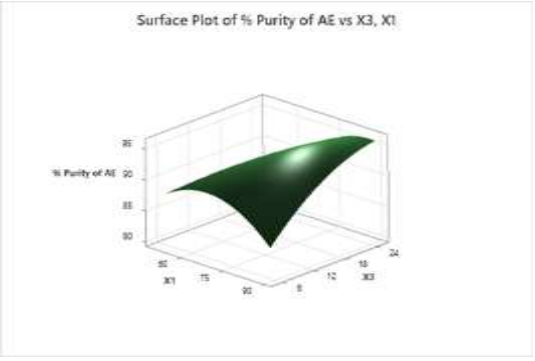

Fig. 06 (d) Surface plot of % Purity of AE vs. X_1 X_3

Table 5 demonstrates the effects of various extraction settings on the quantity and quality of total aloe-emodin content. The contour plots in Fig. 6 (a) indicate the range of purity and the corresponding values of influencing parameters that could be used to predict appropriate ranges of these parameters for higher AE purity. AE contents in dried Aloe Vera plant material ranged from 5.67 mg/g to 37.60 mg/g. The highest AE content was discovered in run 19, while the lowest was discovered in run 5. Finally, surface plots were created based on a model equation to show how AE purity responds to three factors. Table 5 shows the effect of the independent parameters on the response variables, as well as their interaction and regression coefficients, and ANOVA of the experimental results [29].

In order to justify the model's robustness, the coefficient of determination was used to assess the model's quality of fit (R^2). Accounting predictor's actual values, extraction temperature, agitation speed, and solid loading, a second-degree polynomial model for AE purity (mg/g of solid loading) was regressed and developed for optimum AE purity (Table 3). The AE content of the Aloe vera extracts was exceptional, ranging from 35.10 to 36.10 mg/g on average. Figure 7 depicts the optimization plot for investigations. The results of a detailed statistical ANOVA for the models for Aloe-Emodin extraction parameters are mentioned in table 6.

R^2	Adjusted R^2	Predicted R^2	PRESS
96.09 %	92.56 %	69.56 %	28.37

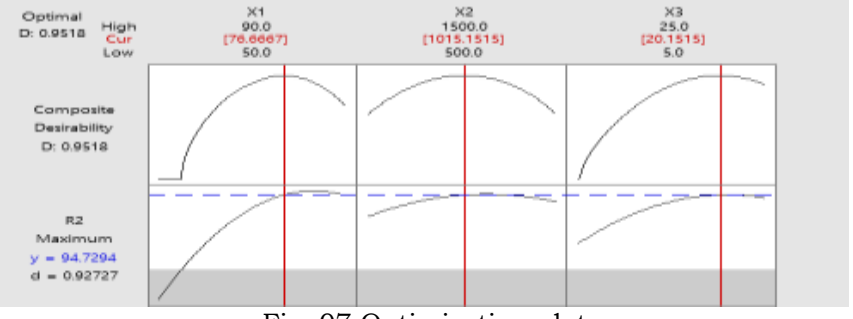

Fig. 07 Optimization plot

Table 06 Optimized values of AE extraction parameters with model predicted purity

Parameters	Temp. ^{0}C	Speed of Agitation rpm	Solid loading (gm)	% Purity
Optimum Conditions	77.66	1015	20.15	94.72

Conclusion

The Aloe Emodin (AE) purity was significantly influenced by temperature maintained, speed of agitation and solid loading i.e. solute concentration in solvent ratio. The maximum AE was obtained at 77.66 ^{0}C temperature, 1015 rpm of Speed of Agitation and 20.15 gm of solid loading, which gives optimum results of the extraction process. As a result, the predicted values were nearer to the experimental value, showing that the model was suitable. Using RSM approach, the extraction of AE from Aloe vera leaves was successfully optimized. As the temperature rises, the target compound's solubility, rate of solvent diffusion, and rate of mass transfer all increases, while the solvent's viscosity and surface tension decrease. Solvents with lower viscosity and surface tension can penetrate deeper into the sample matrix, improving extraction efficiency by exposing more surface area of the sample to the solvent. The experimental data were adequately represented by the second-order polynomial model. Thus, an approach for extracting bioactive components from Aloe-vera sap was designed using RSM with CCD to identify the optimum combination of extraction parameters. The usefulness of study for the development of the industrial extraction process.

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Abbreviation: Active Ingredients (AI), Aloe Emodin (AE), Aloe-Vera latex (AVL), analysis of variance (ANOVA), degree of freedom (DF), Revolution per minutes (rpm), Response function (RF), Response surface methodology (RSM), Central Composite Design (CCD)

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