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Applying an Optimization Technique for The Extraction of Antioxidant Components from *Justicia Adhatoda* Leaves

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Abstract

In this research work, the response surface approach was constructed on a central composite design (CCD) and was applied to control optimization for antioxidant elements extraction from *Justicia adhatoda* shrubberies. Two extraction parameters were assessed in 14 experimental trials as methanol: water (30% to 80%) and the extraction time (4 to 6 days). The quadratic polynomial equation of multiple regression analysis forecasts the extraction process's optimization. The maximum antioxidant extract (AE) was obtained at methanol (55%) and time (6.41 days) 6.48 g/100g on trial 09. The minimum radical scavenging activity (IC_{50}) values were obtained at methanol (55%) and extraction time of 3.59 days on trial 08. It was found that the AE yields and radical scavenging activity (IC_{50}) of *Justicia adhatoda* leaves could be optimized at 6.62 g/100g and 1.66 µg/mL at the optimum condition at a time of 6.41 days and solvent concentration of 76.01% with overall desirability 0.96. According to the response optimizer method, the conditions that maximize the yield and minimize the DPPH are within the established restrictions. The well-fitted actual values to predicted values validate the regression model acceptability. Antioxidant properties of *Justicia adhatoda* leaves using response surface methodology (RSM) confirm this technique's usefulness.

Keywords: Central composite design; Optimization; Quadratic polynomial equation; Analysis of variance; Antioxidant elements.
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1. Introduction

Free radicals are crucial in the oxidative harm that occurs to biological systems.[1–3] In order to determine the antioxidant capabilities of pure substances or extracts, their capacity to capture free radicals is tested. The creation of the energy needed to maintain the efficiency of biological processes depends on oxidation. Continuous production of oxygen-centered free radicals and other reactive oxygen species (ROS) in vivo during metabolic process results in cell death and tissue damage. It has been documented that oxygen radicals have a role in a number of diseases, such as cancer, diabetes, cardiovascular disorders, and aging,[4,5] Accordingly, measuring the antioxidant capacities of pure substances or extracts will lead to substances that may snare free radicals and thereby aid in reversing oxidative damage. Numerous techniques that test a compound's capacity to scavenge free radicals such the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and the 2,20-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) action radical are employed (ABTS+), the superoxide anion radical or the hydroxyl radical.[6–10] The DPPH, often known as the DPPH method, is the most widely used technique. In light of this, we chose to adopt the DPPH technique for our analysis.[11,12] The decrease in absorbance, which is used as an indicator of how quickly the chemicals in the extract are being reduced, is how the rate of change of DPPH is assessed spectrophotometrically. The kinetics of this reaction is used to determine the antioxidant capabilities of the substances under investigation.[4,12–14]

The body is shielded by antioxidants against harm brought on by oxidative stress brought on by free radicals.[15,16] Polyphenols, a type of natural antioxidant found in food and medicinal plants, are particularly good at reducing oxidative damage.[17,18] The core structure of polyphenols contributes to

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their scavenging activity, which aids in the elimination of free radicals. In vitro studies have shown that polyphenols are more potent antioxidants than tocopherols and ascorbate. Polyphenols are excellent antioxidants because of the radical formed from them's capacity to stabilize and delocalize the unpaired electron throughout the structure and their capacity to bind transition metal ions.[19,20] Numerous enzymatic and nonenzymatic antioxidant mechanisms in the human body shield cellular molecules from ROS-caused harm. However, severe or continued oxidative stress makes the innate defense insufficient for total protection. Therefore, certain doses of exogenous antioxidants are continually needed to maintain an acceptable level of antioxidants in order to balance the ROS in the human body. In spite of the fact that several synthetic antioxidants, such butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), are highly effective and employed in industrial processes, they are hazardous and can have negative health consequences on people.[21,22] Hence, compounds from natural sources are capable of protecting against ROS-mediated damage and gives a lot of hope in preventing or treating diseases induced by oxidative damage.[23,24]

A plant from the Acanthaceae family called *Justicia adhatoda* is found all over Bangladesh.[25,26] Vasaka is a common name for it. Basak is local name, Malabar nut is as English name, *Justicia adhatoda* L. is as Scientific name, Adusa, bansa, arusha is as Hindi.[27,28] The Vedas, a repository of Chinese herbal remedies, attest to the use of *J. adhatoda* in the remedy of asthma, chronic obstructive pulmonary disease, whooping cough, colds, and coughs.[29,30] and tuberculosis.[31] More than 2,000 years ago, Indian medicine began using the leaves of *J. adhatoda.*[32,33]

A plant-derived substance with the capability to prevent allowed essential and substratum corrosion could be an antioxidant. Free radicals cause cellular injury, cellular maturation, and long-lasting worsening illnesses like tumors, diabetes, cardiac disease, and cerebrovascular illness.[15,34] It is a fact that process parameter as temperature, period of soaking and solvent system, *etc*. often affects the quality and quantity of active constituents of plants. That is why the quality and quantity of the supplied natural raw materials depend on process parameters like temperature, period of soaking and solvent system, *etc*. which in turn affect the quality and price of the drugs produced.[35]

The biological and pharmacological properties exhibited by many crude plant extracts under experimental conditions are often ambiguous and are not always reproducible. Thus, assessment of the actual pharmacological effects of such extracts often becomes difficult. With regard to the respective laboratory, research is ongoing to find out potential sources of bioactive compounds. Very often it is difficult to find out potential bioactive compounds and their number and volume due to a lack of proper investigation methods *e.g.*, solvent system, temperature, period of soaking, time of collection, and age of the plant. By the traditional method, a huge amount of solvent is required to find out bioactive compounds, sometimes no bioactive compound is found. Investigating the level of bioactivity in the manner of the response surface method is also crucial. If the bioactivity is not significant statistically the result will not be reproducible to use for developing drugs.[36,37]

The one-factor-at-a-time strategy is a term used to describe the traditional experiments in optimization where only one component is changed simultaneously. This method was time-consuming, costly, expensive also unable to calculate the impact of interactions between variables. A convenient method for determining how various aspects and their connections affect one or more independent variables is the response surface approach. Which has been applied effectively to find a grouping of factor levels that produces the best answer. The major benefit of this approach is that it typically takes less experimental trial than standard complete factorial designs while still producing results that are statistically significant.[38,39] It has been applied to improve a number of plant leaf preparing techniques, including grinding, taking out, and restiveness.[40–42]

This present work, therefore, will be conducted to optimize the extraction of DPPH scavenging activity from *Justicia adhatoda* L. plants and their medicinal value using the Statistical Analysis Method (Response Surface Methodology) in the process parameters (soaking period and solvent system).

2. Experimental section

2.1 Handling of samples

Shrubberies of the *Justicia adhatoda* L. (Bengali name: Bashok) plant were collected from BCSIR medicinal garden, Dhaka, Bangladesh. This plant has cultivated by tissue culture section of BCSIR for research purpose. The source of medicinal plants is the cultivated, which is collection of plant material is from land and is subject to local, national, and international guidelines and legislation. The samples were further identified and authenticated from Mst. Nadira Begum, Senior Scientific Officer (Botanist), (Collection Date: 07 October 2018, 16 April 2019, and 08 December 2020 respectively). After collecting fresh plant samples, they are cut into small pieces. Grind the leaves in a blender (LG BL 999SP) and soak the blending portion with a selective concentration of Methanol and Distill water. After a period of a specific time,

evaporate the solvent by the use of rotary evaporator, laboratory freeze dryer and collect the extracts as per procedure of (Fig. 1) for determination of DPPH scavenging activity. Prof. Mohammad Zashim Uddin, a Professor at the University of Dhaka recognized this collection. He gave the collection the accession number DUSH - 10811, which was thereafter stored in the Salar Khan Herbarium at the University of Dhaka.

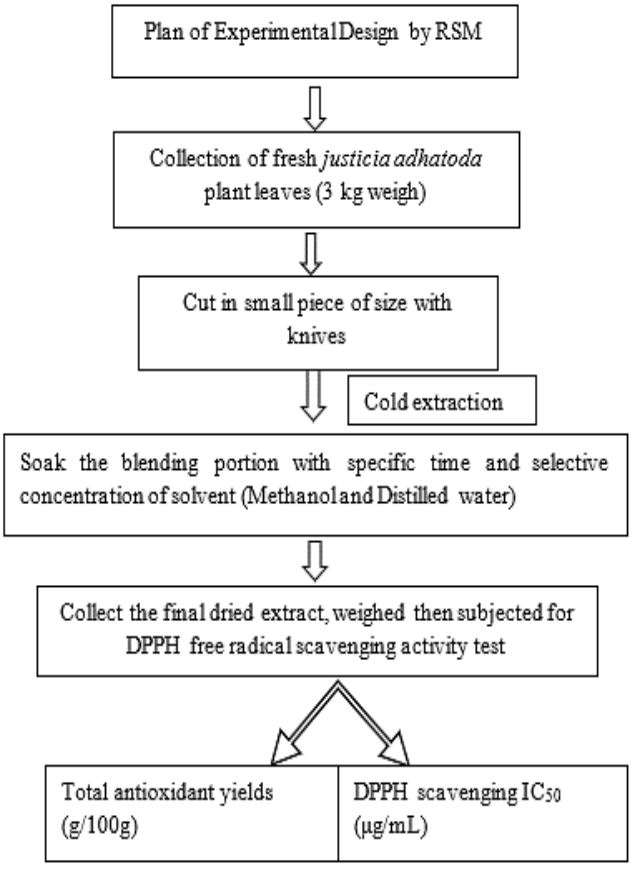

Fig. 1 Schematic diagram for preparation of crude extract of *Justicia adhatoda* by response surface method.

Justicia adhatoda may grow in a wide range of climatic and soil conditions. Alluvial soils are ideal for growing. Soil preparation time is in March–April and planting is done in April–June. It can be propagated vegetatively by taking 15-20 cm long, 3–4 node terminal or lateral stem cuttings, as well as by seed germination. Flowering time is from February to April.

2.2 Chemical and reagents

Sigma-Aldrich Quimica provided DPPH in free radical form (90 percent purity) (St. Louis, MO). Altogether materials must have a more than 99% purity grade. HiMedia supplied the methanol. Vitamin C and BHA have been purchased from HiMedia. Analytical-grade substances were used throughout the analysis. The entire time, doubly purified water from Millipore Co. was used. The remaining altogether of substances remained analytical grade.

2.3 Instrumental

On an ANALYTIK JENA SPECORD 250 PLUS thermostated at air conditioning temperature, absorbance readings were taken.

2.4 Methodology

A plan of experimental design was prepared which had done by Response Surface method (RSM). After collection of fresh *Justicia adhatoda* plant leaves (3.0 kg), cut into small pieces with knives. Samples were blended and soaked the blending portion with specific time and selective concentration of solvent (Methanol and Distilled water). In the present study, cold extraction was selected rather than hot extraction, because in many cases hot extraction turns to be a destructive mode. Some specific phytochemicals may be of thermo labile and decomposes in the hot extraction process. The solvent was evaporated using a rotary evaporator under vacuum, followed by freeze drying. After that, the final dried extract was collected, weighed and then subjected for DPPH free radical scavenging antioxidant activity.

2.5 Determination of DPPH scavenging activity

The extract's ability to scavenge free radicals from stable DPPH was examined using spectrophotometric analysis. The mole ratio of DPPH/BHT was constantly maintained to evaluate how the type of solvent affected the DPPH/antioxidant reaction kinetics. The concentration of unreactive DPPH in DPPH/BHT systems was determined using a slightly modified Brand-Williams method.[6,43] In order to have null adjustments in the spectrophotometer, a blank was made using aliquots of 3 mL of 90 percent aqueous methanol without DPPH and the solvent extract. As a control, the methanolic DPPH solution devoid of antioxidants was utilized. When DPPH interacts with an antioxidant molecule, it produces hydrogen and is reduced. The color transition from deep violet to pale yellow occurred at a wavelength of 517 nm. Amounts of cold and Soxhlet extract that were dose-dependent were separately gathered and dissolved in 90 percent aqueous methanol. 1.5 mL of the 0.1-mM DPPH solution made in 90 percent aqueous methanol was added to each 1.5 mL portion of each extract concentration. The mixture was vigorously shaken before use and stored in darkness; the resulting solution's diminished absorbance was then gauged at 517 nm. The assay is performed 3 times and the data points are expressed as a mean. Scavenging activity (percent) equals (1 - trial absorbance at 517 nm / control absorbance at 517 nm) × 100.[23,43]

2.6 Statistical analysis

The most of commercial software's, *i.e.*, Design-Expert (Stat-Ease, Minneapolis, United States of America), JMP (SAS, Cary, North Carolina) and Minitab *etc*. are usually expensive

and proprietary, whereas; R is an open-source software which allows execution of various statistical techniques and can extend via different packages. To examine the impact of various solvent concentrations and extraction times, a central composite design, response surface approach was applied. In this study, analyses were accomplished by using R environment, *i.e.*, RStudio version 4.1.1 (2021-08-10) developed by.[44]

2.7 Design of an experimental study

The Response Surface approach was used with two response variables, namely solvent ratio and extraction time to evaluate the whole yield and DPPH scavenging IC_{50} (µg/mL) obtained from extraction. A central composite design was applied to create the two response variables' configurations. The point of the ratio of the solvent variable was 55%, while 5 days was the result for the time variable.

The Eq. (1) of the two-factor central composite model is:

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{2-1} \sum_{j=i+1}^{2} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

here expression, Y is the extractions (yield), β_0 is the persistent, β_i, β_{ii}, β_{ij} are the coefficients of the predictor variable (X), and X is the uncoded predictor variable devoid of code (for the extraction time variable, the solvent of extraction (A) at a low level of 30 and high level 80; time of extraction (B) at a low level of 4 and high level 6 days and ε is a random error). In this study, the level of the independent variable (the ratio of solvent and soaking time) is displayed in Table 1. Any of the extraction mainly depends on the texture and water content of the plant materials being extracted. Methanol is suitable for preliminary extraction and was used in the present study. We followed the specific experimental level because the success of the extraction with methanol is directly related to the extent that chorophyll is removed into the solvent and on repeated extraction showed the complete free of green colour as well as it can be predicted that low molecular weight compounds have been extracted.[45]

Table 1. Predictor variable Level, code, and optimized value.

Predictor variable	Units	Low	High	-alpha	+alpha
Time	Days	4	6	3.58579	6.41421
Concentration	Ratio	30	80	19.6447	90.3553

3. Results and discussion

3.1 Optimization

There are numerous reports of the Response Surface Methodology in the earlier literature. The outcomes showed how several parameters, including solvent concentration and extraction duration, affected the yields of antioxidant components in a few herbal extracts.[46–49]

By determining the actual association between the response and the group of independent factors, a mathematical and statistical technique known as the response surface approach can be utilized to examine and enhance multivariable systems. In this work, the solvent % and extraction duration were the two operational variables that were optimized for maximum antioxidant extract (AE) yield and DPPH scavenging IC_{50} (µg/mL). The template for the central composite design (CCD) is revealed in Table 1. The extraction yield was first determined separately from other important parameters like the solvent concentration and extraction time. The DPPH scavenging IC_{50} ranged from 5.28 to 238.67 µg /mL when the method was used, while the AE yield ranged from 2.44 to 6.48 g/100g (Table 2). The solvent

Table 2. Response surface analysis is used in an experimental plan to extract antioxidants from *Justicia adhatoda* shrubberies.

Trial	Procedure parameters		DPPH scavenging IC_{50} (µg/mL)		Antioxidant Extract yields (g/100g)	
	Extraction time (days)	Solvent Ratio Methanol: Water	Experimental	Predicted	Experimental	Predicted
1	4.00	30.00:70.00	116.22	105.95	3.61	3.34
2	6.00	30.00:70.00	238.67	219.14	4.04	4.16
3	4.00	80.00:20.00	31.16	24.73	5.41	5.38
4	6.00	80.00:20.00	16.15	0.46	5.96	6.32
5	5.00	55.00:45.00	40.27	44.46	5.49	5.35
6	5.00	55.00:45.00	45.14	44.46	5.10	5.35
7	5.00	55.00:45.00	44.85	44.46	5.77	5.35
8	3.59	55.00:45.00	5.28	11.72	4.69	4.92
9	6.41	55.00:45.00	55.08	74.60	6.48	6.16
10	5.00	19.64: 80.36	222.31	238.01	2.44	2.57
11	5.00	90.36: 09.64	15.68	25.95	5.75	5.54
12	5.00	55.00:45.00	46.02	44.46	5.03	5.35
13	5.00	55.00:45.00	45.50	44.46	5.71	5.35
14	5.00	55.00:45.00	45.00	44.46	5.00	5.35

(55%) and time (6.41 days) conditions produced the highest AE yield. Also, the minimum DPPH scavenging IC_{50} value was obtained at solvent (55%) and (3.59 days).

The statistical significance of the estimated coefficients and therefore of the effect of the factors, is determined by the values of the *t*-statistic of the *t*-test and the associated p-value (p-value < 0.05 implies a significant effect). From the fit result we can see that the estimators are statistically significant except factor time^2 (p-value < 0.05) in Table 3. But in Table 4 there time: concertration and time^2 are insignificant. Overall, the regression *F* is statistically significant (p-value < 0.05). The adjusted coefficient of determination also presents a high value (R^2adj > 80%).

From the analysis of variance table (ANOVA) of the linear model we can see that the lack of adjustment of this model is not statistically significant (p-value = 0.407998). In conclusion, the model is quite close to the data and is useful for modeling them. Your equation in coded units would be as follows. Table 5 shows the analysis of variance (ANOVA) findings based on a 95% confidence interval. R-squared statistics was applied, though, to evaluate the level of parameter variability. Regression models were evaluated using the *F* statistic and lack of fit test. Table 5 outputs display the first-order models (FO), which specifies the first-order

Table 3. Second order model of DPPH scavenging IC50 (µg/mL) for response surface method.

Factors	Estimate	Std. Error	t value	Pr(>|t|)	Remarks
(Intercept)	44.46334	6.26022	7.1025	0.000102 ***	significant
Time	22.94058	5.42151	4.2314	0.002871 **	significant
Conc.	-76.7426	5.42151	-14.1552	6.04e^{-07} ***	significant
Time:Conc.	-34.365	7.66717	-4.4821	0.00205 **	significant
Time^2	-0.77605	5.64288	-0.1375	0.894013	insignificant
Conc.^2	42.13146	5.64288	7.4663	7.15e^{-05} ***	significant

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1; Multiple R-squared: 0.9736, Adjusted R-squared: 0.957; F-statistic: 58.92 on 5 and 8 DF, p-value: 4.268e^{-06}

Table 4. Second order model of antioxidant extracts yields (g/100g) for response surface method.

Factors	Estimate	Std. Error	t value	Pr(>|t|)	Remarks
(Intercept)	5.35	0.14746	36.2803	3.65e^{-10} ***	significant
Time	0.43893	0.12771	3.437	0.008863 **	significant
Conc.	1.05013	0.12771	8.223	3.58e^{-05} ***	significant
Time:Conc.	0.03	0.1806	0.1661	0.872192	insignificant
Time^2	0.09625	0.13292	0.7241	0.489627	insignificant
Conc.^2	-0.64875	0.13292	-4.8807	0.001223 **	significant

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1; Multiple R-squared: 0.9289, Adjusted R-squared: 0.8844; F-statistic: 20.9 on 5 and 8 DF, p-value: 0.0002114.

Table 5. ANOVA table for central composite design used for optimization.

Source of variance	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Remarks
DPPH scavenging IC_{50} (µg/mL)						
FO(Time, Conc.)	2	51326	25662.8	109.138	1.56e^{-06}	significant
TWI(Time Conc.)	1	4724	4723.8	20.089	0.00205	significant
PQ(Time, Conc.)	2	13228	6614	28.128	0.00024	significant
Residuals	8	1881	235.1			
Lack of fit	3	1859	619.7	140.991	2.99e^{-05}	significant
Pure error	5	22	4.4			
Antioxidant Extract yields (g/100g)						
FO(Time, Conc.)	2	10.3635	5.1817	39.7155	7.01e^{-05}	significant
TWI(Time Conc.)	1	0.0036	0.0036	0.0276	0.872192	insignificant
PQ(Time, Conc.)	2	3.2667	1.6333	12.5188	0.003438	significant
Residuals	8	1.0438	0.1305			
Lack of fit	3	0.4308	0.1436	1.1712	0.407998	insignificant
Pure error	5	0.613	0.1226			

response surface (*i.e.*, a linear function), the canonical analysis of the response surface with two-way interaction model (TWI) and the pure quadratic terms. The table also included lack-of-fit test and the pure error values.

Since the lack of fit of antioxidants extract was statistically insignificant (Pr = 0.40594> 0.05) then the model could predict the response variable appropriately. All the eigenvalues were positive implying the stationary points were minimum optimizing regions which display in Tables 6 and 7.

To determine whether the model was adequate, normal probability plots, comparing residuals and fitted values, histograms, and also comparing residuals and the order of the data of the residuals were utilized (Figs. 2a and 2b). Also, high R^2 of 97.36% and 92.89% for DPPH scavenging IC_{50} and yields respectively indicated that the anticipated vs. real values were displayed in (Fig. 3) to evaluate the best model's

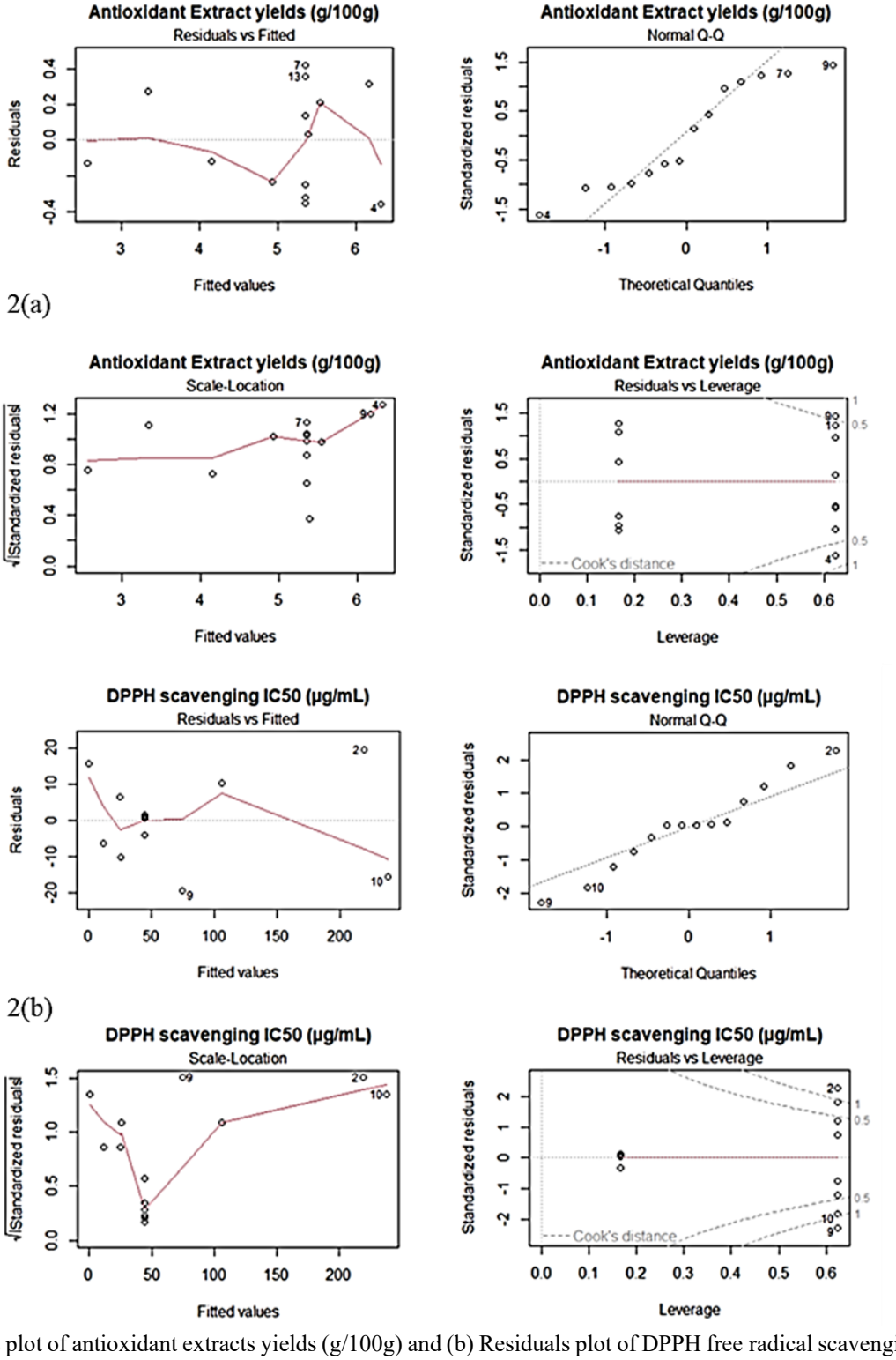

Fig. 2 (a) Residuals plot of antioxidant extracts yields (g/100g) and (b) Residuals plot of DPPH free radical scavenging IC_{50} (µg/mL).

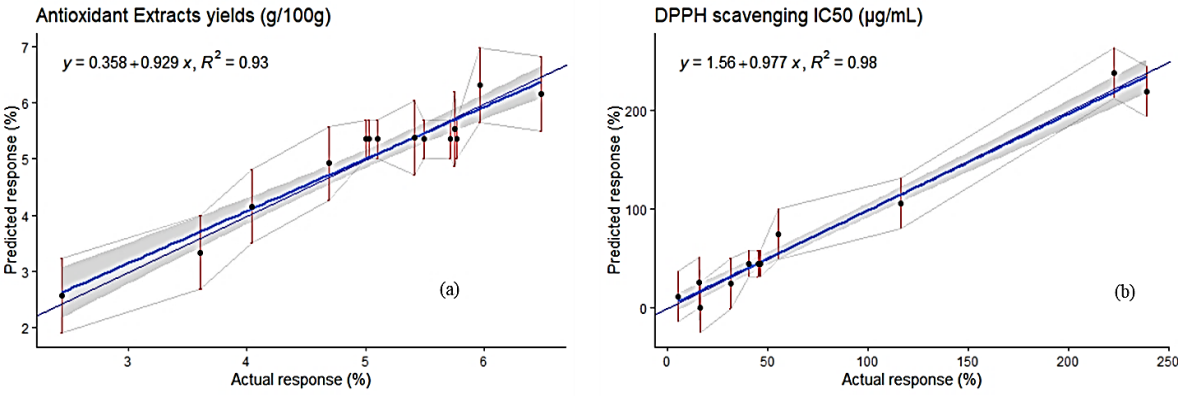

Fig. 3 Predicted versus actual observations of 3(a) antioxidant extracts yields (g/100g) and 3(b) DPPH free radical scavenging IC_{50} (µg/mL).

Table 6. Stationary point of response surface.

Response	Time of extraction	Solvent concentration
DPPH scavenging IC_{50} (µg/mL)	4.463139	72.295096
Antioxidant Extract yields (g/100g)	2.602349	73.847811

performance. Finally, surface plots were employed to demonstrate the relationship between two parameters on the basis of a model equation and DPPH scavenging IC_{50} (µg/mL) and antioxidant extract values.

To identify the predictor's actual values, Time (A) and Methanol (B), a second-degree polynomial model for the AE yield (g/100 g) was regressed using experimental data (Table 2). The quadratic polynomial equation can be used to represent the final empirical regression model of their relationship between responses and the two examined variables for DPPH free scavenging activity IC_{50} and antioxidant extract (AE) yield an Eqs. (2–3):

$$\text{DPPH scavenging } IC_{50} = 44.3223 + 22.9806A - 76.8572B - 34.365AB - 0.7321A^2 + 42.43B \quad (2)$$

$$\text{Antioxidant extract (AE) yield} = 2.64 + 0.2714A + 0.5974B - 0.1022AB + 0.0088A^2 - 0.3580B^2 \quad (3)$$

where A is the extraction time and B is the extraction solvent concentration. According to the equation, DPPH scavenging activity will rise in direct proportion to the lengthening of the extraction process and the dwindling amount of solvent, as shown by the positive and negative constant values, respectively. Similar to this, the equation demonstrated that the yields of antioxidant extract will rise in direct proportion to the lengthening of the extraction process and the solvent, as indicated by the positive constant value.

3.2 DPPH free radical scavenging IC_{50} (µg/mL)

The antioxidant is a bioactive substance that has the potential to reduce allowed radical and substrate oxidation. This process has the potential to produce cell damage, cellular aging, and chronic degenerative illnesses like cancer, diabetes, cardiovascular disease, and neurovascular disease.[34] Analysis of Variance (ANOVA) results showed that the treatment on antioxidant activity had an F compute the value is significant and a p-value of <0.05 except two-way interection model. A significant effect on response was indicated by a p-value of less than 0.05 in the DPPH.

It demonstrates that the aim objective for ideal circumstances has a length of 6.41 days and a solvent concentration of 76.01% methanol. The practicable zone,

Table 7. Eigen analysis.

DPPH scavenging IC_{50} (µg/mL)		$values	
	λ_1 = 48.164097		λ_2= -6.808687
		$vectors	
	Time	-0.33127	-0.94354
	Conc.	0.943537	-0.33127
Antioxidant Extract yields (g/100g)		$values	
	λ_1 = 0.09655185		λ_2= -0.64905185
		$vectors	
	Time	-0.9998	-0.02012
	Conc.	-0.02012	0.999798

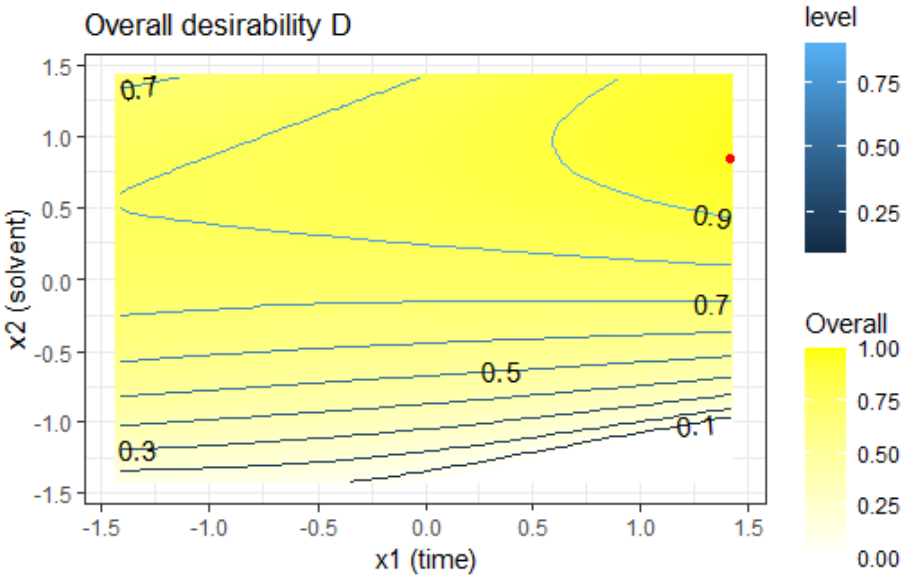

Fig. 4 Response optimizer under ideal conditions; duration of time 6.41 days and solvent concentration is 76.01%.

Table 8. Conditions with maximum value of D.

Time (days)	Solvent Concentration (%)	Prediction of DPPH scavenging IC_{50} (µg/mL)	Prediction of Antioxidant Extract yields (g/100g)	Overall Desirability
6.41	76.01	1.66	6.62	0.96

which is where the target and maximum objectives' ideal conditions are located, can be shown in (Fig. 4) by comparing the contour plots for the target and maximum goals.

According to this method, the conditions that maximize the yield and minimize the DPPH, within the established restrictions, would be the pair of factors with the highest D (Overall = 0.9616). The estimated DPPH free radical scavenging IC_{50} at this point is approximately 1.66 (µg/mL) with a antioxidant extracts yields 6.62 (g/100g), so our restrictions are fulfilled. This can be shown in Table 8. Remember that it should be validated by designing an experiment with these conditions to confirm that it gives values of DPPH and antioxidant extracts yields close to the estimates.

Figures 5 and 6 demonstrate the duration of time (days) and solvent concentration for the extraction of *Justicia adhatoda* leaves using methanol under practicable ideal conditions (percent). The graphical representations of the regression equation that simultaneously display the function of two elements while maintaining the remaining factors at a fixed level are known as the 2D contour and 3D surface plots.[50–52] Plots are used to display values for time (days) and solvent concentration (ratio).

The deep green contour in (Fig. 5) displayed the greatest antioxidant extract yield response of 3.08 g/100g. The lowest response result, 1.13 g/100 g, was represented by the light green color. The contour plot's line of dots graph demonstrated the two components combined in different ratios to provide the same antioxidant extract yield response value. The response surface of the interaction between components can be more easily seen in (Fig. 5), a three-dimensional graph. According to the curve trend, adding more time and less solvent to the extraction process enhanced the yields of antioxidant extract.

The red contour in (Fig. 6) displayed the greatest DPPH scavenging response value of 238.67 µg/mL. The lowest response result, 1.28 µg/mL, was represented by the blue color. The contour plot graph revealed a line made up of dots that combined both components in varying proportions to provide the same value for the DPPH scavenging response. In (Fig. 6), a three-dimensional graph, it is shown how the response surface of the relationship between the components can be more easily seen. The DPPH scavenging value declined when more time and less solvent were added to the extraction process, according to the curve's trend.

3.3 Current challenges

Despite the last two to three decades' worth of successful plant-based drug development programs, future efforts still face numerous obstacles. To keep up with other drug discovery efforts, natural products scientists and the pharmaceutical industry will need to consistently increase the quality and quantity of molecules that enter the drug development phase.

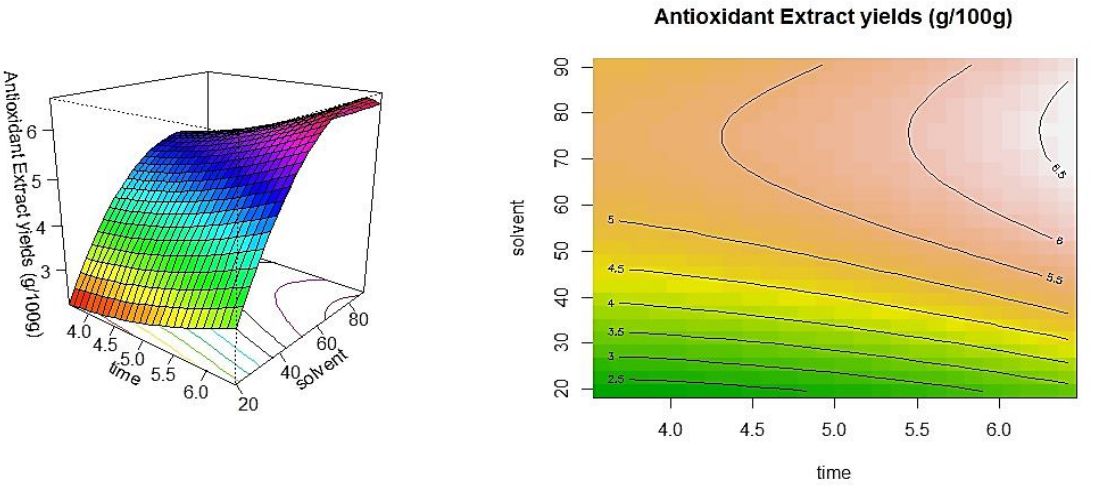

Fig. 5 Contour and response surface of antioxidant extracts yields (g/100g) of *Justicia adhatoda* leaves extract.

Clinical drug development, the procedure before new pharmaceutical goods receive regulatory approval, has been highlighted as the industry's biggest driver of rising prices and is mostly responsible for pharmaceuticals failing to pass the rigorous registration approval process. If drug failure rates are taken into consideration, Dimasi's estimates show that clinical trials cost an average of $124 million per treatment candidate. Research and development expenses as well as amortized losses from time spent out of pocket raise the price to $802 million.[53] The countless leads that are abandoned during the drug discovery process account for a sizable portion of this time and money. Only one out of every 5000 lead compounds is predicted to make it through clinical testing and be licensed for use.[54]

3.4 Potential future perspectives

For the past 15 years, there has been an increase in knowledge of herbal medicines and medicinal plants throughout the subcontinent. As a result, research funding has increased; new departments, such the department of herbal science, as well as new herbal institutes, have been formed or are in the process of doing so. There has been a noticeable increase in publications, but the majority of them are only passable. A significant number of papers published in journals with poor standards also contain substantial amounts of repetition. However, new phytomedicines or conventional chemical entities derived from plants are rarely developed into forms that are both internationally accepted and commercially feasible.[55]

It is projected that Bangladesh sells 12,500 tonnes of dried medicinal plant material. For the rural economy, these goods are valued about Tk. 255 million ($4.5 million) and about Tk. 330 million ($5.8 million) at wholesale prices. Over Tk 480 million ($8 million) was spent on the 5,000 tonnes of imported medicinal plants. There are thought to be 350 inter-district be paris, and 6,000–10,000 local collectors, pikers, and producers

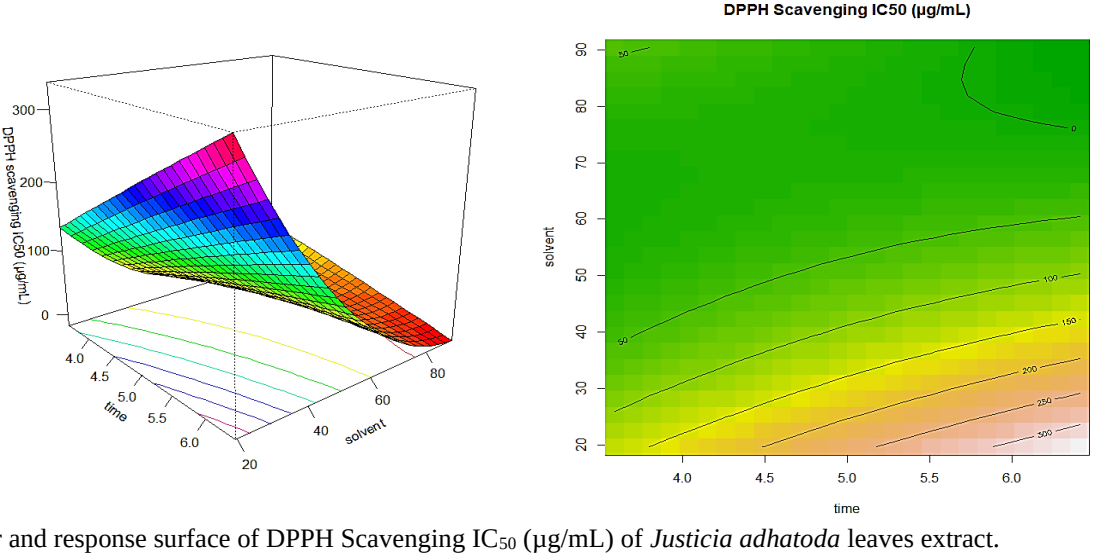

Fig. 6 Contour and response surface of DPPH Scavenging IC_{50} (µg/mL) of *Justicia adhatoda* leaves extract.

herbal practitioners.[56]

supply them. There are reportedly over 200 registered Unani and Ayurvedic manufacturers in total, in addition to about 70 homeopathic companies. They will all have between 2,000 and 4,000 workers between them. In addition, the country is estimated to have 80,000 unlicensed and 5,000 licensed.

3.5 Limitations and/or disadvantages of the proposed study

The limitations of the study can be the specificity of nanoparticles selected for the analysis. Further research is needed to determine the active components in *Justicia adhatoda* leaves as well as the signaling pathway that underpins its anti-oxidant efficacy.

3.6 Innovative description

The results of the antioxidant properties of *Justicia adhatoda* leaves by using response surface methodology (RSM) with Rstudio software confirm this technique's usefulness for the first time.

4. Conclusions

According to the World Health Organization, 75% of the world's populations are using herbs for basic healthcare needs. Since the dawn of mankind, in fact, the use of herbs/plants has offered an effective medicine for the treatment of illnesses. This study demonstrated that the Response Surface approach is a suitable technique for enhancing the extraction of *Justicia adhatoda* leaves. The response surface model was statistically verified using analysis of variance (ANOVA). Each independent variable's computation results had a significant impact ($p< 0.05$) on each response. This result demonstrated that the quadratic polynomial model was accurate in identifying the relationships between all parameters in the study. R^2 is 0.97 for DPPH and 0.97 for antioxidant extracts. *Justicia adhatoda* leaves were best protected under conditions of 76.01% solvent and 6.41 days of extraction time. Fresh leaf DPPH was 1.66 µg/mL after this treatment, and antioxidant extracts were 6.62 g/100 g, with respective desirability of 0.96 or 99.16%. If the desirability value is nearer, optimization will be more desirable. Further research on the bioactive chemical content of *Justicia adhatoda* leaves must be done in order to determine how the extraction technique affects the amount of bioactive compound in the extract from *Justicia adhatoda* leaves.

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Conflict of Interest

There is no conflict of interest.

Supporting Information

Not applicable.

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