Hindawi
International Journal of Chemical Engineering
Volume 2022, Article ID 4933015, 12 pages
https://doi.org/10.1155/2022/4933015

Research Article

Extraction of Catechins from *Aegle marmelos* Fruit Pulp: Statistical Optimization Using Response Surface Methodology and Artificial Neural Networks

Divya Kurimilla,[1] Meena Vangalapati,[1] and Venkata Ratnam Myneni [2]

[1]*Department of Chemical Engineering, AUCE (A), Andhra University, Visakhapatnam, Andhra Pradesh, India*
[2]*Department of Chemical Engineering, Mettu University, Mettu, Ethiopia*

Correspondence should be addressed to Venkata Ratnam Myneni; mvratnam81@gmail.com

Received 25 March 2022; Accepted 16 June 2022; Published 30 June 2022

Academic Editor: Fernanda Casciatori

Aegle marmelos is a medicinal herb that has a variety of biological constituents. "Catechins" are a class of phenolic compounds that have therapeutic value. The present study employs batch extraction with methanol as a solvent to extract the catechins from the pulp of *Aegle marmelos* fruit. Box–Behnken design of response surface methodology (RSM-BBD) is used to optimize the operational parameters impacting catechin extraction, such as solvent concentration, pH, and extraction time. Extraction of 96.5% of catechins was achieved at a methanol concentration of 80%, pH 6.24, and a soaking period of 44.7 hrs (desirability: 0.966). Additionally, MATLAB's artificial neural network (ANN) was used to accurately estimate the extraction yield. The antimicrobial activity of the methanolic extract was tested against five different pathogens, including Streptococcus, Bacillus mega, Pseudomonas putida, Bacillus cerecus, and Staphylococcus aureus, using the agar diffusion method and the tube dilution method. Streptococcus, Bacillus cereus, and pseudomonas putida showed high activity.

1. Introduction

Therapeutic plants can be used in drug development as an indigenous source of new compounds with medicinal promise [1]. According to the World Health Organization (WHO), traditional medicines and primarily natural plant products are used by 80% of the developing world's population for basic health care [2]. In comparison to synthetic pharmaceuticals, which are susceptible to adulteration and side effects, they have minimal toxicity, are cost-effective and pharmacologically active, and provide a simple solution to many human issues. Plants and their products are significant inceptions for food and medicine, both of which are extremely beneficial to humanity [3]. There are several herbs that are commonly used to treat cardiovascular difficulties, liver disorders, central nervous system disorders, and digestive and metabolic disorders. Therapeutic plants include both primary and secondary metabolites [4]. These metabolites have therapeutic properties and are used to treat a wide range of disorders. Therapeutic medicinal herbs are not only safe, but they are also cheap, effective, and readily available.

Aegle marmelos (Bilvam) is a medicinal herb plant that is one of the 250,000 living terrestrial plant species in the world [1]. Bilvam is a member of the Rutaceae family and is also known as Indian quince, bael, bilva, golden apple, and a number of other names. More than 100 phytochemical compounds have been found in the plant's various portions. Phytochemicals discovered in bilvam include alkaloids, cardiac glycosides, coumarins, polysaccharides, saponins, steroids, flavonoids, fatty acids, tannis, and terpenoids. These compounds are widely known for their biological and pharmacological effectiveness against a wide range of chronic ailments, including cancer, cardiovascular disease, and gastrointestinal issues [2, 3, 5]. Aside from these properties, there have been reports of antioxidant, antiulcer, antidiabetic, cancer-preventive, antihyperlipidaemic, anti-inflammatory, antibacterial, and antispermatogenic effects.

It serves as a climatic cleanser in comparison to other plants since it emits a big amount of oxygen. Bilvam is an important plant for reforestation, especially in dry peripheral soils [6]. Every part of the *Aegle marmelos* plant has therapeutic characteristics and is used to treat a range of eye and skin disorders. Bilvam seeds contain a unique fatty acid (12-hydroxyoctadec-cis-9 enoic acid, commonly known as ricinoleic acid) that has the potential to be turned into biodiesel. The *Aegle marmelos* fruit extract was used as a fuel in the synthesis of zinc ferrite nanoparticles [7]. The fruit pulp of Indian bael (*Aegle marmelos*) was used as a new fuel for the solution combustion production of zinc oxide nanoparticles (ZnO NPs) [8].

Many plant-based foods, including fruits, vegetables, and drinks, contain significant concentrations of a type of polyphenolic chemicals known as catechins [9]. Catechins offer a number of physiological advantages, including the capacity to scavenge free radicals and delay the degradation of the extracellular matrix. Because of the functional properties of catechins, effective strategies for extracting and purifying its ingredients from natural plants are regarded to be necessary. Catechins are antioxidant chemicals that have been extensively researched. Catechins have been researched to increase their stability and rate of absorption in the human body [9]. Tea catechins are often used in a range of neutraceuticals, medications, and cosmetics to either lengthen product shelf life or enhance human health [10]. It has been proven that caffeine and polyphenols can be extracted from Iranian green tea leaves [11]. Catechins in Camellia sinensis leaves were extracted using an effective microwave-assisted extraction (MAE) method that utilized green deep eutectic solvents [12].

Physiologically active polyphenol compounds extracted from botanical materials have been the subject of several investigations in recent years. The aim of this study is to investigate the antimicrobial activity of the catechins extracted from *Aegle marmelos* fruit pulp. Agar diffusion and tube dilution procedures were used to study the extract's antimicrobial activity. The process parameters that impact the extraction process are optimized using response surface methodology (RSM) and artificial neural networks (ANN).

2. Experimental

2.1. Materials and Methods. Analytical-grade chemicals are employed in the course of the research. *Aegle marmelos* fruit pulp was gathered in Vizianagaram, Andhra Pradesh, India. The plant's taxonomic classification has been verified. Throughout the research, double distilled water has been used.

2.2. Processing of Plant. After washing with tap water, the plant material was rinsed in distilled water. The washed material was sun-dried for two to three days. The dried pulp material was powdered and used as a raw material. This powder was sieved to different particle sizes ranging from 354 to 205 μm and stored in an airtight container until further usage.

2.3. Quantitative Determination of Catechins in Aegle marmelos. Catechin content of the plant extract was determined by using buffer *c* (5% triethanolamine (v/v), 5% (w/v) and $FeCl_3$ reagent. For the plant extract, 1.725 ml of buffer and 375 μl of ferric chloride were added, and the absorbance of the resulting mixture was measured at 510 nm using UV-vis spectrophotometer.

2.4. Extraction Studies. The preliminary extraction experiments are carried out to find a suitable solvent. The solvents tested are methanol, acetone, and water. 1 g of the fruit pulp powder prepared was taken in a conical flask containing the methanol solvent. The solution was diluted to 50 mL. The solutions were soaked for a period of time before being filtered using Whatman No. 1 filter paper. Similar procedure was applied to the remaining solvents. The extraction samples collected were analyzed with UV spectrophotometer for catechin content. Then for the solvent selected, other process parameters like extraction time, pH, solvent concentration, and size of the powdered sample were varied one at a time to find the best conditions.

2.4.1. Response Surface Methodology (RSM). Response surface methodology (RSM) is one such combination of mathematical and statistical methods that may be used to assess quantitative data and their interaction terms from analytical experiments to create and solve multivariant equations concurrently [13, 14]. RSM is based on a polynomial equation to establish a model between the dependent and independent elements, as well as a symmetrical model to anticipate and define the experimental optimal circumstances [15]. The Box–Behnken design (BBD) is a well-known RSM design for improving bioactive chemical extraction. The Box–Behnken design is made up of rotating lower-dimensional designs that estimate all linear, quadratic, and two-way interactions. It does not allow for design reduction. They have no corner points in the design space. The axial points are beyond the design space box established by the factorial element of the design, which is rotatable. This enables the projected reaction to be approximated with identical variance, regardless of direction from the design space's center. As indicated in Table 1, three independent variables, methanol concentration (A), pH (B), and extraction time (C) were explored using BBD in preliminary extraction trials. Stat-Ease Design Expert Software ver.12 was used to predict the extract yield (response) using a 2^{nd}-order polynomial equation.

2.4.2. Artificial Neural Network (ANN). Modeling and simulation of extraction process is well recognized as it can predict the extraction process and complicated functional relationships. Artificial neural network (ANN) is a valuable tool to interpret the relationship between the input and output data of augmented experimentations [16]. The ANN model is capable of learning the pattern of the underlying process from prior data and generalizing the acquired information (or the complex mathematical relationship

Table 1: Factors and its levels used for the present study.

Factor	Name	Units	Minimum	Maximum
A	Methanol %		20.00	80.00
B	pH		3.00	8.00
C	Extraction time	hr	12.00	48.00

between input and output data). The ANN model is comprised of three distinct layers of input, hidden, and output layers known as neurons, which may be used to predict the connection between input and output layers [17]. All of the data are randomly divided into three categories: training (70%), testing (15%), and validation (15%). The performance metrics of mean square error (MSE) and coefficient of determination (R^2) are used to evaluate the outcome of the ANN model.

$$\mathrm{MSE} = \frac{1}{N}\sum_{i=1}^{N}\left(y_{\mathrm{prd},i} - y_{\mathrm{exp},i}\right)^2,$$
$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(y_{\mathrm{prd},i} - y_{\mathrm{exp},i}\right)}{\sum_{i=1}^{N}\left(y_{\mathrm{prd},i} - y_m\right)}, \quad (1)$$

where $y_{\mathrm{prd},i}$ is the ANN forecasted value, $y_{\mathrm{exp},i}$ is the experimental value, N is the number of data points, and y_m is the average of experimental data.

The ANN computations in this study were carried out using the Toolbox of MATLAB Version R2021. A three-layer ANN with a tangent sigmoid transfer function (tansig) at the hidden layer, a linear transfer function (purelin) at the output layer, and a Levenberg–Marquardt backpropagation algorithm with 1000 iterations were used. The input layer has three nodes that represent the influencing elements of the extraction process such as ethanol concentration, pH, and extraction time.

2.5. FTIR Studies. Fourier transform infrared spectroscopy (FTIR) is a technique which is used to determine the functional groups of the active components within the extract based on the peak values in the region of IR radiation. When the extracts were passed into the FTIR, the functional groups of the components were separated based on its peak quantitative relation. FTIR spectrum is recorded between 4000 and 400 cm^{-1}.

2.6. Antimicrobial Activity. The methanolic extract of the plant material was prepared using the cold percolation technique. Different quantities of dry powder were soaked in the same amount of solvent and soaked for 24 hrs at room temperature with continuous agitation at 150 rpm. Filter the aforementioned extract and store it in the refrigerator for later use. The susceptibility of harmful microorganisms to drugs varies greatly between strains [2, 9]. As a result, knowing the susceptibility of these bacteria is extremely beneficial not only in therapy but also in epidemiological studies. The antimicrobial susceptibility test may be performed in two ways: diffusion (agar well diffusion method)/test tube dilution method. The agar well diffusion method is commonly used to determine the antibacterial activity of plant or microbial extracts. The agar plate surface is inoculated by spreading a volume of microbial inoculums over the whole agar surface. Then, using a sterile cork borer or tip, an aseptically punched hole with a diameter of 6 to 8 mm is made, and a volume of the antimicrobial agent or extract solution at the required concentration is placed in the well. The agar plates are then incubated under proper conditions, depending on the test microorganism, and the diameter of the inhibitory growth zones is measured. The dilution technique employs various quantities of plant extract into test tubes containing nutrient broth and a standard suspension of the test organism inoculation. The test tubes are then incubated overnight at 37°C. Each sample's absorbance is measured after incubation.

$$\%\ \text{inhibition} = \frac{\text{absorbance (control)} - \text{absorbance (sample)}}{\text{absorbance (control)}} \times 100. \quad (3)$$

3. Results and Discussion

3.1. Extraction Studies. The selection of extraction solvent has a major role in extraction of the bioactive components. Experiments were conducted with three solvents i.e., water, acetone, and methanol. The methanol used as a solvent provides good yield of catechin from *Aegle marmelos* pulp powder. The results are shown in Table 2.

Methanol was used as a solvent in subsequent extraction tests. Experiments were carried out in OFAT (one factor at a time) optimization methodology. Catechins were extracted using methanol concentrations ranging from 0% to 100%. According to the results of the trials, the maximum catechin extraction was 48.6% for 80% ethanol. The investigations were carried out to determine the effect of pH on extraction, and the maximum catechin extraction of 58.32% was attained at pH = 4. The finer the particle size, the greater will be the % extraction. For extraction, several sizes of plant powder (354, 328, 250, and 205 μm) were utilized. At 205 μm, the maximum percentage extraction of 92.6% was recorded. The pulp powder was soaked in the solvent for different time periods like 1 day, 2 days, and 3 days at pH 4, with 80% methanol and 205 μm particle size yielded a maximum extraction of 94.5% for the time duration of 2 days. Figure 1 depicts the extraction procedure in a visual manner.

3.1.1. Response Surface Methodology (RSM). Box–Behnken design (BBD) applied based on 17 experimental runs with three independent variables, methanol concentration (A), pH (B), and extraction time (C), was conducted to investigate their main and interaction contribution on catechin extraction. Replicates were used to avoid experimental errors. The analysis of variance (ANOVA) was used to realize the diagnostics checking test for adequacy of the proposed model based on the Fishers F test. The regression coefficient (R^2) indicates the amount of variation around the mean explained by the model. The

Table 2: Effect of different solvents on catechin extraction %.

S.no.	Solvent	Concentration of catechin (mg/l)	Catechin extraction (%)
1	Water	20.6	4.12
2	Acetone	130.84	26.168
3	Methanol	206.13	41.226

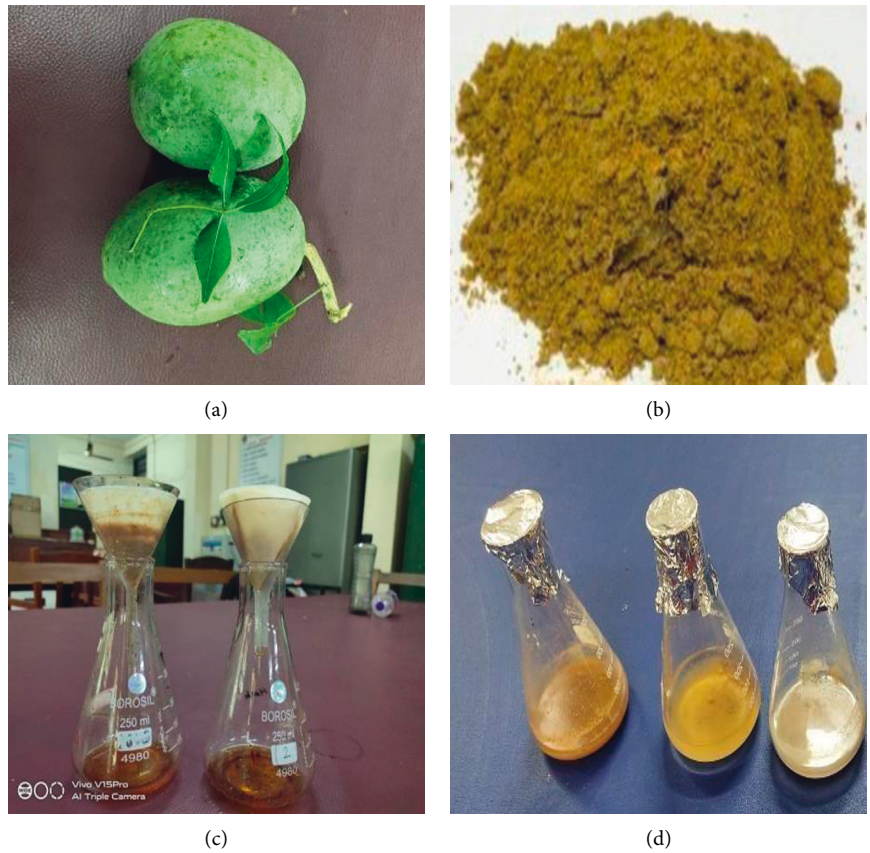

Figure 1: (a) *Aegle marmelos* fruit; (b) *Aegle marmelos* fruit powder; (c) extract prepared; (d) extract prepared with different solvents.

coded and uncoded levels of independent factors according to 17 experiments correspond to BBD along their responses are shown in Table 3. The quadratic model in terms of coded factors is expressed as

$$\begin{aligned}\text{Catechins Yield} = {} & +4.07324 - 0.318208\,\text{Methanol\%} + 14.57030\text{pH} + 0.827815\,\text{Extraction time} - 0.023333\,\text{Methanol\%}^{*}\text{pH} \\ & + 0.005060\,\text{Methanol\%}^{*}\text{Extraction time} \\ & + 0.063722\text{pH}^{*}\text{Extraction time} + 0.007894\,\text{Methanol\%}^{2} - 1.24080\text{pH}^{2} - 0.018248\,\text{Extraction time}^{2}\end{aligned} \tag{4}$$

3.2. Analysis of Variance (ANOVA). The Model F-value of 168.96 indicates that the model is statistically significant (Table 4). There is a 0.01% probability that such a large F-value occurs as a result of noise. *P* values less than 0.0500 indicate the existence of significant model terms. A, B, C, AB, AC, BC, A^2, B^2, and C^2 are all significant model terms in this situation. The *F*-value for lack of fit is 3.09, indicating that the lack of fit is not significant in comparison to the pure error. A negligible lack of fit is desirable. The predicted R^2 of 0.9466 is reasonably close to the adjusted R^2 of 0.9895 (Table 5); in other words, the difference is less than 0.2. The signal-to-noise ratio is determined by Adeq Precision. A ratio greater than four is preferred, so that the design space can be navigated.

Table 3: Designed variables and the response using BBD.

Run	Methanol%	pH	Time (hrs)	Response (% extraction)
1	50	3	48	55.7
2	80	5.5	12	78
3	50	5.5	30	70.24
4	50	5.5	30	69.6
5	50	5.5	30	70.6
6	20	5.5	48	60.12
7	20	5.5	12	54.6
8	50	3	12	48.2
9	50	5.5	30	70.3
10	20	3	30	49.6
11	50	8	12	52.43
12	80	3	30	83.6
13	20	8	30	59.8
14	50	8	48	71.4
15	50	5.5	30	72.26
16	80	8	30	86.8
17	80	5.5	48	94.45

Table 4: ANOVA for quadratic model.

Source	Sum of squares	df	Mean square	*F*-value	*P* value	
Model	2861.72	9	317.97	168.96	<0.0001	Significant
A-methanol %	1762.10	1	1762.10	936.35	<0.0001	
B-pH	138.86	1	138.86	73.79	<0.0001	
C-extraction time	293.30	1	293.30	155.86	<0.0001	
AB	12.25	1	12.25	6.51	0.0380	
AC	29.87	1	29.87	15.87	0.0053	
BC	32.89	1	32.89	17.48	0.0041	
A^2	212.55	1	212.55	112.95	<0.0001	
B^2	253.22	1	253.22	134.56	<0.0001	
C^2	147.19	1	147.19	78.21	<0.0001	
Residual	13.17	7	1.88			
Lack of fit	9.20	3	3.07	3.09	0.1525	Not significant
Pure error	3.98	4	0.9938			
Cor total	2874.89	16				

Table 5: Fit statistics.

Std. dev.	1.37
Mean	67.51
C.V.%	2.03
R^2	0.9954
Adjusted R^2	0.9895
Predicted R^2	0.9466
Adeq precision	43.9380

3.3. Response Surface Plots and Interaction between the Parameters for Catechin Extraction. The generated surface contour plots will aid in visualizing the statistical significance of the independent variables on the dependent variables. The 3D response plot was applied to the experimental data and the effect of parameters such as time, pH, and methanol concentration was observed to determine the extract yield. To maximize the extraction efficiency and energy savings, the period of extraction was tuned in this study. As illustrated in Figures 2(b) and 2(c), increasing time had a beneficial influence on the overall extraction yield. Additionally, the plots demonstrated that when the time of the extraction was increased beyond 40 hrs, the overall extraction yield remained relatively constant. As illustrated in Figures 2(a) and 2(b), pH has a significant effect on extraction. Another critical element impacting extraction efficiency is solvent concentration. A linear effect was seen and was statistically significant when methanol% was studied. This indicates that increasing the methanol concentration results in an increase in extraction, as illustrated in Figures 2(a) and 2(c). Catechin was shown to have an optimum methanol concentration of 80%. Indeed, a methanol concentration of 80% has been shown to be effective in extracting a variety of phenolics [18].

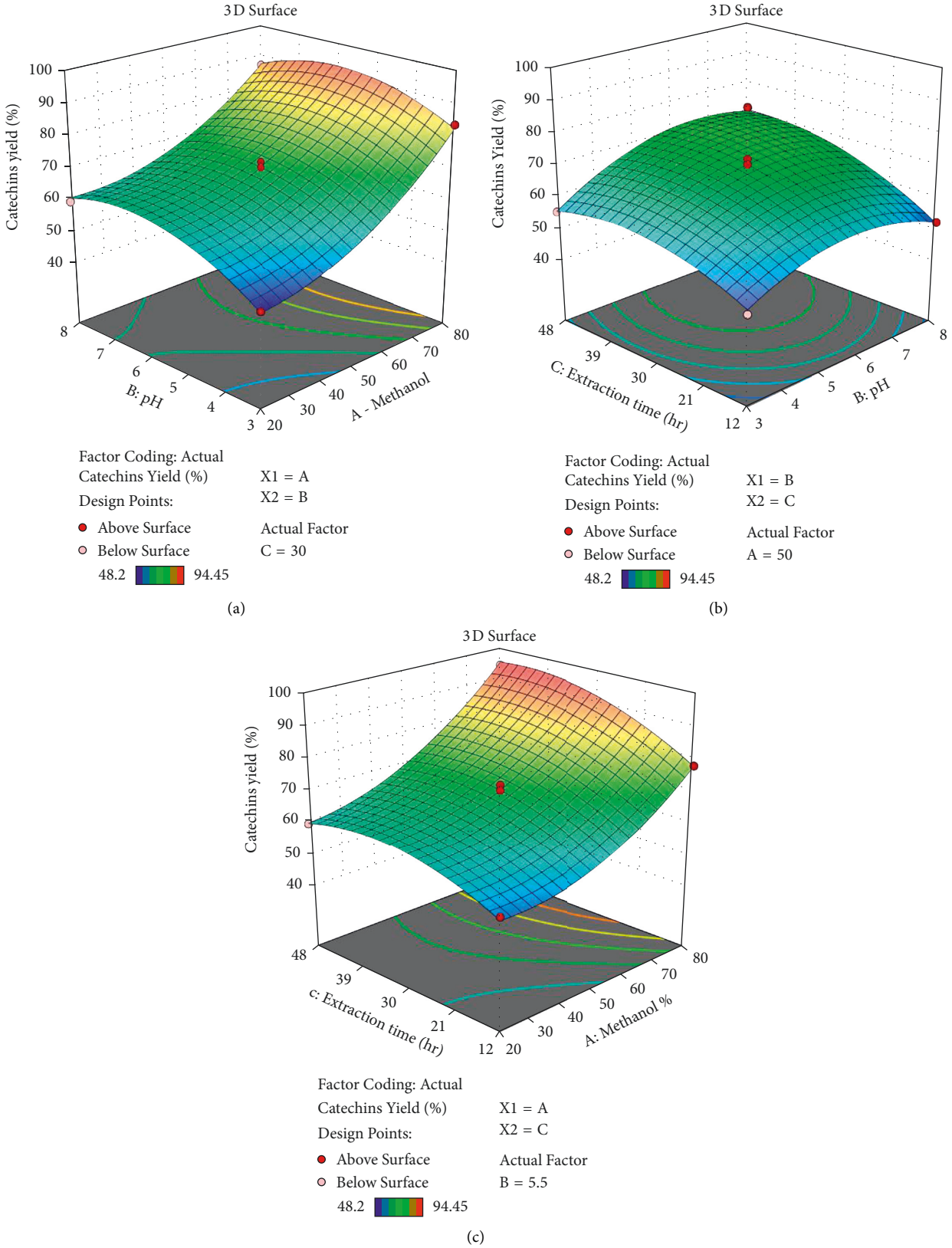

Figure 2: (a) 3D response surface plot of methanol % and pH. (b) 3D response surface plot of extraction time and pH. (c) 3D response surface plot of methanol % and extraction time.

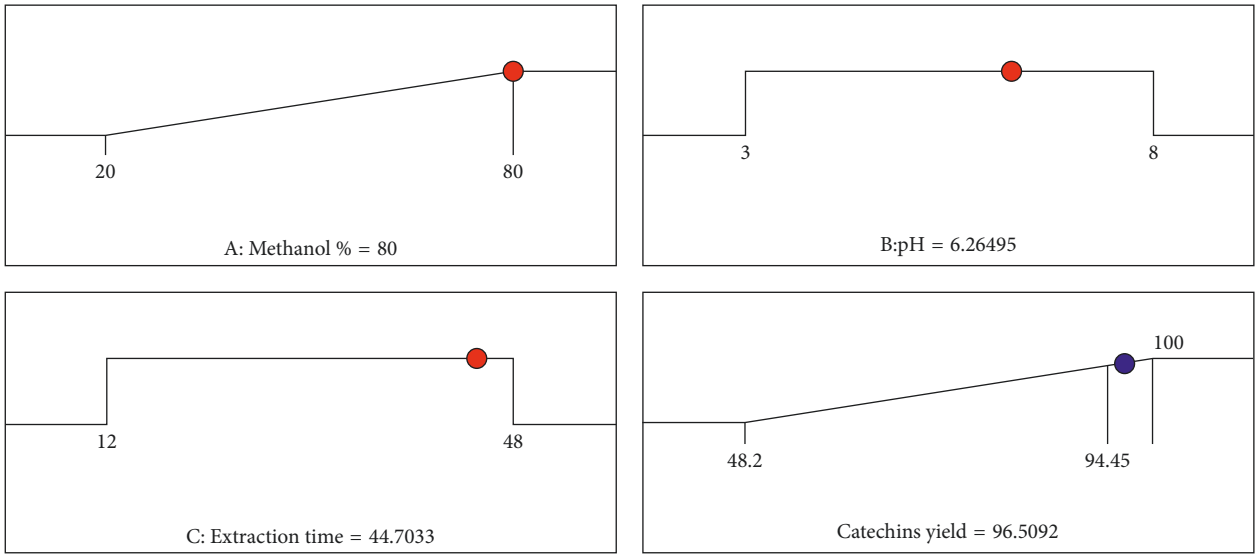

Figure 3: Ramp plot of optimization.

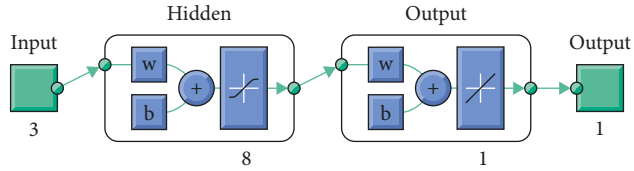

Figure 4: Optimum ANN architecture.

3.4. Optimization. The objective used in this study is to maximize the response across a set of variables. For this aim, the design expert software selects the factors necessary to get the most ideal outcomes. At optimal conditions of 80% methanol, 44.7 hrs extraction duration, and pH 6.24, the maximum catechin extraction was 96.5% with a desirability of 0.966, as shown in Figure 3. The predicted extraction percentage was confirmed using the experimental response obtained under the given ideal conditions.

3.4.1. ANN Modeling Using MATLAB. The maximum value of R^2 and the minimum value of MSE of the testing dataset were used to determine the ideal design of an ANN model. The optimization approach for the training process was the backpropagation algorithm. The best adsorption topology was found to be 3-8-1, and the architecture is shown in Figure 4.

The MSE values are 0.00848, 1.407, and 1.424, respectively, for training, validation, and testing. The R^2 for the ANN model is 0.9615 and the model obtained a good connection with the optimum structure from the training data, as shown in Figure 5, and it worked rather well in validation and testing, despite some data scattering. The ANN model worked admirably for the dataset. The MSE for the optimized ANN model is plotted against the epoch number in Figure 6. After 4 epochs, the training process was found to have come to an end. These findings revealed that the experimental data and the anticipated data from the ANN model were in good agreement.

3.4.2. Comparison of RSM and ANN. Figure 7 shows the RSM and ANN predictions for the designed experiments in comparison to each other. Both models were shown to be useful in predicting the experimental data. It was determined that the ANN correlation coefficient was 0.961, while the RSM correlation coefficient was 0.995.

3.5. FTIR Studies. To decipher the infrared spectrum, it is necessary to tie the absorption bands to the sample's chemical composition. Studying *Aegle marmelos* fruit pulp powder, the FTIR spectroscopic results revealed the existence of numerous functional groups, as shown in Figure 8. The stretched-OH bond of alcohol groups is responsible for the large peak at 3872.74 cm^{-1}, which shows the presence of hydroxyl (-OH) group. Stretch vibrations of the N-H bond provide a peak at 3446.05 cm^{-1}, while the stretching vibrations of C=O produce a peak at 1601.99 cm^{-1} [19]. Aromatic cyclic ethers with C-O stretch at 1071.66 cm^{-1}were found. The results confirm the presence of different phytochemical ingredients and are in agreement with earlier studies [20]. The FTIR of the ethanolic extract shows a peak at 2947.56 cm^{-1}caused by the stretching

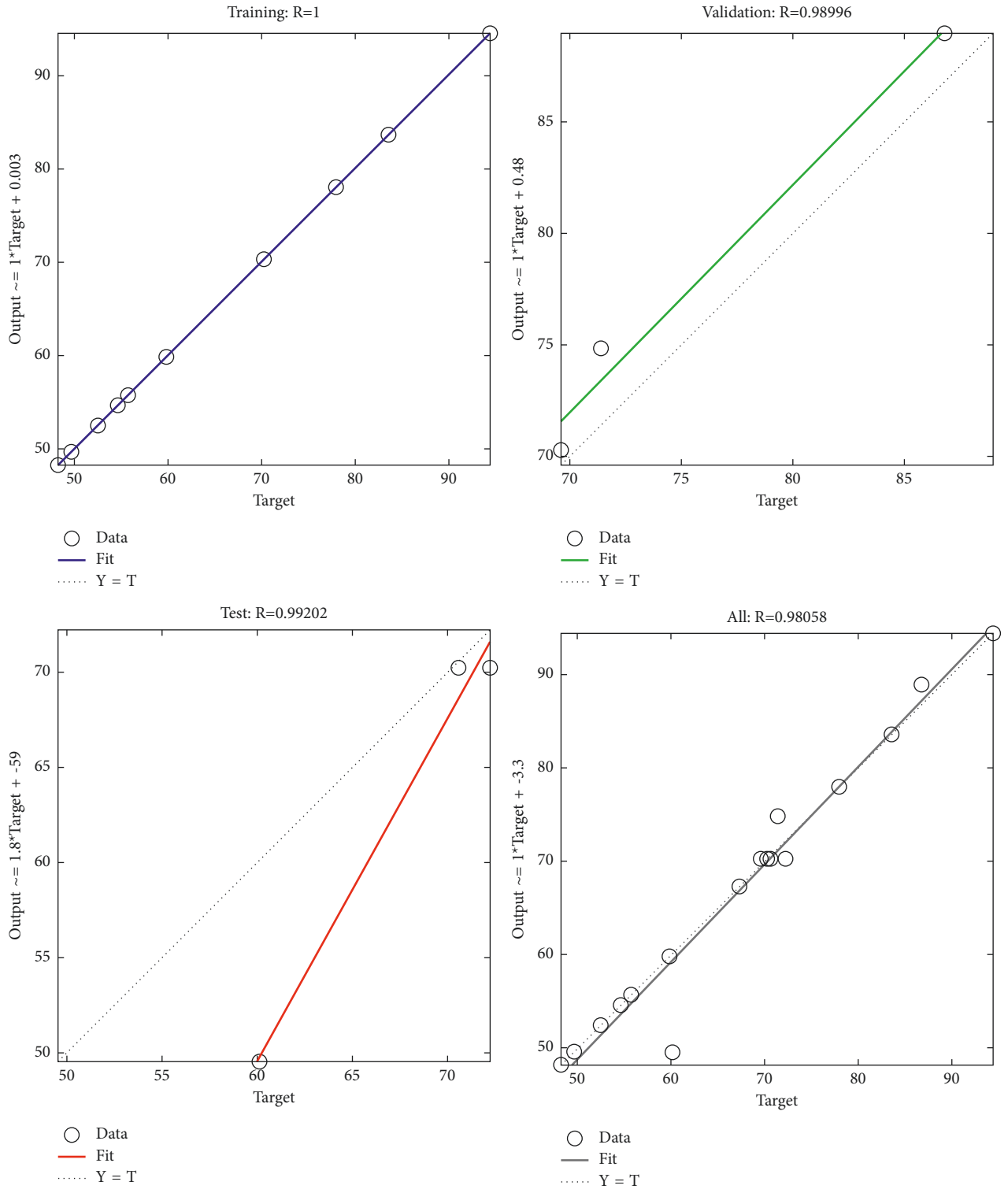

Figure 5: Output of ANN model for training, validation, testing, and complete data.

vibration of-OH, further a band at 1618 cm^{-1} due to the stretch of the aromatic ring quadrant, and another band at 1090 cm^{-1} due to the stretch of the aromatic alcohol. A secondary aliphatic alcohol, the C-O stretch, may be seen at 990 cm^{-1}.

3.6. Antimicrobial Activity. The antimicrobial activity of the test material (plant extract) was determined in vitro using the agar diffusion technique (Figure 9). Streptococcus, *Bacillus* mega, *Pseudomonas* putida, *Bacillus* cerecus, and *Staphylococcus aureus* were the bacteria studied. For 24 hrs,

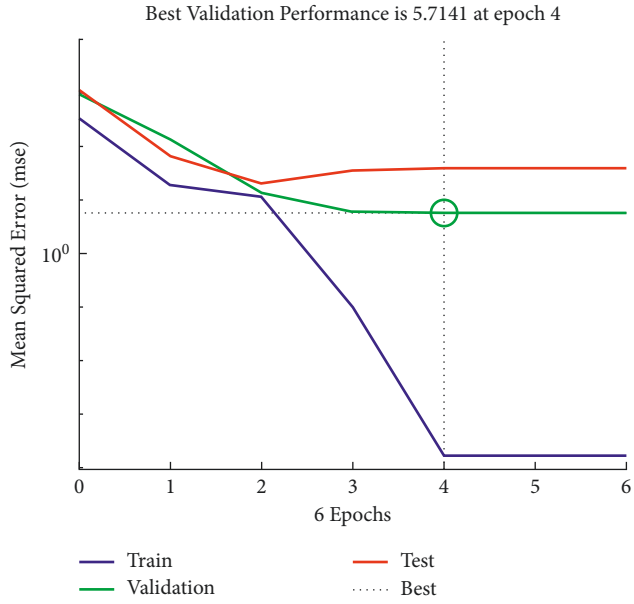

Figure 6: MSE vs. the number of epochs in the hidden layer.

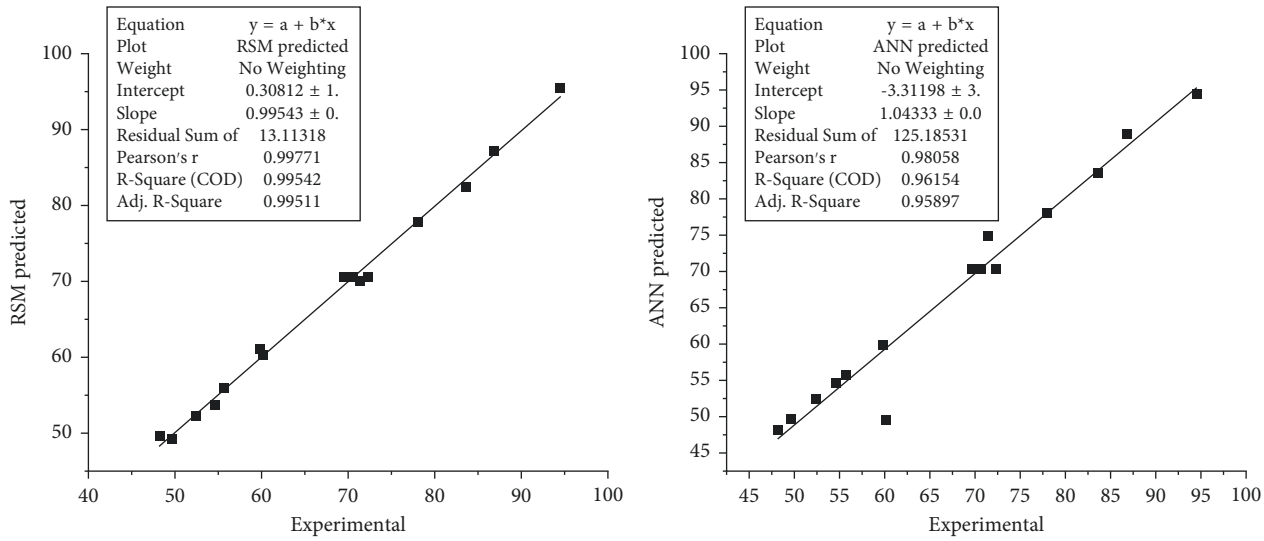

Figure 7: Experimental vs. predicted values for RSM and ANN.

the plates were incubated at 37°C in an incubator. The zone of inhibition was used to assess antibacterial activity. The tube dilution test is the standard method for determining levels of resistance to an antibiotic. Tables 6 and 7 present the findings of the agar diffusion method and tube dilution method, respectively. Out of the tested microorganisms, the plant extract showed high antimicrobial activity towards Streptococcus, Pseudomonas putida, and Bacillus cerecus.

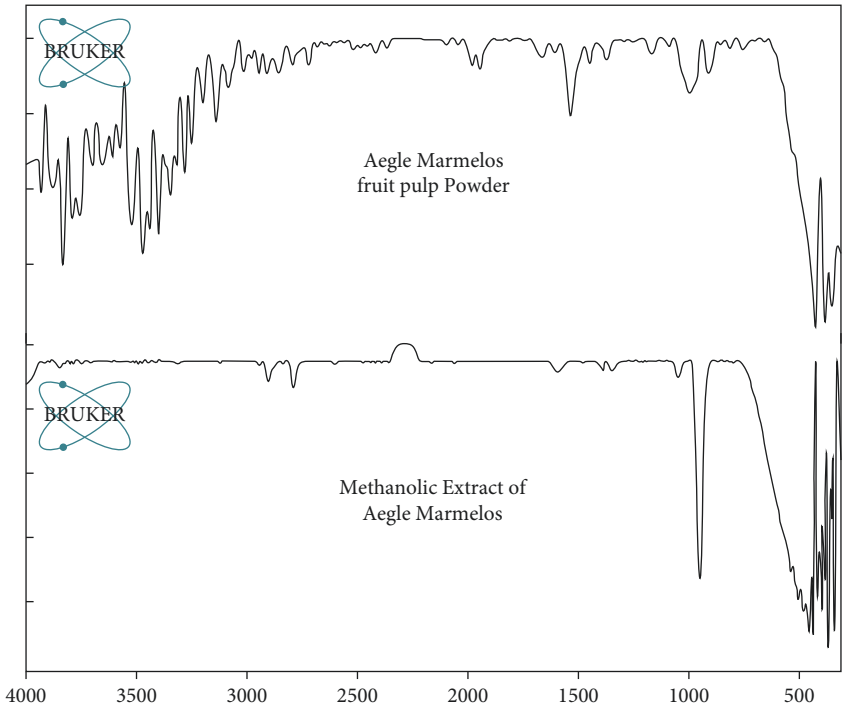

Figure 8: FTIR images of the *Aegle marmelos* fruit pulp powder and methanolic extract.

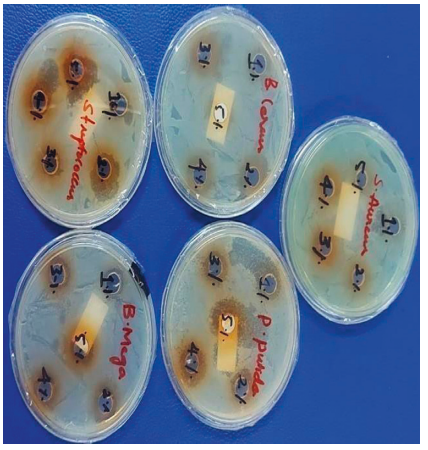

Figure 9: Zone of inhibition of ethanolic extract by agar diffusion method.

Table 6: Zone of inhibition by agar diffusion method.

Concentration (g/ml)	Zone of inhibition (cm)				
	Streptococcus	Bacillus mega	Pseudomonas putida	Bacillus cerecus	Staphylococcus aureus
1	1.6	1.2	1.8	1	0.9
2	2.1	1.7	2.0	1.5	1.3
3	2.0	1.9	2.4	1.8	1.4
4	2.3	2.0	2.6	2	1.9
5	2.8	2.3	2.7	2.4	2.0

Table 7: % inhibition by tube dilution method.

Concentration (g/ml)	% inhibition				
	Streptococcus	Bacillus mega	Pseudomonas putida	Bacillus cerecus	Staphylococcus aureus
1	17	11.76	12.42	7.15	10.2
2	11.87	29.4	29.35	24.44	11.04
3	35.9	39.79	35.3	33.68	27.7
4	45.3	47.16	38.6	53.5	41.35
5	67.6	65.25	65.4	69	51.34

4. Conclusions

Plant-based medicines have made significant contributions to human health improvement and serve as a source of inspiration for the development of new medication molecules. As a result of the findings from the present study, it may be inferred that catechins derived from *Aegle marmelos* fruit pulp could be exploited in pharmacology. The key findings of the study are as follows:

(i) Methanol serves as the extraction solvent

(ii) RSM-BBD research indicated a 96.5 percent catechin output at a methanol concentration of 80%, time of 44.7 hours, and pH of 7.4. (6.24)

(iii) In both the RSM and the ANN models, the optimum extract yield was accurately predicted, as well as the connection between the dependent and independent variables

(iv) Antibacterial activity against microorganisms was remarkably strong in the extract

Data Availability

All the data are available in the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] N. A. Ibrahim, F. M. El-Sakhawy, M. M. D. Mohammed, M. A. Farid, N. A. M. Abdel-Wahed, and D. A. H. Deabes, "Chemical composition, antimicrobial and antifungal activities of essential oils of the leaves of Aegle marmelos (L.) correa growing in Egypt," *Journal of Applied Pharmaceutical Science*, vol. 5, 2015.

[2] B. Manandhar, K. R. Paudel, B. Sharma, and R. Karki, "Phytochemical profile and pharmacological activity of Aegle marmelos Linn," *Journal of Integrative Medicine*, vol. 16, pp. 153–163, 2018.

[3] S. Patil and P. Muthusamy, "A bio-inspired approach of formulation and evaluation of Aegle marmelos fruit extract mediated silver nanoparticle gel and comparison of its antibacterial activity with antiseptic cream," *The European Journal of Integrative Medicine*, vol. 33, Article ID 101025, 2020.

[4] S. Rahman and R. Parvin, "Therapeutic potential of Aegle marmelos (L.)-An overview," *Asian Pacific Journal of Tropical Disease*, vol. 4, pp. 71–77, 2014.

[5] V. M. Mani, A. P. G. Soundari, D. Karthiyaini, and K. Preethi, "Bioprospecting endophytic fungi and their metabolites from medicinal tree Aegle marmelos in Western Ghats, India," *Mycobiology*, vol. 43, pp. 303–310, 2015.

[6] M. Devi, S. Devi, V. Sharma, N. Rana, R. K. Bhatia, and A. K. Bhatt, "Green synthesis of silver nanoparticles using methanolic fruit extract of Aegle marmelos and their antimicrobial potential against human bacterial pathogens," *Journal of Traditional and Complementary Medicine*, vol. 10, pp. 158–165, 2020.

[7] V. Lakshmi Ranganatha, S. Pramila, G. Nagaraju, Udayabhanu, B. S. Surendra, and C. Mallikarjunaswamy, "Cost-effective and green approach for the synthesis of zinc ferrite nanoparticles using aegle marmelos extract as a fuel: catalytic, electrochemical, and microbial applications," *Journal of Materials Science: Materials in Electronics*, vol. 31, pp. 17386–17403, 2020.

[8] C. Anupama, A. Kaphle, Udayabhanu, and G. Nagaraju, "Aegle marmelos assisted facile combustion synthesis of multifunctional ZnO nanoparticles: study of their photoluminescence, photo catalytic and antimicrobial activities," *Journal of Materials Science: Materials in Electronics*, vol. 29, pp. 4238–4249, 2018.

[9] J. Bae, N. Kim, Y. Shin, S.-Y. Kim, and Y.-J. Kim, "Activity of catechins and their applications," *Biomedicine Dermatology*, vol. 4, pp. 1–10, 2020.

[10] Q. V. Vuong, J. B. Golding, M. Nguyen, and P. D. Roach, "Extraction and isolation of catechins from tea," *Journal of Separation Science*, vol. 33, pp. 3415–3428, 2010.

[11] V. Ghasemzadeh-mohammadi, B. Zamani, M. Afsharpour, and A. Mohammadi, "Extraction of caffeine and catechins using microwave-assisted and ultrasonic extraction from green tea leaves: an optimization study by the IV-optimal design," *Food Science and Biotechnology*, vol. 26, pp. 1281–1290, 2017.

[12] J. Li, Z. Han, Y. Zou, and B. Yu, "Efficient extraction of major catechins in *Camellia sinensis* leaves using green choline chloride-based deep eutectic solvents," *RSC Advances*, vol. 5, pp. 93937–93944, 2015.

[13] V. R. Myneni, N. R. kanidarapu, F. Shaik, and M. vangalapati, "Response surface modeling of the removal of methyl orange dye from aqueous solution using magnesium oxide nanoparticles immobilized on chitosan," *Iranian Journal of Chemistry and Chemical Engineering*, vol. 36, 2021.

[14] V. R. Myneni, N. R. Kanidarapu, and M. Vangalapati, "Methylene blue adsorption by magnesium oxide nanoparticles immobilized with chitosan (CS-MgONP): response surface methodology, isotherm, kinetics and thermodynamic studies," *Iranian Journal of Chemistry and Chemical Engineering*, vol. 39, pp. 29–42, 2020.

[15] M. Venkata Ratnam, C. Karthikeyan, K. Nagamalleswara Rao, and V. Meena, "Magnesium oxide nanoparticles for effective photocatalytic degradation of methyl red dye in aqueous

solutions: optimization studies using response surface methodology," *Materials Today Proceedings*, vol. 26, pp. 2308–2313, 2019.

[16] V. R. Myneni, T. Punugoti, N. S. Kala, N. R. Kanidarapu, and M. Vangalapati, "Modelling and optimization of methylene blue adsorption onto magnesium oxide nanoparticles loaded onto activated carbon (MgONP-AC): response surface methodology and artificial neural networks," *Materials Today Proceedings*, vol. 18, pp. 4932–4941, 2019.

[17] B. Gudeta, S. Solomon, and M. V. Ratnam, "Bioinsecticide production from cigarette wastes," *International Journal of Chemical Engineering*, vol. 2021, Article ID 4888946, 15 pages, 2021.

[18] F. Mujeeb, P. Bajpai, and N. Pathak, "Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of aegle marmelos," *BioMed Research International*, vol. 2014, Article ID 497606, 11 pages, 2014.

[19] N. Bhardwaj, D. Prasad, and R. Haldhar, "Study of the aegle marmelos as a green corrosion inhibitor for mild steel in acidic medium: experimental and theoretical approach," *Journal of Bio-Tribo-Corrosion*, vol. 4, 2018.

[20] T. G. Deokar, S. S. Pawar, and M. G. Jadhav, "FESEM and FTIR spectroscopic characterization of Aegle marmelos (L.) unripe fruit," *International Research Journal of Biological Sciences*, vol. 6, pp. 34–39, 2017.