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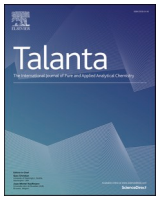

Deep eutectic solvent-based green extraction of *Strychnos potatorum* seed phenolics: Process optimization via response surface methodology and artificial neural network

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ABSTRACT

The current research focused on extraction optimization of bioactive compounds from *Strychnos potatorum* seeds (SPs) using an eco-friendly glycerol-sodium acetate based deep eutectic solvent (DES). The optimization was accomplished using response surface methodology (RSM) and artificial neural networking (ANN). The independent variables included shaking time (A), temperature (B), and solvent-to-feed ratio (C), and the responses were the extraction yield, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (DPPH), and antidiabetic activity (α-amylase inhibitory activity). The SPs extracts obtained under optimal conditions (29 min, 40 °C and 30 mL/g of A, B, and C parameters, respectively) had 30.43 mg gallic acid equivalents (GAE)/g of dry weight (DW) TPC, 10.99 mg rutin equivalents (RE)/g DW TFC, 26.16 % antioxidant activity and 46.95 % α-amylase inhibitory activity. For all the outputs, the ANN percentage error was less than the RSM percentage error for the predicted values against the experimentally measured values. The results were further supported by the %AAD (% absolute average deviation) and R^2 values obtained from RSM and ANN methods. The %AAD for TPC, TFC, DPPH, and α-amylase inhibitory activity by RSM was 7.31, 4.80, 4.03, and 4.36, while by ANN, it was 1.18, 3.90, 1.99, and 2.97, respectively. It is worth noting that despite no statistical difference between the two predictive models, ANN gave closer results to the experimental values. Correlation among various response types showed that TPC and TFC were strongly correlated. This research highlights the efficiency of glycerol-sodium acetate DES as an extractant.

1. Introduction

Strychnos potatorum L. (Loganiaceae family) is a medicinal plant highly regarded for its bioactive properties. It is often referred to as Nirmali or Clearing Nut due to its traditional use in water purification, where seeds are utilized for filtering impurities. The plant is primarily found in tropical regions, including India, Sri Lanka, Myanmar, and parts of Southeast Asia [1]. It thrives in dry forests, scrublands, and tropical woodland environments. Its occurrence is less widespread in other parts of the world, but its medicinal properties have gained attention internationally, leading to interest in its phytochemical constituents and therapeutic potential. In Ayurveda, Unani, and traditional medicine systems, *S. potatorum* has long been recognized for its healing potential [2]. It has been widely utilized in traditional medicine to treat various ailments. The seeds, mainly, are renowned for their antidiabetic, anti-inflammatory, and antioxidant activities. They have also been used to address arthritis [3] liver disorders and remedy for eye infections [4, 5].

The seeds of *S. potatorum* are rich in a diverse array of bioactive compounds, including flavonoids and other polyphenolics, which are responsible for many of its medicinal activities [6]. Polyphenols are known for their antioxidant potential and play a critical role in neutralizing free radicals and reducing oxidative stress, making them valuable in preventing diseases like diabetes, cancer, and cardiovascular

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disorders [7].

Antioxidants play a crucial role in mitigating oxidative stress, which occurs when there is an imbalance between free radicals and the body's ability to neutralize them. Free radicals, highly reactive molecules generated through normal metabolic processes or external factors such as pollution and UV radiation, can cause cellular damage, leading to various chronic conditions such as cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders [8]. Phenolic compounds, known for their potent antioxidant activity, help scavenge these free radicals, preventing oxidative damage to cells. *S. potatorum*, rich in phenolic and antioxidant compounds. These bioactive compounds are believed to have potential for treating oxidative stress and related ailments [5]. By optimizing the extraction of these bioactives from *S. potatorum* seeds using an eco-friendly deep eutectic solvent (DES), this research may contribute to developing natural antioxidant therapies that can offer protection against free radical-induced diseases, highlighting their importance in promoting health and combating oxidative damage.

Apart from oxidative stress, a closely associated disorder, Diabetes is a global health concern, affecting millions of people and posing serious health risks if left unmanaged. According to the World Health Organization (WHO), the prevalence of Diabetes has been steadily increasing, with over 422 million people diagnosed worldwide, and the numbers continue to rise [9]. Diabetes, particularly type 2, is characterized by insulin resistance or inadequate insulin production, leading to high blood sugar levels and complications such as cardiovascular disease, kidney failure, and neuropathy [10]. While pharmaceutical treatments are widely used to manage blood sugar levels, there is growing interest in herb-based treatments and dietary supplements for their potential in controlling and preventing diabetes [11]. Medicinal plants, rich in bioactive compounds like polyphenols, have demonstrated significant antidiabetic properties, aiding in blood sugar regulation and insulin sensitivity. *S. potatorum*, with its abundant polyphenols and antioxidant activity, shows promise in complementing conventional treatments. Thus, optimizing the extraction of these compounds from *S. potatorum* could lead to effective, natural antidiabetic supplements that offer a safer and more holistic approach to managing diabetes.

With the growing awareness of environmental sustainability, extraction processes for recovering bioactive metabolites from plant sources are now expected to be efficient and environmentally friendly. In this regard, the selection of an appropriate solvent plays a critical role. In the early 21st century, a novel class of solvents emerged as a greener alternative to traditional organic solvents. These are known as deep eutectic solvents (DES). They are promising extractants for bioactive compounds due to their desirable properties such as biodegradability [12], low toxicity [13], ease of synthesis [14], and low cost [15]. By selecting suitable precursors, customized deep eutectic solvents (DESs) can be formulated to meet specific requirements. A DES is usually prepared by heating a mixture of two (or sometimes more) compounds, where one acts as a hydrogen bond donor (HBD) and the other as a hydrogen bond acceptor (HBA). HBAs typically include alcohols, amines, or carboxylic acids, while HBDs are often quaternary ammonium salts or other salts capable of accepting hydrogen bonds. DESs have gained prominence as a sustainable alternative to conventional hazardous and volatile solvents, offering a greener solution for various extraction processes [16,17]. They have been found to be very efficient in extracting polyphenolic compounds. The DES used in the current study consisted of sodium acetate and glycerol (1:3), which has been ranked an efficient extractant in previous studies [18].

Apart from the nature of the solvent, an extraction process also depends on various experimental parameters, such as time, temperature, solvent-to-feed ratio, solvent viscosity, surface tension, and density. For an efficient extraction process, these parameters must be optimized and valiadated [19]. To this end, many researchers have studied the effectiveness of some hydrolytic enzymes [20], surfactants [21], acids [22], and alkali [23] in combination with organic solvents [24]. The techniques included ohmic heating [25], microwave-assisted extraction [26], and ultrasound-assisted extraction [27] have been foun effective to extract bioactive compounds from solid samples especially seeds.

With this approach in mind, we opted for an eco-friendly and easy-to-prepare glycerol and sodium acetate-based DES to extract phenolic bioactives from SPs. Key extraction parameters, such as extraction time, temperature, and liquid-to-solid ratio, were tested at various levels and optimized using response surface methodology (RSM), with results further validated through artificial neural networks (ANNs). The final extraction conditions were counter-validated to ensure reliability. RSM, a powerful tool for multi-response optimization, was instrumental in this research. Its strength lies in its ability to analyze variable interactions, providing a comprehensive understanding of the system [28]. On the other hand, artificial neural networks (ANN) are an emerging form of nonlinear computational modeling, inspired by biological neural networks, such as those in the human brain. Leveraging principles of artificial intelligence, ANNs are increasingly used to tackle complex optimization problems. In this model, raw data is introduced into the input layer and then processed through one or more hidden layers, where intricate relationships and patterns are identified. The final output layer delivers the ultimate prediction, derived from the computations in the hidden layers, providing a robust and adaptive approach to problem-solving in various fields [29].

Thus, by using a green extraction medium and optimizing extraction parameters through advanced techniques like response surface methodology (RSM) and artificial neural networks (ANN), the current research not only improves extraction efficiency but also provides a green alternative for the recovery of valuable phenolic compounds from SPs. This approach addresses the demand for greener, safer, and more effective extraction processes in natural product chemistry.

2. Material and method

2.1. Chemicals

In all the experimental work, analytical-grade chemicals were used. Glycerol (>85 %) and sodium acetate anhydrous (>99.9 %) (Merck-Dramstadt, Germany), were used for the preparation of DES. Folin-Ciocalteu was received phenol (≥98 %) from Scharlau (Barcelona, Spain). Ascorbic acid (≥99.0 %) (Sigma-Aldrich), rutin (>80.0 %) (Sigma-Aldrich), sodium hydroxide (>98 %) (Sigma-Aldrich), ferrous sulfate heptahydrate (>99 %) (Sigma-Aldrich), anhydrous sodium carbonate (99.9 %) (Sigma-Aldrich), aluminum chloride (99 %) (Sigma-Aldrich), and DPPH (≥95 %) (Steinheim, Germany) were used for TPC, TFC and for antioxidant activity assays. Porcine pancreatic α-amylase (≥90 %) (Sigma-Aldrich), disodium hydrogen phosphate (≥99 %) (Sigma-Aldrich), sodium chloride (≥99 %) (Sigma-Aldrich), 3,5-dinitrosalicylic acid (≥97 %) (Sigma-Aldrich), sodium potassium tartrate (≥99 %) (Sigma-Aldrich), DMSO (≥99.9 %) (Fisher Chemicals), acarbose (≥95 %) (Sigma-Aldrich), potato starch (≥99 %) (Sigma-Aldrich) were used for anti-diabetic activity.

2.2. Sample preparation

Strychnos potatorum seeds (SPs) were purchased from the local market in Lahore, Pakistan, and were identified by a Botanist from the Department of Botany, Government College University Lahore by Dr. Tehreema Iftikhar (voucher # 3080). The SPs samples were washed, dried under shade, pierced into coarse powder, and then passed through an 80-mesh sieve to obtain uniform-sized particles.

2.3. Preparation of DES

Glycerol (Gly) and sodium acetate (NaOAc) were mixed in a 3:1 ratio at 60 °C. The mixture was stirred for 45–50 min and at 600–700 rpm on a hot plate under vacuum until a clear transparent light brown colour

solution was formed. Characterization of DES was done by monitoring its FTIR spectrum, density, and sound velocity using Density Sound Velocity (DSA) meter [30].

2.4. Extraction procedure

To extract phytochemicals from SPs, 1 g of sample is dissolved in different volumes (10, 20, and 30 mL) of Gly/NaOAc DES according to the treatment layout assembled in Table 1. The sample was subsequently subjected to a heating stirrer to extract phenolics. The heating shaker had a temperature range of 05–380 °C and an overheat protection of 420 °C. Temperature delay accuracy is ±0.1 °C, whereas external temperature sensor accuracy is ±0.5 °C. Digital speed control is available with a 200–1500 rpm range and ±20 rpm sensor precision. Power is 510 W, 100–120/200-240V, 50/60Hz. The acceptable ambient temperature is 05–40 °C, with 80 % RH humidity.

2.5. Extraction parameters

The factors tested for the extraction of phenolics from SPs are time, temperature and solvent to solid ratio. The experimental and coded levels given in Table 1, are generated following rotatable central composite design (CCD). To optimize TPC, TFC, % radical scavenging activity, and inhibition of alpha-amylase, a total of 17 random runs were conducted and outcomes were sent to Design-Expert version 13 (Stat-Ease Inc Minneapolis, USA) for analysis of variance and modulate the responses (Eq. (1)).

$$y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \Sigma_{i=1}^{k} \sum_{j=i+1}^{k-1} \beta_{ij} X_i X_j + \varepsilon \quad \text{(Eq. 1)}$$

Where y is the response, X_i and X_j are input factors, β represents regression coefficients and k shows the no of responses.

2.6. Artificial neural networking (ANN)

Artificial neural networking (ANN) was used to determine non-linear correlation between the input variables (time, temperature, and solvent to solid ratio) and the target responses (TPC, TFC, DPPH, and α-amylase inhibitory activity) [31]. The experimental data based on RSM was used for artificial neural networking (ANN) estimation. The base of neural network training is a feedforward backpropagation (FFBP) network [32]. The criteriafor the selection of neural networks is based on regression and root mean square error (RMSE) analysis. The number and function on the hidden layer was chosen through trial and error method [33]. The model mean square errors (MSE) in the three data sets—training-70 % (11 samples), testing-15 % (3 samples), and validation-15 % (3 samples), and the training function applied was LM (Levengerg-Marquadt)—formed the basis for the performance choice. The adjustment of weights and bias was done using the neural network tools gradient descent function. The trial and error-based approach was used to determine the total number of hidden neuron layers (3:20–10:1). Various algorithms were checked for the transfer functions of hidden layers to achieve optimal response, the best fit transfer functions (Logsig and Tansig) were applied on two hidden layers. The linear function (Purelin) was the output function. ANN was generated using Neural Network toolbox-TM in MATLAB R2019a.

Table 1
Experimental factor (and their levels) applied for the Deep eutectic solvent Based Extraction of Phenolics from affecting the extraction of phenolics from Strychnos potatorum seeds.

Factors	Levels		
	−1	0	+1
Time of extraction (min)	10	25	40
Temperature (°C)	40	45	50
Solvent to solid Ratio g/mL	10	20	30

2.6.1. Total phenolic content (TPC) assay

The assay reported by Waterhouse (2002) was followed to determine plant extract's total phenolic content. 1 mL of the heating shaker's extract was dissolved in 10 mL ethanol. Reagents were prepared using the reported method. 0.06 mL of DES extract, 4.5 mL of water, and 0.3 mL of Folin-Ciocalteu reagent were mixed using a multi-mode micropipette. This solution was incubated for 8 min at room temperature. After incubation, 0.9 mL of 7.5 % sodium carbonate solution was added to the mixture, and the solution was again incubated for 30 min at 40 °C. A pure DES solution was used to prepare the blank. After incubation, absorbance was measured with a spectrophotometer at the wavelength of 765 nm. The gallic acid curve was used as a standard [34].

2.6.2. Total flavonoid content (TFC) assay

To find the overall flavonoid content, 0.5 M sodium nitrate and 0.3 M aluminum chloride were added to the 300 μL of each extract that had been extracted independently in their respective test tubes. This was done after 3.4 mL of 30 % aqueous methanol was added to the 300 μL of each extract. After that, 150 μL of each substance was added. 1 mL of sodium hydroxide with a concentration of one million was added to the mixture after waiting for 5 min. An ultraviolet–visible spectrophotometer was then used to take an absorbance reading at a wavelength of 506 nm. An identical experiment was carried out, with the sole difference being that this time, the blank was generated by employing DES rather than extract as the method of production. The rutin was used as reference standards (50–200 mg/mL) and results were expressed as Rutin Equivalent (RE)/g of extract [35].

2.7. Free radical scavenging capacity (FRSC) assay

Following is the procedure we used to determine the percentage of radical scavenging activity The SPs extract were treated with solution containing 0.015 g of DPPH dissolved in 100 mL of methanol. The stock solution of DPPH was left to cool before use and diluted with methanol to lower its absorbance to 0.98 at 517 nm. The 400 μL of different concentrations of SPs extracts were combined with 4 mL of DPPH working solution without light. The mixture was then baked for half an hour at 37 °C. The absorbance of every sample was measured at 517 nm [36].

2.8. α-Amylase inhibitory activity assay

The antidiabetic activity of SPs extracts was assessed in terms of their ability to inhibit α-amylase. Acarbose (Standard) dilutions were prepared between 20 and 100 ppm. 0.5 mL of enzyme solution and 0.5 mL plant sample were mixed in a test tube. The mixture was incubated for 30 min at 25 °C in dark. 1 mL of starch is then added and again incubated for 3 min. After incubation, 1 mL of DNS was added and heated in a water bath for 15 min at 85 °C. After heating, 9 mL of distilled water was added, and the absorbance was measured at 540 nm. Acarbose is used as a standard drug in this experiment. For the preparation of control, all the procedures are the same except DMSO is added in place of the plant sample [37].

% Inhibition was calculated using the formula given below (Eq. (2)).

$$\% \text{ Inhibition} = \frac{Absorbance\ of\ control - Acsorbance\ of\ sample}{Absorbance\ of\ control} \text{ x } 100 \quad \text{(Eq. 2)}$$

2.9. Statistical analysis

The Design-Expert 13 and MATLAB R2019a were used for all statistical analysis and modeling. Several statistical measures, including the

ANOVA, F-test, regression (R^2), the percentage absolute average deviation (%AAD), and the root mean square error (RMSE), were utilized to investigate the prediction capabilities of both the RSM and the ANN. For the analysis of the significance of difference among the two statistical models (RSM and ANN) applied for the prediction, a paired sample *t*-test was applied [38].

$$AAD(\%) = \left[\frac{\sum_{1=1}^{n} |Y_i - y_i| \Big/ Y_i}{n}\right] \times 100 \quad (3)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (y_i - Y_i)^2}{\sum_{i=1}^{n} (Y_i - \bar{y})^2} \quad (4)$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n} (y_i - Y_i)^2} \quad (5)$$

$$\%\ \text{Prediction error} = \frac{Predicted\ Value - Measured\ VAlue}{Predicted\ value} * 100 \quad (Eq.\ 6)$$

3. Results and discussion

3.1. DES formation and characterization

Formation of DES was achieved by heating a mixture of sodium acetate (NaOAc) and glycerol (Gly) in a 3:1 ratio as per a reported method [39]. The DES was characterized following the transitions within 3250-3300 cm^{-1} of of FTIR spectral analysis. The physicochemical properties of DES were measured by DSA (Density and Sound Velocity meter). The density was 1.28 g/cm^3, while the kinematic and dynamic (absolute) viscosities were 354.8 mm^2/s and 454.2 mPa s, respectively. All the measurements were done at 25 °C.

3.2. Extraction optimization

Based on previous literature on the extraction of phenolics [40–42], the factors affecting the extraction of SPs phenolics were set within extraction time of 10–40 min, temperature 40–50 °C, and solvent-to-solid ratio of 10–30 mL/g. Central Composite Design (CCD) tested these factors and their ranges at different levels. The outcomes of the model are illustrated in Table 2. The data were fitted into 2nd degree polynomial equation to obtain suggestions for response extraction models. The significance of the model terms was determined by analyzing variance (ANOVA). The results of ANOVA are shown in Table 3.

From the R^2 values, the impact of input variables on the output variables can be determined, and thus, it is considered a powerful tool for model fitness estimation. The predicted and adjusted R^2 are in satisfactory agreement, indicating that the generated models fit the experimental observations (Table 3).

3.2.1. Effect of the parameters on the responses

According to the experimentally obtained values, the maximal TPC yield of 30.31 mg/mL and TFC yield of 15.09 mg/mL was obtained at 25 min time (A) with 50 °C temperature (B) while the solvent-to-solid (SS) ratio (C) maintained was 30 mL/g. The minimum yield for TPC and TFC was 3.16 mg/mL and 4.02 mg/mL at A = 10, B = 45, and C = 10. It was observed that the maximum TPC and TFC did not align with higher DPPH radical scavenging and α-amylase activities at the same parametric conditions; the values obtained were 43.08 % and 34.37 %, respectively. The maximum DPPH free radical scavenging activity was 62.02 % at A = 25, B = 40, and C = 10, while the minimum percentage observed was 15.33 at A = 25, B = 50, and C = 10. A maximum α-amylase inhibition percentage of 63.57 % was observed at A = 25, B = 50, and C = 10, while minimum percentage values were determined to be 25.82 at A = 25, B = 45, and C = 20.

$$Y_{TPC\ (SHA)} = 14.82 + 2.68B + 6.85C - 1.82BC - 9.01A^2 + 2.13B^2 + 5.67C^2 \quad (5)$$

$$Y_{TFC\ (SHA)} = 8.54 + 0.8169A + 2.77C - 1.22A^2 + 1.13B^2 \quad (6)$$

Equations (5) and (6) are the model equations for TPC and TFC, respectively. According to these equations, the total phenolic and flavonoid content of the SPs extracts increases with increase in temperature and liquid to solid ratio , which might be due increase in mass transfer rates at elevated temperature and solvent to solid ratio. Still, the yields substantially decrease as the temperature rises above the degradation points of phytochemicals or provides the temperatures for a prolonged time. The combined effect of B and C is impacting negatively because heating at high temperatures can also denature the phytochemicals, as mentioned earlier [43].

$$Y_{\text{antioxidant (SHA)}} = 34.80 - 8.16B - 2.83C - 7.83AC + 16.03BC + 4.11C^2) \quad (7)$$

Equation (7) is the model equation for antioxidant potential (DPPH inhibitory activity) of SPs extracts. According to this equation, the

Table 2
Comparison of total phenolic and flavonoid content, antioxidant and α-amylase inhibitory activity by using experimental data further assisted with RSM and ANN approaches.

Run	A:Time	B: Temp	C:Solvent-Sample Ratio'	Responses: TFC			TPC			Antioxidant			Alpha-amylase		
	'min'	'°C'	'mL/g'	ACTUAL	ANN	RSM	Actual	ANN	RSM	Actual	ANN	RSM	Actual	ANN	RSM
1	40.00	50.00	20.00	9.54	9.55	10.11	10.32	10.32	10.95	19.87	19.80	21.87	45.85	45.80	45.28
2	40.00	45.00	30.00	11.16	12.97	11.17	19.40	19.40	18.73	26.64	26.04	26.05	42.97	43.54	41.51
3	10.00	45.00	10.00	4.02	4.02	4.00	3.16	3.16	3.83	32.61	32.60	33.18	40.89	40.80	42.15
4	40.00	40.00	20.00	8.39	8.40	8.41	4.95	4.95	6.12	38.16	38.04	38.53	35.97	36.01	37.28
5	25.00	45.00	20.00	9.35	8.20	8.54	14.61	14.84	14.82	32.69	35.10	34.80	30.53	30.86	30.87
6	25.00	45.00	20.00	7.25	8.20	8.54	13.70	14.84	14.82	37.13	35.10	34.80	31.07	30.86	30.87
7	10.00	40.00	20.00	8.78	8.77	8.21	5.02	4.95	4.39	41.84	41.80	39.66	29.73	29.70	30.21
8	10.00	45.00	30.00	10.30	10.29	*10.90*	16.80	16.80	17.93	41.25	41.20	43.17	27.04	26.92	26.51
9	25.00	45.00	20.00	9.50	8.20	8.54	15.59	14.84	14.82	35.68	35.10	34.80	29.51	30.86	30.87
10	10.00	50.00	20.00	7.05	7.06	7.04	11.46	11.46	10.29	24.19	24.10	23.68	33.03	33.07	31.55
11	40.00	45.00	10.00	7.59	7.60	6.99	6.56	6.56	5.42	49.39	49.08	47.37	47.47	47.40	47.95
12	25.00	40.00	10.00	8.12	8.12	8.70	11.30	11.30	11.27	62.04	62.00	63.55	44.69	44.60	42.66
13	25.00	50.00	10.00	6.97	6.41	7.00	19.77	19.72	20.28	15.39	15.30	15.16	63.64	63.40	63.58
14	25.00	45.00	20.00	8.39	8.20	8.54	15.14	14.84	14.82	31.87	35.10	34.80	37.56	30.86	30.87
15	25.00	40.00	30.00	12.30	12.30	12.27	29.12	29.12	28.61	25.74	25.70	25.82	47.96	47.90	47.88
16	25.00	45.00	20.00	8.20	8.20	8.54	15.07	14.84	14.82	36.86	35.10	34.80	25.87	30.86	30.87
17	25.00	50.00	30.00	15.09	15.10	14.51	30.31	30.31	30.34	43.11	42.39	41.56	34.44	32.91	36.3

Table 3
Regression coefficients, ANOVA, ANN, and % C.V. values for TPC, TFC, antioxidant, and α-amylase inhibitory activity using the heating shaker method.

Factors	TFC		TPC		Antioxidant (DPPH)		α-amylase inhibitory activity	
	Coefficients	p-values	Coefficients	p-values	Coefficients	p-values	Coefficients	p-values
Intercept	8.54		14.82		34.80		30.87	
Linear Terms								
A	0.8169*	0.0343	0.5984	0.1912	−0.7337	0.4448	5.20*	0.0044
B	0.1337	0.6808	2.68**	0.0003	−8.16**	<0.0001	2.34	0.1058
C	2.77**	<0.0001	6.85**	<0.0001	−2.83*	0.0167	−5.52*	0.0032
Linear Combined Terms								
AB	0.7169	0.1478	−0.2652	0.664	−0.1700	0.8982	1.67	0.3801
AC	−0.678	0.1678	−0.1986	0.7442	−7.83**	0.0005	2.30	0.2371
BC	0.9843	0.0606	−1.82*	0.017	16.03**	<0.0001	−8.12*	0.0026
Quadratic Terms								
A^2	−1.22*	0.0246	−9.01**	<0.0001	−1.47	0.2768	−1.43	0.4358
B^2	1.13*	0.034	2.13*	0.0073	−2.39	0.0967	6.64*	0.0065
C^2	0.9543	0.0617	5.67**	<0.0001	4.11*	0.0133	10.09**	0.0007
***p-value* Model**		0.0014		<0.0001		<0.0001		0.0015
p-value Lack of Fit		0.55		0.0801		0.3988		0.8215
RSM R^2		0.9427		0.9897		0.9423		0.9423
adjusted R^2		0.8691		0.9765		9.35		0.8680
Predicted R^2		0.5974		0.8674		0.8044		0.7545
C·V. %		9.86		8.21		7.34		9.35

Significance: *The variables having $p < 0.05$ are significantly affecting the model equation.
**The variables having $p < 0.001$ are highly significant in determining the output equation.

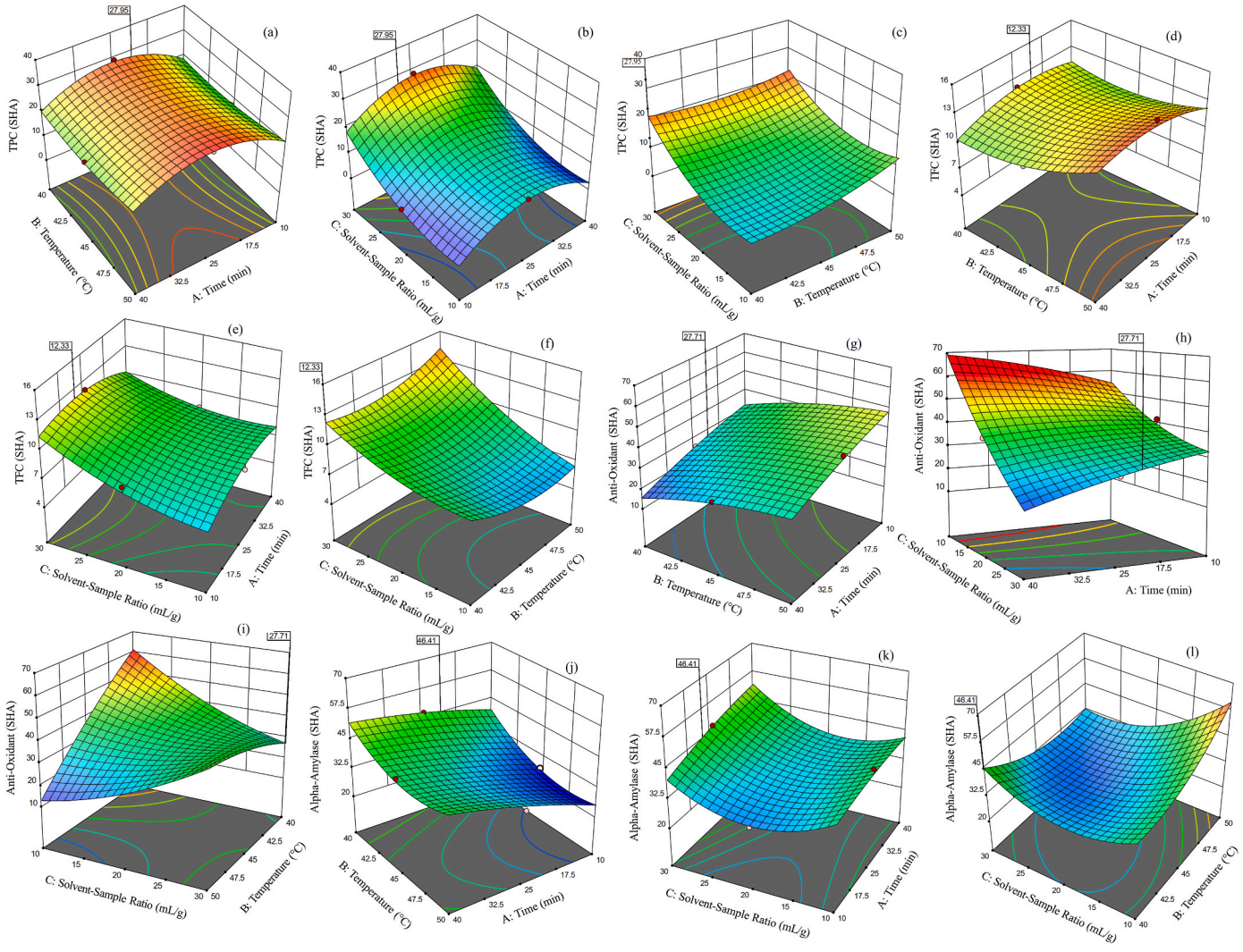

Fig. 1. Response surface graphs showing the combined effects of time, temperature and solvent-to-solid ratio for total phenolic and flavonoid contents, antioxidant and α-amylase inhibitory activity.

quadratic term C^2 and interaction term BC positively affected the extraction of antioxidant phytochemicals as more solvent helps in better diffusion of plant phytochemicals (Fick's diffusion law) [44], while the combined effect of temperature and solvent-to-solid ratio gives better antioxidants. Temperature, in this case, is helpful in extraction, but when time (A) is increased for extraction, the DPPH activity decreases because of the degradation of the free radical scavengers, which may be decomposed during extraction [45].

$$Y_{\alpha\text{-amylase (SHA)}} = 30.87 + 5.20A - 5.52C - 8.12BC + 6.64B^2 + 10.09C^2 \quad (8)$$

Equation (8) shows the model equation for α-amylase inhibitory activity. The quadratic terms B^2 and C^2 and linear term A are impacting positively on overall % inhibitory activity because the reason that good extraction of phytochemicals is observed at high temperatures and more SS ratio [46] but the combined effect of BC is adverse as the increasing temperature may cause degradation of phytochemicals regardless of the increase in extraction efficiency due to more solvent [47].

In Fig. 1(a–f), the extraction yield increases initially by increasing time, but after a specific time, both the TPC and TFC start decreasing, which may be due to the degradation of plant material for prolonged heating [27,48]. By increasing the solvent, the TPC and TFC are increased, which can be explained based on the contact surface area of the seeds; with more solvent, it is more excellent, which also influences the mass transfer phenomenon [30]. It can also be explained based on the dielectric constant of the highly polar DES used. Both temperature and solvent-to-solid ratio affect the extraction of TPC due to more effective collisions, which ultimately lead to increased surface area and washing efficacy [48]. On the other hand, little effect of temperature is observed on the extraction yield of TFC. The contradictory effects of temperature on the two activities may be routed to the fact that the poly-phenols or phenols are reactive at high temperatures and may lead to the change in functionalization of the –OH groups, on the other hand, flavonoids are comparatively stable at high temperatures.

In Fig. 1(g–i), the DPPH activities were prominent (40 %) for the extracts which were run on lower temperature values of 40 °C for the time of 10 min (10–40 min; least amount of time given for extraction) at constant SS of 25 mL. This might be be due to the fact that more time at elevated temperatures may decrease the phytochemicals' radical scavenging activities. Less solvent 10 mL and more time 40 min favors the DPPH radical scavenging activity (42.6 %) at a constant temperature of 45 °C because less solvent with more time would yield more concentrated phytochemicals. Contrarily, more solvents can cause a micellar effect around phytochemicals, depriving their availability as free radical scavengers. On the other hand, this effect is compensated at lower temperature (40 °C) for the extract preparation with a lesser quantity of solvent (10 mL) which causes a concentrated amount of phytochemicals. It is consistent with the fact that DPPH is a concentration-dependent activity [48].

In Fig. 1(j–l), it was observed that the greater extraction time (40 min), along with the combined effect of elevated temperature (50 °C) and lower solvent-to-solid (SS) ratio (10 mL), effectively increased the overall yields of the phytochemicals which are thus inhibiting the α-amylase (62.794 %), because increased time and less solvent helps in better diffusion of phytochemicals [49], while less temperature with less time (AB) and increased temperature with increased solvent (BC), both negatively affected the activities, 30.21 %, and 36.13 %, respectively, for % inhibition of alpha-amylase. This is due to the increase in temperature by also increasing the solvent may degrade phytochemicals; in this case, the deciding factor would shift towards the temperature parameter rather than the solvent parameter [50].

3.3. Artificial neural networking (ANN)

For prediction and optimization, RSM and ANN were used to generate a model based on the target/output variable. The ANN model optimal values of weights and bias of the layers were based upon the MSE of the Lavenberg-Marquardt (LM) algorithm [51]. The training results were selected on the basis of MSE values and Correlation coefficients (R) generated by the ANN. ANN, when compared with RSM, is considered a powerful tool for modeling and optimization because it can process the input variables to conclude nonlinear complex modeling relationship [52,53]. Besides ANN can adapt and learn patterns directly from data without needing fixed models. It is more accurate, handles noise well, and works effectively with unseen data, making it great for dynamic and data-heavy tasks.

The current study compares the efficiency of the statistical tools (in the present study, RSM and ANN) to generate reality-based models for yield predictions and optimizations and analyzes the correlation among the response variables.

TPC based on ANN gave the training, testing, and validation correlation coefficient greater than 0.9901. The best validation performance was achieved at epoch 0, and the MSE obtained was equal to 4.619e-7. The validation checks were performed at epoch 2, where the maximum gradient obtained was 1.1699e-09. For TFC, the correlation coefficient (R^2) obtained was greater than 0.9912 for all three sets of training, testing, and validation, and the value of R^2 for all the data sets was 0.9815. The best validation performance was obtained at epoch 0, which was 0.7394 for mean square error. A Max gradient of 2.8373e-10 was achieved at epoch 3. Similar observations based on correlation coefficients were made on the data set of antioxidants (DPPH radical scavenging activity). The correlation coefficient (R) obtained was greater than 0.9804 for all three sets of training, testing, and validation, while the value of R for all the data sets was 0.9937. The best validation performance was obtained at epoch 0, which came out to be 1.0621 for mean square error. A Max gradient of 1.3619e-12 was achieved at epoch 3. The values for the correlation coefficient were greater than 0.98 for the α-amylase activities performed on the extracts. The correlation coefficient (R^2) obtained was greater than 0.98 for all three sets of training, testing, and validation, while the value of R^2 for all the data sets was 0.9875. The best validation performance was obtained at epoch 0, which was 0.0065333 for mean square error. A max gradient of 3.2067e-11 was achieved at epoch 3. The correlational charts for training, testing, and validation of the model biases and weights compared with the actual (target data) experimental results can be visualized in Fig. 2.

3.4. Comparison between RSM and ANN predicted results

The predicted and actual results are depicted in the following graphs which reveal that for both RSM and ANN prediction, the points are close to the straight line which gives an insight about the significance of the regression models (Fig. 3).

The RSM-based experimental data was used to estimate ANN (see Fig. 4). The training of the neural network was based on a feedforward backpropagation (FFB) network. The selection of the neural network was based on regression and root mean square error (RMSE) analysis. The training function selected was LM (Levengerg-Marquadt), and the performance selection was based on the mean square errors (MSE) in the three data sets (training-70 %, testing-15 %, and validation-15 %). The weights and bias were adjusted by the gradient descent function in *nntools*. An approach based on trial and error was utilized in order to determine the total number of hidden layers and neurons (3-20-10-1). Log-Sigmoid (Logsig) and Tan-sigmoid (Tansig) transfer functions were applied to the two consecutive hidden layers, while linear (Purelin) transfer functions were the output Neural network toolbox-TM in MATLAB R2019a was used for the construction of ANN [32].

3.5. Comparison of RSM and ANN using SPSS

Comparison between the two statistical methods used as a tool for prediction was analyzed based upon different parameters, including R^2, AAD, and RMSE. For TPC, the R^2 value obtained by ANN was 0.9978,

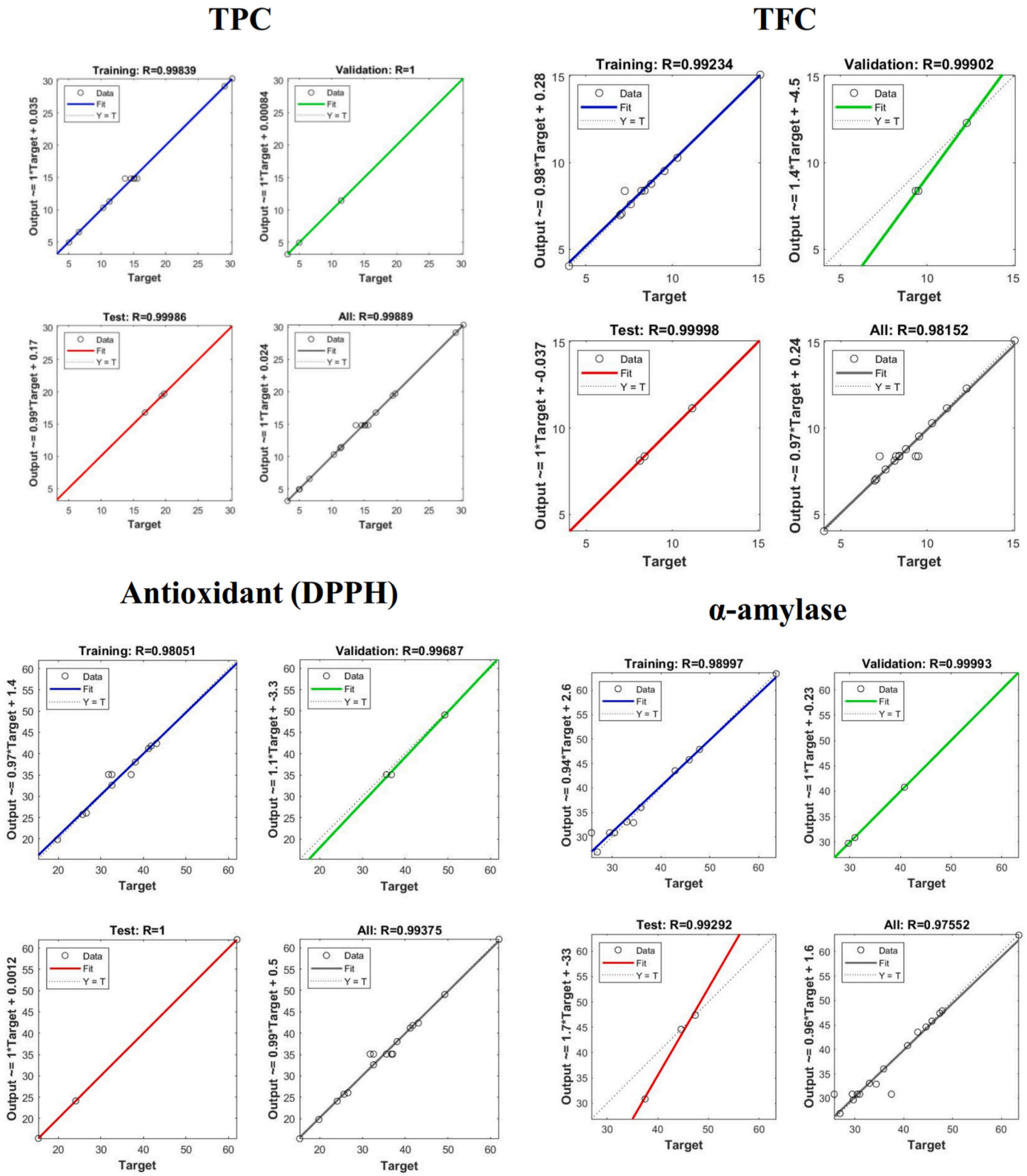

Fig. 2. Artificial neural networking (ANN- based regression graphs for total phenolic and flavonoid contents, antioxidant and α-amylase inhibitory activity.

which was greater than the RSM predicted model's R^2 value, which came out to be 0.9897. The absolute average deviation (AAD) of ANN is 1.18, and the RSM is 7.31, which shows that the ANN value is less than the RSM. For TFC, the R^2 value obtained by ANN was 0.9634, which was greater than the RSM predicted model's R^2 value, which came out to be 0.9427. The opposite observation was made in the AAD, where ANN gave the value of 3.90, which is less than the RSM value observed to be 4.80. For DPPH radical scavenging activity, the R^2 value obtained by ANN was 0.9875, which was greater than the RSM predicted model's R^2 value of 0.9772. The absolute average deviation of ANN is 1.99, and RSM is 4.03, which shows that the ANN value is less than RSM. For α-amylase, the R^2 value obtained by ANN was 0.9516, which was greater

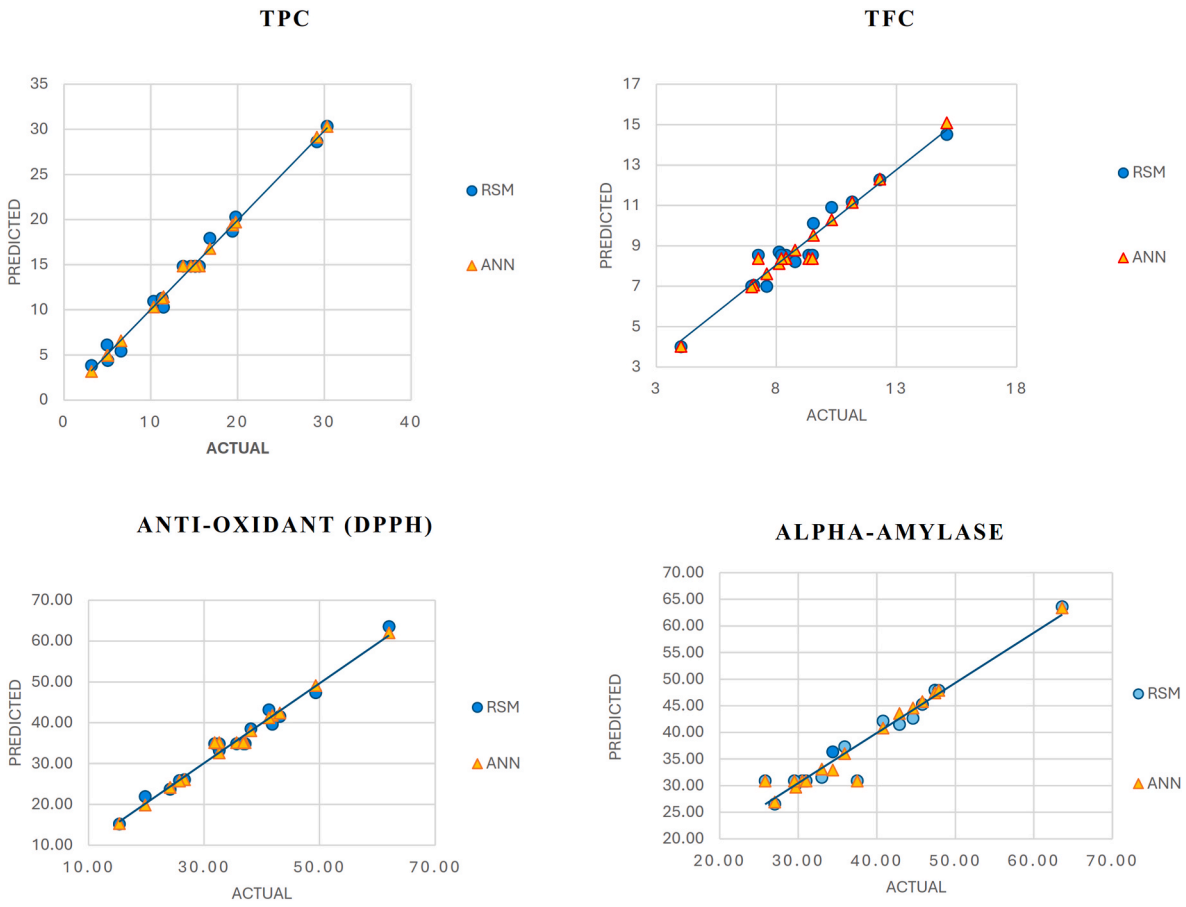

Fig. 3. Predicted RSM and ANN value graphs compared with the experimental values of total phenolic and flavonoid contents, antioxidant (DPPH), and α-amylase inhibitory activity.

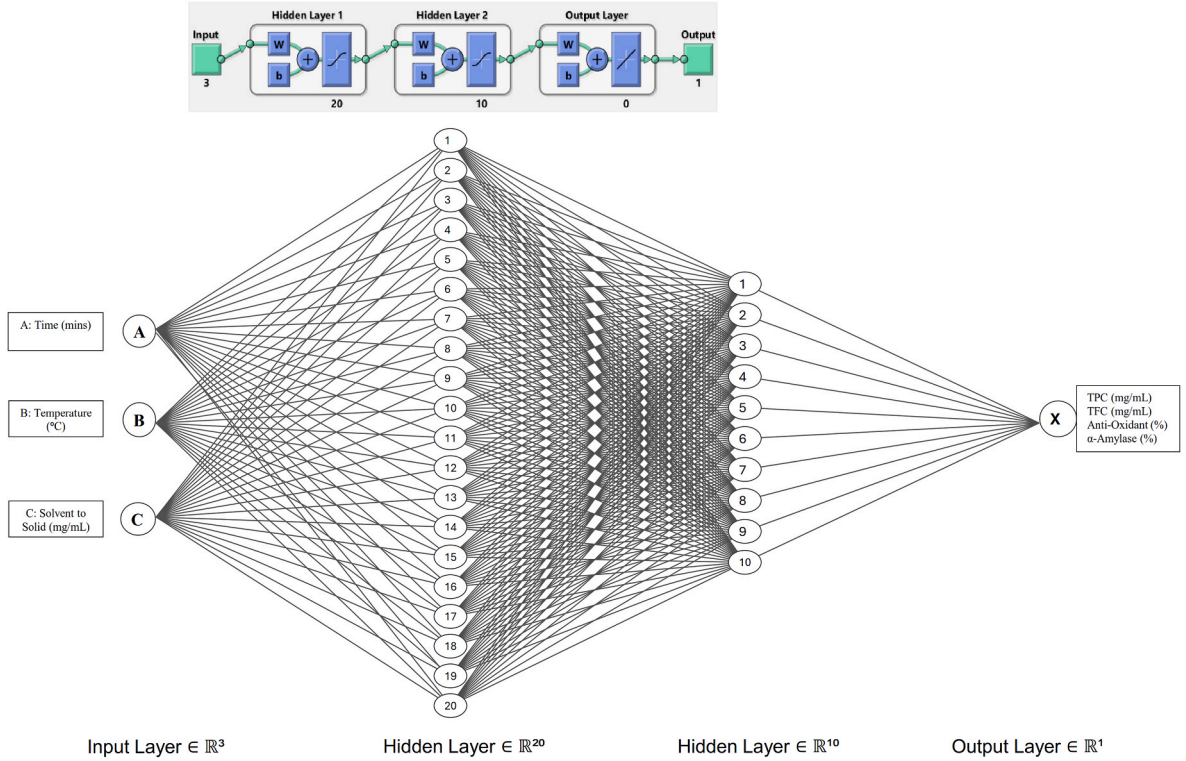

Fig. 4. Representation of artificial neural network representing an input layer, two hidden layers, and an output layer.

than the RSM predicted model's R^2 value, which came out to be 0.9423. The absolute average deviation of ANN is 2.97 and RSM is 4.36, which shows that the ANN value is less than RSM's. Greater R^2 and less AAD suggest a good approximation of predicted values with the actual ones. The RMSE was calculated to analyze the efficiency of two methods. RMSE for TPC obtained from ANN-generated outputs was 0.35 while that obtained from RSM was 0.75; for TFC RMSE for ANN-generated outputs was calculated to be 0.66 while that obtained from RSM was 0.56; for DPPH the RMSE obtained was 1.21 and 1.64 for ANN generated output and RSM predicted outputs respectively; for α-amylase, RMSE from ANN was 2.10 and from RSM was 2.28. It was observed that overall, ANN gave better results (lower RMSE) than RSM for all of the outputs (Table 5).

The optimization values also gave similar results as the overall model results where ANN gave lesser percentage prediction errors as compared to RSM (Table 4)

Upon further analysis to test whether the difference between the absolute errors was significant or not, a paired sample *t*-test was applied on the absolute errors obtained for ANN and RSM. It was found that the ANN generated output was statistically significantly better for TPC ($p < 0.001$), and for both DPPH and α-amylase ($p = 0.003$) than the RSM predicted outputs. While for all other pairs, the results were not significantly different.

3.6. Optimization and validation

The optimization solution provided by RSM was validated experimentally, and simulations using ANN were performed with the same parametric inputs. The percentage prediction errors (PPE) of the values predicted by RSM and ANN were calculated using Equation (4). At the optimized parameters of A = 29, B = 40, and C = 30 (as suggested by RSM), the predicted total phenolic content (TPC) from RSM was 28.23 mg/mL (PPE = 7.33 %), while ANN predicted 29.14 mg/mL (PPE = 4.26 %). The actual experimental yield, however, was 30.43 mg/mL (Table 6).

For TFC, the actual yield was 10.99 mg/mL, compared to the RSM prediction of 12.05 mg/mL (PPE = 9.69 %) and the ANN prediction of 11.64 mg/mL (PPE = 5.92 %). Similarly, the actual percentage inhibition of α-amylase activity was 46.95 %, while RSM predicted 49.23 % (PPE = 4.86 %) and ANN predicted 48.78 % (PPE = 3.91 %).

Regarding the radical scavenging activity (measured as DPPH inhibition), the actual experimental value was 26.16 %, while RSM predicted 23.65 % (PPE = 9.95 %) and ANN predicted 25.21 % (PPE = 3.65 %).

All predictions from both RSM and ANN fell within acceptable limits of percentage prediction error, demonstrating that both tools were in reasonable agreement with the experimental results.

3.7. Correlation among activities using SPSS

In this study, correlations between the experimental responses were also assessed (Table 7). The values of TPC, TFC, DPPH antioxidant activity, and α-amylase inhibitory activity were analyzed using SPSS statistical software. The correlation analysis of activities derived from the same extraction method provided valuable insights into the efficiency of phytochemical extraction and its relation to α-amylase inhibition and DPPH radical scavenging activities. Pearson's correlation analysis revealed a moderately positive correlation (r = 0.775) between TPC and TFC, which was highly significant ($p < 0.001$). However, weak and mild correlations were observed among the other variables, and these correlations were statistically insignificant ($p > 0.05$).

Table 5
Paired sample correlations among RSM and ANN-based absolute errors.

Pair	Correlation Variables	N	Correlation	Sig.
Pair 1	TPC (ANN) TPC(RSM)	17	0.169	0.516
Pair 2	TFC (ANN) & TFC (RSM)	17	0.264	0.305
Pair 3	DPPH (ANN) & DPPH (RSM)	17	0.636	0.006
Pair 4	α-amylase (ANN) & α-amylase (RSM)	17	0.948	0.000

Table 6
Predicted and Experimental values of Response variables under optimal conditions suggested by RSM.

Responses	Experimental	Predicted	
		RSM	ANN
TPC	30.43 ± 0.21	28.23	29.13
TFC	10.99 ± 0.14	12.05	11.64
Antioxidant	26.16 ± 0.36	25.65	25.21
α-Amylase	46.95 ± 0.11	49.23	48.78

Table 7
Correlation among the total phenolic content (TPC), total flavonoid content (TFC), DPPH, and alpha-amylase activities.

		TPC	TFC	DPPH SHA	Alpha-amylase
TPC	Pearson Correlation	–	0.775**	−0.227	0.137
	Sig. (2-tailed)		0.000	0.380	0.599
	N	17	17	17	17
TFC	Pearson Correlation	0.775**	–	0.050	−0.118
	Sig. (2-tailed)	0.000		0.848	0.652
	N	17	17	17	17
Antioxidant	Pearson Correlation	−0.227	0.050	–	−0.328
	Sig. (2-tailed)	0.380	0.848		0.199
	N	17	17	17	17
Alpha-amylase	Pearson Correlation	0.137	−0.118	−0.328	–
	Sig. (2-tailed)	0.599	0.652	0.199	
	N	17	17	17	17

** Correlation is significant at 0.01 level (2- tailed).
* Correlation is significant at 0.05 level (2-tailed).

4. Conclusions

The study described in this article successfully demonstrated that glycerol-sodium acetate deep eutectic solvent (DES) is an effective, and

Table 4
Predictive capacity comparison of RSM and ANN models.

Parameters	TFC		TPC		Antioxidant (DPPH)		α-Amylase	
	RSM	ANN	RSM	ANN	RSM	ANN	RSM	ANN
R^2	0.9897	0.9978	0.9427	0.9634	0.9423	0.9875	0.9423	0.9516
AAD (%)	4.80	3.90	7.31	1.18	4.03	1.99	4.36	2.97
PPE (%)	9.69	5.91	7.24	4.26	9.59	3.64	4.86	3.91
RMSE	0.42	0.35	0.64	0.16	1.40	0.69	1.48	0.96

eco-friendly medium for extracting bioactive compounds from *Strychnos potatorum* seeds (SPs). Optimization of extraction conditions using response surface methodology (RSM) and artificial neural networking (ANN) revealed that ANN provided superior predictive accuracy in modeling key extraction outcomes, including total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (DPPH), and antidiabetic (α-amylase inhibitory) activity. Under optimal extraction conditions (29 min, 40 °C, and a solvent-to-feed ratio of 30 mL/g), the SPs extract yielded high concentrations of phenolics and flavonoids, with significant antioxidant and antidiabetic effects. The comparative analysis of ANN and RSM further highlighted ANN's lower percentage error and greater alignment with experimental data, making it a more reliable tool for predicting extraction outcomes. This research underscores the potential of glycerol-sodium acetate DES as a sustainable and efficient alternative to conventional solvents. These findings provide a valuable framework for future studies aiming to scale up eco-friendly extraction processes for natural products with pharmaceutical and nutraceutical applications.

CRediT authorship contribution statement

Haroon Iftikhar: Writing – original draft, Methodology, Investigation, Formal analysis. **Sumia Akram:** Writing – review & editing, Software, Resources, Data curation. **Noor-ul-Ain Khalid:** Writing – original draft, Formal analysis. **Dildar Ahmed:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Masooma Hyder Khan:** Software, Methodology, Formal analysis. **Rizwan Ashraf:** Validation, Resources, Methodology, Formal analysis. **Muhammad Mushtaq:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors of this monograph declare no conflict of Interest.

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

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