



Combined experimental design based-PROMETHEE approach to evaluate antimicrobial activity and optimization of polyphenol content of *Prunus spinosa* L. fruit

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Abstract

Present research was undertaken to assess total phenolic content (TPC) and antimicrobial activity of *Prunus spinosa* L. Combined design including mixture design and response surface methodology (RSM) was used to create experimental points and to determine the best solvent composition for extraction of bioactive components of *P. spinosa*. To construct model, water, methanol, acetone (A, B, C, 0–100%) were selected as solvents and extraction time (D, 30–60 °C) was used to be categorical process factor. The TPC analysis results for each experimental point ranged from 5.40 ± 0.32 to 108.70 ± 2.14 mg GAE/g. The optimal condition for the highest phenolic compounds was obtained with the point including the mixture containing water: acetone (61.28:38.72, v/v) and 56.27 °C of extraction temperature, which was the most suitable for maximum TPC (108.74 ± 2.806 mg GAE/g). The ANOVA analysis of the combined design showed that TPC response were statistically significant, with determination coefficients of 98.85%, and fit the quadratic x linear regression models. Extracts obtained from the experimental point were evaluated to assess the antimicrobial activity of *P. spinosa*. The results were evaluated through PROMETHEE. The PROMETHEE results indicate that the extract demonstrating the most significant efficacy against all five bacteria is composed of 50% water and 50% methanol, processed at 60 °C. In conclusion, bioactive compounds from *P. spinosa* were successfully extracted using the chosen temperatures and solvents, and the antibacterial efficacy of the experimental results derived from the design expert program was thoroughly analyzed using PROMETHEE. The findings indicate that PROMETHEE is applicable for comparing and optimizing the extraction of bioactive chemicals from botanical sources. Moreover, it can be utilized in the development of a product aimed at particular microorganisms or to assess the synergy among various phytochemicals based on their antibacterial and antioxidant characteristics.

Keywords Total phenolic content · *P. spinosa* · Antimicrobial activity · Response surface methodology · PROMETHEE

Introduction

Prunus spinosa, a member of the Rosaceae family commonly referred to as blackthorn or sloe, recognized for its richness in various substances, including tocopherols (α -tocopherol, β -tocopherol, γ -tocopherol, δ -tocopherol), ascorbic acid, and β -carotene. Furthermore, it is noted that it contains polyphenolic and flavonoid compounds, such as quercetin and rutin [1]. Moreover, it is well-known for its antioxidant, antidiabetic, antimicrobial, anti-inflammatory,

and anticancer properties, and contains a wealth of polyphenols including anthocyanins, phenolic acids, and flavonoids [2]. Moreover, it is very important in preventing cardiovascular disease since it includes a lot of anthocyanins, which are known for their antioxidant capabilities. Thus, it is commonly utilized in the medical, pharmaceutical, and food industries [3].

Numerous studies have been reported regarding the antioxidant, antibacterial, and anti-inflammatory effects of *P. spinosa* [4–6]. These properties are attributed to the phenolic compounds that are naturally present in the structure of *P. spinosa* and are classified as secondary metabolites [7]. Given the significant biological potential of *P. spinosa* and its extensive diversity in phenolic compounds, the implementation of an effective extraction method is crucial [8]. The primary parameters that affect the performance of the

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extraction process include extraction time, temperature, solvent type and composition, solvent-to-solid ratio, and the number of extraction stages [9]. However, there is no literature consensus on a single, successful standard extraction procedure or the best extraction solvent. Several investigations have shown that solid–liquid extraction with different solvents is most effective, and higher polarity solvents usually dissolve polyphenols better [10]. Polar solvents, including methanol, ethanol, and acetone, along with their aqueous mixes, are frequently employed, yielding favorable extraction results [11].

Response surface methodology (RSM) is a rigorous mathematical and statistical technique effectively utilized in assessing various process components and their interactions [12, 13]. RSM has been effectively utilized for the optimization of food processing conditions, including temperature, time, and solvent concentration [14, 15]. Mixture design is an additional method employed for the optimization of solvent components for a specified response [16]. It minimizes the consumption of raw materials, solvents, and time, while enhancing the selectivity and optimization necessary for precise plant and food analysis [17]. Mixture designs, including simplex-lattice and simplex-centroid designs, have demonstrated efficacy in various areas. Numerous research studies have employed this statistical method to enhance the extraction of phenolic chemicals from various plant matrices [18, 19].

To optimize the solvent for extracting phenolic compounds from fruits, it is essential to employ RSM in conjunction with mixture design, particularly when temperature is a factor. This study employed a combined design of RSM and mixture design to acquire comprehensive information regarding the relationship between independent and dependent variables under defined conditions. The RSM component of the combined design employed a D-optimal design type. D-optimal design methodologies can integrate both quantitative and qualitative elements within a single experimental framework [20]. The combined design enables the simultaneous optimization of mixture and factor responses, offering advantages in raw material usage, time, and cost, as it significantly reduces the number of required experimental points. This study aimed to extract phenolic compounds from *P. spinosa* utilizing a conventional extraction method informed by D-optimal design. The antimicrobial activity results from each experimental point derived from the combined design on microorganisms were then assessed using PROMETHEE, a method commonly employed in multi-criteria decision-making evaluations. The PROMETHEE is the abbreviation of preference ranking organization method for enrichment evaluation [21].

The PROMETHEE approach is widely recognized as one of the greatest approaches for making decisions based on multiple criteria at once, and the GAIA plane, a geometric

depiction of the process, is utilized to capitalize on the visual benefits provided to the decision maker. This is because PROMETHEE methodologies allow for both visual assessments and rankings of criteria based on relevant variables [22]. The PROMETHEE technique explains the distinctions among criteria by highlighting the advantages and disadvantages of each alternative [23]. It is well structured for decision-making scenarios with a limited set of alternatives that require assessment based on multiple conflicting criteria [24]. Ülengin et al. [25] identify a minimum of three advantages of the PROMETHEE method. The primary advantage is its intuitive outranking methodology. The second advantage is the efficacy of PROMETHEE in addressing real-world planning challenges. Another feature of PROMETHEE is its comprehensive ranking capability. The PROMETHEE I and PROMETHEE II facilitate partial and total rating of alternatives, respectively. PROMETHEE I is utilized for partial ranking, whilst PROMETHEE II is employed for total ranking. The PROMETHEE approach has been extensively utilized across several applications due to its efficacy in outranking alternatives and its availability in multiple iterations [26–28]. Nonetheless, its application within chemometric approaches in the food sector is limited.

The literature indicates that a combined design utilizing various solvents and temperatures for the extraction of bioactive substances from *P. spinosa* has not been employed. The antimicrobial activity of the experimental points derived from the combined design on microorganisms remains unexamined. Furthermore, although previous studies on *P. spinosa* and its antimicrobial properties focused on the effects of a single solvent [4, 29–31], this research aimed to provide a more comprehensive evaluation of the similarities and differences in antimicrobial effects of *P. spinosa* across single, binary, and triple mixtures of various solvent compositions, utilizing the PROMETHEE method for analysis. In other words, the innovative aspect of this study is that the PROMETHEE method was used to rank the experimental points obtained from the combined design considering the antimicrobial activity of *P. spinosa*.

Material and methods

Materials

The *Prunus spinosa* used in the study was sourced from a local enterprise in Çanakkale province. The *P. spinosa* has been preserved at -18°C till put to use. The fruits were employed directly as fresh produce without any prior treatment. All microorganisms utilized in the study were sourced from the repositories of the Food Engineering Department at Yıldız Technical University. All media, solutions, and chemicals employed in the investigations were obtained from the

MERCK Ltd. (Darmstadt, Germany) corporation and are of analytical grade.

Experimental design

The experimental design was executed employing Design Expert version 7.0.0 software (State-Ease Inc., USA). The experiment was structured as a combined method, employing the mixture design alongside response surface methodology (RSM). The components of the mixture were identified as water (X_1), methanol (X_2), and acetone (X_3). The extraction temperature (X_4) was chosen as the numeric process factor in the RSM section. D-optimal design was conducted in the RSM component of the combined design. Table 1 presents a summary of the design employed. The response was determined as the total phenolic content (TPC) of the extracts obtained under the circumstances outlined by the combined design. A total of 24 experimental points were derived from the design, as illustrated in Table 2. The square root function was employed to transform the data, as the maximum TPC to minimum TPC was greater than 10. The proposed combined model mixture process for the TPC responses has been identified as quadratic concerning mixture order and linear in terms of process order. The backwards elimination technique was employed to remove non-significant parameters (ABD, ACD, BCD, $p > 0.1$) from the established model.

At each experimental point, the subsequent polynomial equation of function x_i was fitted for each variable. This polynomial model lacks a constant term (intercept equal to zero), distinguishing it from complete polynomial models due to this characteristic. The equation of the polynomial model was:

$$Y = \sum_{i=1}^4 \beta_i X_i + \sum_{i=1}^4 \sum_{j=i+1}^4 \beta_{ij} x_i x_j \quad (1)$$

$$= \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{14} X_1 X_4 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4$$

The optimization process utilized desire functions $d_i(Y_i)$ to evaluate the extraction parameters, specifically x , in order to attain the "most desirable" response value, which is the maximum TPC value. The functions were predicated on a specific response Y_i intended for maximization in this investigation [32].

$$d_i(\hat{y}_i) = \begin{cases} 0 & \hat{y}_i(x) < L_i \\ \left(\frac{\hat{y}_i(x) - L_i}{T_i - L_i} \right)^Z & L_i \leq \hat{y}_i(x) \leq T_i \\ 1 & \hat{y}_i(x) \geq T_i \end{cases} \quad (2)$$

Table 1 Design summary used for the extraction of *P. spinosa* according to TPC response

Design summary																
Study type: Combined																
Initial design: D-optimal/ Point exchange																
Design model: Quadratic x Quadratic																
Runs: 24																
Component	Name	Units	Type	Low Actual	High actual	Low coded	High coded	Mean	SD							
A	Water	mL	Mixture	0.00	100.00	0.000	1.000	31.250	35.782							
B	Methanol	mL	Mixture	0.00	100.00	0.000	1.000	33.333	37.268							
C	Acetone	mL	Mixture	0.00	100.00	0.000	1.000	35.417	38.584							
D	Temperature	°C	Numeric	30.00	60.00	-1.000	1.000	45.313	12.147							
Response	Name	Units	Observation	Analysis	Minimum	Maximum	Mean	SD	Ratio	Trans	Model					
Y	TPC	mg/g	24	Polynomial	5.40	108.70	47.13	29.04	3.411	Square Root	Quadratic x Linear					

Table 2 The experimental points for the combined design and the results of TPC for each experimental point

Design points	Coded values				Actual values				TPC (mg GAE/ g raw fruit)	
	A	B	C	D	A	B	C	D	Experimental data	Predicted data
1	0	0	0	1	0	100	0	60	36.88±0.38	41.36
2	1	0	0	-1	100	0	0	30	31.37±0.24	29.34
3	0	0	1	1	0	0	100	60	13.18±0.31	11.99
4	0	0	1	0	0	0	100	45	7.96±0.74	9.25
5	0	0	1	-1	0	0	100	30	5.40±0.32	6.51
6	0.5	0	0.5	0	50	0	50	45	103.82±2.86	102.09
7	0	1	0	-1	0	100	0	30	34.10±0.10	32.21
8	0	0.5	0.5	0.83	0	50	50	45	48.58±0.45	44.43
9	0	0	1	1	0	0	100	60	12.27±0.28	11.99
10	0	1	0	0	0	100	0	45	35.02±0.36	36.79
11	1	0	0	1	100	0	0	60	37.27±0.86	39.29
12	0.5	0.5	0	1	50	50	0	60	61.51±0.82	63.15
13	1	0	0	0	100	0	0	45	34.13±0.19	34.32
14	0	0.5	0.5	-1	0	50	50	30	41.77±0.80	40.78
15	0	0	1	-1	0	0	100	30	6.85±0.41	6.51
16	0	0.5	0.5	1	0	50	50	60	44.87±0.74	48.09
17	0.666	0.167	0.167	-0.80	66.67	16.67	16.67	37.50	68.37±0.23	75.45
18	0.5	0.5	0	0	50	50	0	45	65.87±0.26	58.37
19	0.5	0.5	0	-1	50	50	0	30	50.06±0.76	53.60
20	0.666	0.167	0.167	0.88	66.67	16.67	16.67	52.50	87.96±0.79	82.07
21	0	1	0	1	0	100	0	60	46.48±0.71	41.36
22	0.17	0.666	0.167	-0.80	16.67	66.67	16.67	37.50	53.93±1.15	57.94
23	0.5	0	0.5	-1	50	0	50	30	94.72±1.21	93.54
24	0.5	0	0.5	1	50	0	50	60	108.70±2.14	110.63

A (Water, mL), B (Methanol, mL) and C (Acetone, mL), D (Temperature, °C),

where L_i and T_i represented the lower and target values, respectively, sought for the response Y_i corresponding to L_i and T_i .

Desirability values were aggregated into a single index, D , by translating each response into a partial desire function d_i . A dimensionless function was used to achieve the ranges of each response. Thus, a 0–1 scale represents the spectrum from worst to best. The weighted geometric mean of n desire functions was calculated using a global desirability function D [33]:

$$D = (d_1^{p_1} \times d_2^{p_2} \times d_3^{p_3} \times \dots \times d_n^{p_n})^{1/\sum p_i} \\ = \left[\prod_{i=1}^n d_i^{p_i} \right]^{1/\sum p_i} \quad (3)$$

where, p_i represents the weight of the i th element, and it is normalized such that $\sum_{i=1}^n p_i = 1$

The maximum target in the current work was the desired response of TPC. Design-Expert Software (Stat Ease Inc.®, Version 11. Minneapolis) was used for analysis,

modeling, optimization of the experimental data, and 3D graph plotting.

Extraction of bioactive components and determination of TPC

Extraction was performed at the experimental points presented in Table 2. The extraction process included the selection of solvent variables, namely water, methanol, and acetone. The temperature was recognized as a process variable. Five gr of the raw fruit were measured and placed into a 100 mL amber reagent bottle, followed by the addition of 50 mL of the solvent. The fruits in the mixture underwent a fine chopping process utilizing the ultra-turrax (IKA ULTRA-TURRAX T18 digital, Germany) at 15,000 rpm for 5 min. The mixtures were subsequently maintained for 30 min at the temperatures outlined in Table 2 by using magnetic stirrer equipment with a heated circulating bath (Velp Scientifica, Italy) and thermostat (Cetinkaya, CP5022, Türkiye). Following centrifugation at 6500 rpm for 10 min, the

supernatant was subjected to filtration using a 0.45 µm polyethylene polytetrafluoroethylene (PTFE) filter (Membrane Solutions). The extracted samples were preserved at −18 °C until utilized.

The TPCs of the filtrates were assessed using the modified method outlined by Singleton et al. [34]. In summary, 0.5 mL of the extract, 2.5 mL of diluted Folin-Ciocalteu's phenol reagent (diluted 1:10 with distilled water), and 2.0 mL of 75 g/L Na₂CO₃ were mixed and then the tubes were permitted to rest for 30 min in a dark location at room temperature. The absorbance of the samples was measured at 760 nm using a Shimadzu UV-1800 spectrophotometer from Kyoto, Japan. TPC was quantified as mg/g (mg of gallic acid equivalent (GAE) per 1 g of the raw fruit).

Microorganisms and growth conditions

Three gram-positive (*Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 19118, *Streptococcus mitis*) and two gram-negative bacteria (*Escherichia coli* O157:H7 ATCC 33150 and *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028) were employed to evaluate the antibacterial efficacy of the *P. spinosa* extracts. All bacterial strains underwent activation twice in Nutrient Broth [35]. Inoculum were prepared by culturing for 18 h in Nutrient Broth at 37 °C.

Determination of in-vitro antimicrobial activity

Based on the technique detailed Durak and Ucak [36], the antibacterial activity of *P. spinosa* extracts was ascertained using the agar diffusion method. Autoclaved Nutrient Agar was injected with each 18-h bacterial strain to achieve a inoculum size of 10⁶–10⁷ CFU/mL at a temperature range of 43–45 °C, and then poured into petri dishes. Upon the solidification of agar, four wells for each extract (n=4) were created in the agar using a sterile cork borer (4 mm). The extracts, as outlined in Table 2, were dispensed into the wells at a volume of 50 µL. The relevant solvent used in each extract was considered as negative control. The petri dishes underwent incubation at 37 °C for a duration of 24 h. Antimicrobial activity was assessed by measuring the zone of inhibition against test organisms relative to negative control.

Bioactive properties for optimum point of *P. spinosa*

Further investigations were conducted to ascertain the bioactive characteristics of the optimal point identified using the experimental design. The antioxidant capacity of extracts was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) technique, the cupric reducing antioxidant capacity (CUPRAC) assay and the ferric reducing antioxidant ability (FRAP). In DPPH assay, 0.1 mL of extract was mixed with

4.9 mL of 0.1 mmol/L DPPH• in methanol and incubated in the dark for 20 min [37]. The absorbance of the samples was measured using a UV-vis spectrophotometer. The results were expressed as milligrams of Trolox equivalent per g of raw fruit (mg TE/g raw fruit).

The CUPRAC assay was performed by adding 0.1 mL of the sample to a mixture of 1 mL CuCl₂ (10 mmol/L), 1 mL neocuproine (7.5 mmol/L), and 1 mL NH₄Ac (1 mol/L), followed by the addition of 1 mL distilled water to achieve a total volume of 4.1 mL [38]. The absorbance at 450 nm was recorded following a 1 h incubation of the samples in darkness. The results were presented as mg of Trolox equivalents (TE) per g of raw fruit (mg TE/g raw fruit).

The FRAP assay was conducted utilizing the FRAP reagent, composed of 3 mM acetate buffer (pH 3.6), 10 mM TPTZ solution, and 20 mM FeCl₃·6H₂O in a 10:1:1 (v/v/v) ratio. The sample (0.1 mL) was combined with 0.9 mL of water and 2 mL of FRAP reagent, then incubated at room temperature for 30 min in the dark [39]. The absorbance of the resulting solution was quantified at 593 nm with a spectrophotometer. The data were presented as mg of Trolox equivalent (TE) per g of raw fruit (mg TE/g DM).

The total flavonoid content (TFC) of the samples was evaluated using the method outlined by Zhishen et al. [40]. The extracts (1 mL) were combined with 4 mL of distilled water, 0.3 mL of a 5% NaNO₂ solution, and 0.3 mL of a 10% AlCl₃ solution, then permitted to remain at room temperature for 6 min. After the incorporation of 2 mL of NaOH (1 M), the total volume was augmented to 10 mL using distilled water. A spectrophotometer measured the absorbance at 510 nm. The results were expressed in milligrams of catechin equivalent (CE) per g of raw fruit (mg CE/g raw fruit).

The concentration of anthocyanins was evaluated by examining the absorbance values measured at different pH levels [41]. The absorbance of the extracts was measured at 520 and 700 nm. The concentration of anthocyanins in the samples was ascertained using the subsequent equation:

$$\text{Anthocyanin content} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{A \times MW \times DF \times 1000}{\epsilon \times 1} \quad (4)$$

The results were quantified as cyanidin-3-glucoside equivalents for *P. spinosa* by utilizing the absorption value (A) of the samples, the molecular weight (MW) of 449.2 g/mol, an absorptive coefficient (ε) of 26,900 L/mol, and a dilution factor (DF) of the samples.

Determination of individual phenolic compound profile of optimum point

The specific phenolic component in optimum point of *P. spinosa* was analyzed using High-Performance Liquid Chromatography (HPLC). Standard calibration curves were established utilizing gallic acid, protocatechuic acid,

4-hydroxybenzoic acid, caffeic acid, syringic acid, p-coumaric acid, rutin, ferulic acid, m-coumaric acid, d-coumaric acid, myricetin, quercetin, kaempferol, and chrysin. The samples were filtered using a 0.45 μm syringe filter.

The evaluation was conducted using a Shimadzu HPLC system, which includes a pump (LC-20AD), a DAD detector (SPD-M20A), an oven (CTO-20A), an automatic injector (SIL-20AHT), a degasser unit (DGU-20A5R), and a communication bus module (CMB-20A). Separations were conducted at 40 °C using a C18 Intersil® ODS column (5 μm , 250 $\times$ 4.6 mm). The mobile phase comprised solution A (distilled water with 0.1% (v/v) acetic acid) and solution B (acetonitrile with 0.1% (v/v) acetic acid). The samples were eluted using the subsequent gradient: 10% B (0–2 min), 10–30% B (2–27 min), 30–90% B (27–50 min), 90–100% B (51–60 min), and returned to initial conditions at 63 min. The flow rate was 1 mL/min. The injection loop volume was 20 μL . Compounds were identified by comparing their retention time values to those of standards. The HPLC–DAD results were presented in mg/g.

Statistical analysis

The data from TPCs was presented as the mean $\pm$ SD of triplicate assays. The regression analysis and optimization of the responses were conducted using Design Expert Statistical Software package 7.0.0 (Stat Ease Inc., Minneapolis, USA).

PROMETHEE I-II was employed as a statistical tool to analyze the differences and similarities in the antimicrobial effects of each experimental point generated through the combined design on microorganisms, facilitating a more

comprehensive interpretation of the findings. Alternatives were established by appending ‘S’ to the beginning of each experimental point. Microorganisms were determined as the criteria of the study. A total of 24 alternatives and 5 criteria were used.

The PROMETHEE approach is a multi-criteria decision-making technique that facilitates the evaluation of alternatives through the application of preference functions selected according to the established criteria. The evaluation of alternatives is obtained by performing paired comparisons [22]. In this study, the PROMETHEE method was employed to rank the alternatives according to their antimicrobial inhibition effects.

The PROMETHEE analysis was conducted with the Visual PROMETHEE program. The weights implemented in the analysis are obtained from the entropy analysis. The PROMETHEE I method is employed to derive a partial ranking of experimental points according to their antimicrobial effects. The ranking is established using the PROMETHEE II methodology.

The PROMETHEE method consists of four consecutive steps [42, 43]:

Step 1. Evaluate the n solutions ($a_1, a_2, \dots, a_n$) within set A according to m criteria C_k , resulting in the decision matrix $X = (x_{ik})$ where ‘ i ’ ranges from 1 to n and ‘ k ’ ranges from 1 to m . When $G_k(d_{ij})$ equals zero, schemes a_i and a_j are indistinguishable. When $G_k(d_{ij})$ equals 1, scheme a_i is granted absolute precedence over scheme a_j .

Table 3 ANOVA results of the combined design for TPC of *P. spinosa* extract

Source	Sum of Squares	DF	Mean square	F Value	p-value	Remarks
Model	120.91	8	15.11	160.63	<0.0001	Significant
Linear Mixture	38.29	2	19.14	203.48	<0.0001	
AB	6.05	1	6.05	64.34	<0.0001	
AC	72.59	1	72.59	771.55	<0.0001	
AD	0.42	1	0.42	4.51	0.0506	
BC	10.44	1	10.44	110.95	<0.0001	
BD	0.35	1	0.35	3.71	0.0733	
CD	1.13	1	1.13	11.99	0.0035	
Residual	1.41	15	0.09			
Lack of Fit	1.08	12	0.09	0.82	0.6530	
Pure Error	0.33	3	0.11			Not significant
Correction Total	122.32	23				
R^2	0.9885					
Adjusted R^2	0.9823					
Predicted R^2	0.9731					
Adequate precision	42.5924					

A (Water, mL), B (Methanol, mL) and C (Acetone, mL), D (Temperature, °C), DF, degree of freedom, $p < 0.05$ indicates statistical significance, ANOVA refers to analysis of variance

$$G_k(d_{ij}) = P_k(a_i, a_j) \in [0, 1] \quad (5)$$

Step 2. A multi-criteria preference ranking index (H) is calculated using the weight (W) assigned by the decision maker.

$$H(a_i, a_j) = \sum_{k=1}^m W_k P_k(a_i, a_j) \quad (6)$$

Step 3. The sequence of A_i 's preferences is dictated by $\Phi^+(a_i)$ and $\Phi^-(a_i)$, which denote the positive and negative orientations, respectively.

$$\Phi^+(a_i) = \sum_{j=1}^n H(a_i, a_j) \text{ and } \Phi^-(a_i) = \sum_{j=1}^n H(a_j, a_i) \quad (7)$$

$$\Phi(a_i) = \Phi^+(a_i) - \Phi^-(a_i) \quad (8)$$

Step 4. The exact ordering of the alternatives is established according to the values of $\Phi(a_i)$.

Results and discussion

The objectives of the current study was (i) to designate an appropriate solvent mixture and extraction temperature for the effective extraction of phenolic from *P. spinosa* utilizing a combined design, (ii) to optimize key independent variables through RSM employing a D-optimal combined design based on TPC results, (iii) to determine the bioactive properties of optimum point of *P. spinosa*, (iv) to assess the antibacterial properties of *P. spinosa* extracts at each experimental point via the PROMETHEE method, (v) to compare TPC data obtained from the optimum extraction method and antimicrobial results of PROMETHEE.

Phenolic concentration extracts obtained from blackthorn

The TPCs of *P. spinosa* extracts are presented in Table 2. The total phenolic contents of the extracts ranged from

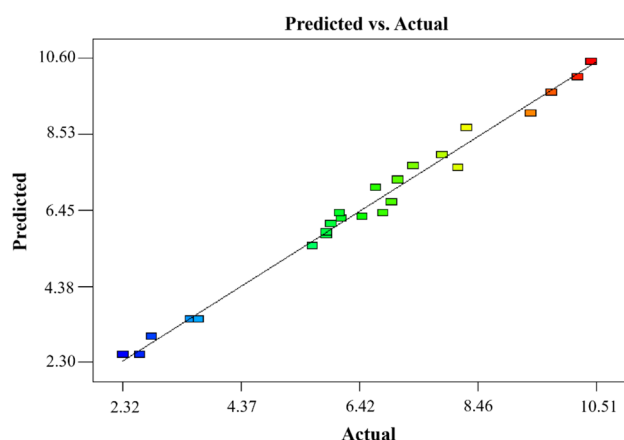


Fig. 1 Experimental versus predicted phenolic contents of the *P. spinosa* extracts

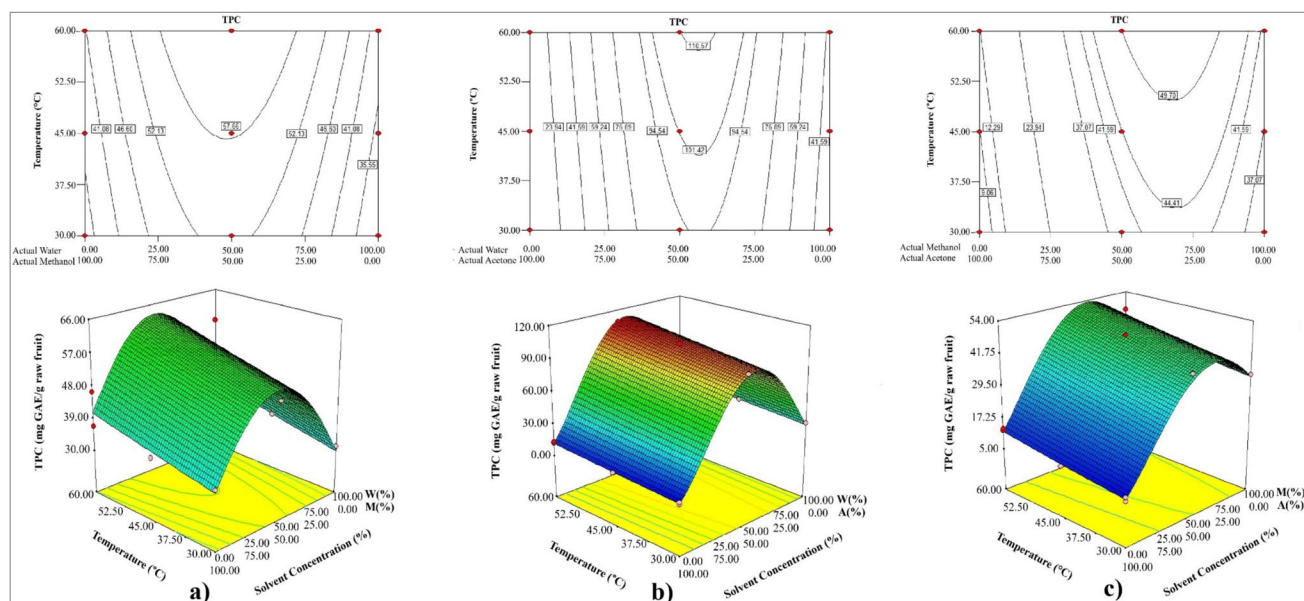


Fig. 2 Contour and three-dimensional response surface plots showing the influences of extraction temperature (°C) and solvent concentration (%) on TPC amount of *P. spinosa*

5.40 mg GAE/g of raw fruit to 108.70 mg GAE/g of raw fruit. The highest content for TPC was achieved at design point 24, utilizing water: acetone (50:50, v/v) mixture and an extraction temperature of 60 °C. The results indicate that both the extraction solvent and temperature significantly affected the quantity of phenolic extracted from *P. spinosa*. The TPC values of *P. spinosa* were observed to be elevated in comparison to earlier studies. TPC values were reported to be 7.02–11.97 mg GAE/g by Bayram [44] and 2.16–3.43 mg GAE/g FW in the study of Damar and Yilmaz [45]. Studies indicate that varying TPC values are achieved through the use of different solvents. Koraqi et al. [46] identified the maximum TPC at 134 ± 1.6 mg GAE/g by employing a ternary mixture of lactic acid, maltose, and water (66:16:16 v/v) for the extraction of *P. spinosa* in their research. In a study, TPC of *P. spinosa*, extracted with methanol, was found to range from 3.72 to 5.05 mg GAE/g [6]. Studies indicate that varying TPC values are produced depending on the solvents employed.

The results of combined design for TPC amount of *P. spinosa*

The combination of mixture design and RSM was employed to examine the concurrent impacts of independent mixture and process factors on the TPC of *P. spinosa* extracts. Properties such as surface porosity and phenolic profile are key factors influencing the selection of the optimal solvent combination for extraction purposes [36]. This study identified the quadratic $\times$ linear model as the optimal representation of the effects of solvent type and extraction temperature on the TPC of *P. spinosa*. Table 3 presents the ANOVA results for the established quadratic $\times$ linear model concerning TPC. The model includes only the significant results, as the insignificant ones (ABD, ACD, BCD interaction terms) were excluded through the backwards elimination procedure. Initially, the experimental data underwent square root transformation to ensure the validity of ANOVA. The F-value of the model was determined to be 160.63, and the quadratic

$\times$ linear model demonstrated significance ($P < 0.05$). This indicates that the model is suitable for optimizing the solvent mixture to maximize extraction yield from the samples.

The TPC, represented as square root transformed values, can be predicted based on solvent concentration and temperature using the following equation:

$$\begin{aligned} \text{Sqrt(TPC)} = & 4.73853A + 5.15770B + 1.54564C \\ & + 6.59549AB + 22.65188AC + 0.024702AD \\ & + 8.51143BC + 0.020326BD + 0.032082CD \end{aligned}$$

where A, B, C, and D were water, methanol, acetone and temperature, respectively.

The established model has a determination coefficient of 0.9885, indicating that it could be used to estimate TPC of the sample based on mixture component concentrations (water, methanol, and acetone) and temperature. The adj- R^2 value was calculated to be 0.9823, indicating a close proximity to the R^2 value. This suggests that all parameters incorporated in the developed model were essential for optimization. The pred- R^2 statistic provides insight into the prediction efficacy of the regression model [47]. All R^2 values were greater than 0.9, indicating that the models demonstrated adequacy and possessed sufficient predictive capability (Fig. 1). Adequate precision value of model was determined to be 42.5924. The results indicate that the models employed in this study effectively determined the TPC values of *P. spinosa* through multiple regression and ANOVA analysis. Analysis of variance results indicated that all linear mixture components and interaction terms (AB, AC, BC, CD) significantly affected the TPC of *P. spinosa* ($P < 0.1$).

Figure 2 illustrates how temperature and solvent combinations affect the TPC of extracts. The response surface plots, and contour graphs illustrate the variation in phenolic extraction from *P. spinosa* as a function of extraction temperature and solvent concentration. Figure 2a illustrates that the TPC increases with rising temperature in the model utilizing water and methanol as solvents. The graph clearly indicates that the maximum TPC is achieved at 60 °C with

Fig. 3 Desirability value and defined factor values for the most desirable ($D = 1.000$) solution for maximization of TPC

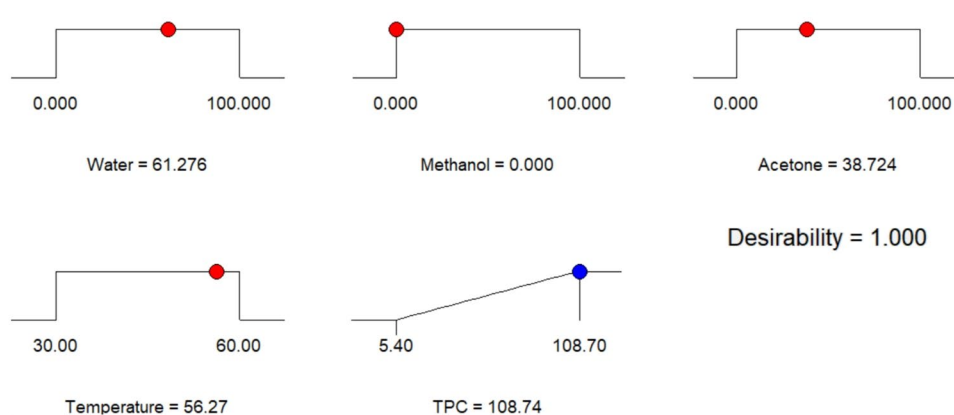


Table 4 Bioactive properties and antimicrobial activity of optimum point

Total bioactive compound						
TPC (mg GAE/g)	Total antioxidant capacity (mg TE/g)			Total flavonoid content (mg CE/g)		Total anthocyanin content (mg/L)
	DPPH	CUPRAC	FRAP			
Optimum point	110.97 ± 2.806	395.22 ± 3.921	86.55 ± 1.686	80.55 ± 1.110	46.82 ± 0.438	
Polyphenol profile (mg/g)						
Gallic acid	Protocatechuic acid	Caffeic Acid	p-Coumaric acid	Rutin	m-Coumaric acid	Myricetin
					d-coumaric	Chrysin
Optimum point	1690.69 ± 1.086	1275.00 ± 2.205	2605.13 ± 1.430	55.63 ± 0.199	543.29 ± 0.085	4213.13 ± 2.769
Antimicrobial activity (mm)						
<i>E. coli</i> O157:H7	<i>Salmonella</i> Typhimurium			<i>S. aureus</i>		<i>S. mitis</i>
						<i>L. monocytogenes</i>
Optimum point	3.55 ± 0.071	4.00 ± 0.141	1.35 ± 0.212	6.1 ± 0.141		6.25 ± 0.354

a 50:50 (W:M, v/v) ratio. Two-dimensional contour plots and three-dimensional plots of TPC responses for *P. spinosa* as a function of extraction temperature-W:A concentration is presented in Fig. 2b. The figure demonstrates a gradual increase in the TPC of *P. spinosa* with rising temperature. The most desirable TPC value was achieved with a 50:50 (W:A, v/v) ratio. The relationship between the TPC amount of *P. spinosa* and varying levels of extraction temperature and M:A concentration was illustrated using two-dimensional contour plots and three-dimensional plots in Fig. 2c. The plots clearly illustrate that the TPC amount rose as the temperature increased. The evaluation of the three graphs indicates a clear positive correlation between temperature and the TPC amount. A study conducted by V. González-de-Peredo et al. [8] employed a Box–Behnken design to investigate various process variables, including methanol composition, pH, temperature, cycle, amplitude, and sample–solvent ratio, for the extraction of *P. spinosa* under optimal conditions. The primary parameters influencing the extraction of TPC (4.5015 mg GAE/g) include the extraction cycle, methanol percentage, and pH of the solvent, with optimal values identified as 0.2 s, 67%, and pH 7, respectively. The TPC obtained from the current study exceeds that of the referenced study. Temperature is a significant parameter for extraction, as evidenced by the comparison of both studies. Various studies also reported an increase in the quantity of extracted bioactive components as the extraction temperature increased [48].

One of the primary objectives of the current study was to identify the optimal solvent mixture composition and extraction temperature for the extraction of TPC from *P. spinosa*. Figure 3 illustrates the desirability value and specified factor values for the optimal solution (D = 1.000) aimed at maximizing TPC. The optimization results indicated that the optimal solvent mixture consists of acetone and water in a ratio of 38.72% acetone to 61.28% water, with a temperature of 56.27 °C. Another study on solvent optimization for extracting phenolic compounds from *Pistacia terebinthus* L. identified a 39% water and 61% acetone mixture at room temperature as the optimal solvent, resulting in the maximum extraction of phenolic compounds [36]. A separate study identified the optimal solvent for extracting phenolic compounds from *Prunus mahaleb* with a mixture of 36% acetone and 64% water at room temperature [49]. The findings of previous studies indicate that the combination of acetone and water is identified as the most effective solvent for extracting phenolic compounds from various materials.

The optimization results revealed that the suggested solvent mixture would extract a TPC of approximately 108.74 mg GAE/g of raw fruit. To assess the accuracy of the constructed model, an extraction of blackthorn was performed utilizing the optimized solvent mixture, and the TPC of the sample was determined experimentally for validation

Table 5 Antimicrobial activity of *P. spinosa* extracts

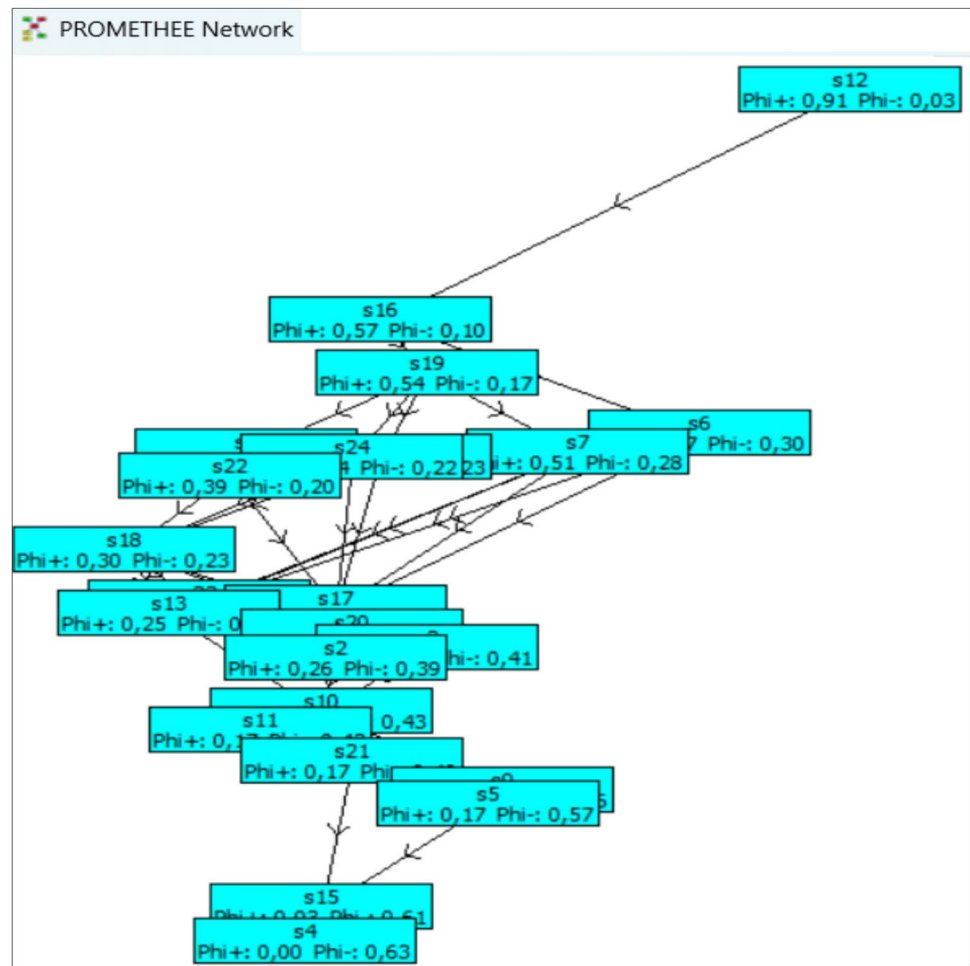
Mixtures	<i>Escherichia coli</i> O157:H7	<i>Salmonella</i> Typhimurium	<i>Staphylococcus aureus</i>	<i>Listeria monocytogenes</i>	<i>Streptococcus mitis</i>
1	4.00 ± 0 ^{Cb}	4.00 ± 0 ^{Eb}	1.00 ± 0 ^{Bc}	6.00 ± 0 ^{Ca}	NZ ^{Cd}
2	2.00 ± 0 ^{Eb}	4.50 ± 0.5 ^{Ea}	NZ ^{Cc}	4.00 ± 0 ^{Da}	NZ ^{Cc}
3	6.00 ± 0 ^{Ba}	NZ ^{Gc}	1.00 ± 0 ^{Bb}	NZ ^{Fc}	NZ ^{Cc}
4	NZ ^{*Ga}	NZ ^{Ga}	NZ ^{Ca}	NZ ^{Fa}	NZ ^{Ca}
5	NZ ^{Gb}	1.50 ± 0.5 ^{Fa}	1.00 ± 0 ^{Bab}	NZ ^{Fb}	NZ ^{Cb}
6	2.00 ± 0 ^{Ec}	2.00 ± 0.0 ^{Fc}	2.00 ± 0 ^{Ac}	6.00 ± 0 ^{Cb}	10.00 ± 0 ^{Ba}
7	4.00 ± 0 ^{Cc}	NZ ^{Ge}	1.00 ± 0 ^{Bd}	6.00 ± 0 ^{Cb}	10.00 ± 0 ^{Ba}
8	4.00 ± 0 ^{Cb}	6.00 ± 0 ^{Da}	1.00 ± 0 ^{Bc}	4.00 ± 0 ^{Db}	NZ ^{Cd}
9	1.00 ± 0 ^{Fa}	1.00 ± 0 ^{F^{Ga}}	1.00 ± 0 ^{Ba}	NZ ^{Fb}	NZ ^{Cb}
10	6.00 ± 0 ^{Ba}	NZ ^{Gc}	NZ ^{Cc}	2.00 ± 0 ^{Eb}	NZ ^{Cc}
11	1.00 ± 0 ^{Fb}	4.00 ± 0 ^{Ea}	NZ ^{Cc}	4.00 ± 0 ^{Da}	NZ ^{Cc}
12	6.00 ± 0 ^{Bc}	8.00 ± 0 ^{Cb}	2.00 ± 0 ^{Ad}	8.00 ± 0 ^{Bb}	15.00 ± 0 ^{Aa}
13	4.00 ± 0 ^{Ca}	4.00 ± 0 ^{Ea}	NZ ^{Cb}	4.00 ± 0 ^{Da}	NZ ^{Cb}
14	4.00 ± 0 ^{Cb}	4.00 ± 0 ^{Eb}	NZ ^{Cc}	6.00 ± 0 ^{Ca}	NZ ^{Cc}
15	NZ ^{Gb}	NZ ^{Gb}	NZ ^{Cb}	2.00 ± 0 ^{Ea}	NZ ^{Cb}
16	4.00 ± 0 ^{Cc}	10.00 ± 0 ^{Ba}	2.00 ± 0 ^{Ad}	6.00 ± 0 ^{Cb}	NZ ^{Cc}
17	2.00 ± 0 ^{Ec}	12.50 ± 0.5 ^{Aa}	NZ ^{Cd}	4.00 ± 0 ^{Db}	NZ ^{Cd}
18	4.00 ± 0 ^{Cb}	4.00 ± 0 ^{Eb}	NZ ^{Cc}	6.00 ± 0 ^{Ca}	NZ ^{Cc}
19	12.50 ± 0.5 ^{Aa}	6.00 ± 0 ^{Db}	NZ ^{Cc}	6.50 ± 0.5 ^{Cb}	NZ ^{Cc}
20	2.00 ± 0 ^{Eb}	2.00 ± 0 ^{Fb}	NZ ^{Cc}	8.00 ± 0 ^{Ba}	NZ ^{Cc}
21	3.00 ± 0 ^{Da}	2.00 ± 0 ^{Fb}	NZ ^{Cd}	2.00 ± 0 ^{Eb}	NZ ^{Cd}
22	4.00 ± 0 ^{Cb}	4.00 ± 0 ^{Eb}	NZ ^{Cc}	10.00 ± 0 ^{Aa}	NZ ^{Cc}
23	3.00 ± 0 ^{Dc}	4.00 ± 0 ^{Eb}	NZ ^{Cd}	6.00 ± 0 ^{Ca}	NZ ^{Cd}
24	3.00 ± 0 ^{Dc}	4.00 ± 0 ^{Eb}	2.00 ± 0 ^{Ad}	6.00 ± 0 ^{Ca}	NZ ^{Cc}

A–G The same superscript uppercase letters in a column mean no significant ($p > 0.05$) differences. a–g The same superscript lowercase letters in a row mean no significant ($p > 0.05$) differences. NZ*: No zone (mm)

purposes. The experimental values of the predicted total phenolic content of the extract obtained with the optimized solvent were determined to be 110.977 ± 2.806 mg GAE/g raw fruit. The experimental results were found to align well with the estimated TPC derived from the constructed model. The optimized solvent mixture was employed to re-extract the fruit of *P. spinosa*, and several bioactivity properties were characterized. For this aim, the fruit of *P. spinosa* was examined for its antimicrobial activity, individual polyphenol profile, total phenolic, total flavonoid, total anthocyanin content, and total antioxidant capacity (DPPH, CUPRAC, and FRAP). According to Table 4, TPC, DPPH, CUPRAC, FRAP, total flavonoid content, and total anthocyanin content for optimum point were found to be 110.97 ± 2.806 mg GAE/g, 55.07 ± 0.041 mg TE/g, 395.22 ± 3.921 mg TE/g, 86.55 ± 1.686 mg TE/g, 80.55 ± 1.110 mg CE/g, and 46.82 ± 0.438 mg/L, respectively. The optimization study by Kotsou et al. [50] revealed that the TPC was measured at 30.74 mg GAE/g, the total flavonoid content was recorded at 3.23 mg RtE/g, and the total anthocyanin content was quantified at 125.2 g cyanidin-3-O-glucoside equivalents/g.

These results were obtained from an extraction process utilizing 75% ethanol, with a duration of 30 min and a temperature of 80 °C. The evaluation of present study results indicates that the acetone–water mixture demonstrated greater efficiency in the extraction of bioactive substances. The *P. spinosa* fruits were found to contain several polyphenol chemicals. The components were accurately identified and quantified using a previously developed chromatographic method for the efficient and thorough analysis of phenolic chemicals. 4-Hydroxybenzoic acid, syringic acid, ferulic acid, quercetin, and kaempferol were not detected. The main phenolic compound of the *P. spinosa* fruit was caffeic acid (2605.13 ± 1.430 mg/g) while the lowest one was *p*-coumaric acid (55.63 ± 0.199 mg/g). Damar and Yilmaz [45] identified protocatechuic acid (38.60 ± 1.87 mg/kg FW) as the primary component in the hydrolyzed sample. The disparity observed between the two studies may be attributed to the methods employed for extract acquisition and the chromatographic techniques utilized. The optimization results indicate that *P. spinosa* exhibited the most significant inhibitory effect on *S. mitis*, while demonstrating the least

Fig. 4 PROMETHEE network



effect on *S. aureus*. In a separate investigation, *P. spinosa* demonstrated a consistent inhibitory effect across all tested microorganisms. The observed difference between the two studies could be attributed to the specific growing conditions and geographical region of the wild fruit utilized, in addition to the bacterial strains employed [51]

Evaluation of the in-vitro antibacterial activity results of *P. spinosa* fruits extracts with PROMETHEE

Antimicrobial tests were conducted on five microorganisms for each experimental point derived from the expert design program. The antimicrobial effect for each point is presented in Table 5. The acquired real data underwent normalization within the range of 0–1 for the purpose of PROMETHEE analysis. The criteria were assigned equal weight. The interface of the decision matrix of normalized data in the Visual PROMETHEE program is given in Fig. S1.

In the subsequent phase, the results underwent a partial evaluation utilizing PROMETHEE-I, based on Φ^+ and Φ^- values. PROMETHEE presents pairwise comparisons of samples that have been calculated to yield positive or negative superiority values, resulting in three possible outcomes for the compared samples. The outcomes indicate that one sample demonstrates superior performance across all criteria, one sample is comparable to another, and one sample exhibits inferior performance relative to the others. Figure 4 shows a partial sequence of PROMETHEE-I methods under the PROMETHEE Network title. Each alternative is depicted as a node on the PROMETHEE Network screen, with preferences illustrated through arrows. The arrangement of the nodes highlights the closeness of the flow values. The findings from the PROMETHEE Network demonstrate that S12 outperforms all criteria, with S16 and S9 following in rank. The S4 is the most distant from the S12. The PROMETHEE-II method was employed to establish a comprehensive ranking.

The results of PROMETHEE-II offer a comprehensive ranking of samples by considering the net advantages value, which is assessed through an assessment of both positive and

Table 6 Flow rates, scores and rankings according to PROMETHEE-II method results

Rank	Action	Phi	Phi +	Phi –	Scores
1	S12	0.8783	0.9130	0.0348	100
2	S16	0.4696	0.5739	0.1043	17.96
3	S19	0.3739	0.5391	0.1652	14.22
4	S6	0.2696	0.5652	0.2957	11.27
5	S1	0.2348	0.4174	0.1826	10.46
6	S7	0.2348	0.5130	0.2783	10.46
7	S8	0.2261	0.4522	0.2261	10.27
8	S24	0.2261	0.4435	0.2174	10.27
9	S22	0.1913	0.3913	0.2000	9.55
10	S14	0.0609	0.2957	0.2348	7.32
11	S18	0.0609	0.2957	0.2348	7.32
12	S23	– 0.0348	0.2696	0.3043	6.05
13	S17	– 0.0435	0.3043	0.3478	5.94
14	S13	– 0.0522	0.2522	0.3043	5.84
15	S20	– 0.0870	0.2870	0.3739	5.44
16	S3	– 0.1130	0.2957	0.4087	5.16
17	S2	– 0.1304	0.2609	0.3913	4.99
18	S10	– 0.2261	0.2087	0.4348	4.09
19	S11	– 0.2609	0.1739	0.4348	3.8
20	S21	– 0.3130	0.1739	0.4870	3.39
21	S9	– 0.3652	0.1913	0.5565	3.01
22	S5	– 0.3913	0.1739	0.5652	2.84
23	S15	– 0.5739	0.0348	0.6087	1.75
24	S4	– 0.6348	0.0000	0.6348	1.45

negative superiority values. Table 6 indicates that S12 exhibits the highest net phi value compared to all other samples. The other prominent samples on the list include S16, S19, S6, and S1, in that order. The scores and rankings derived from the PROMETHEE-II method are also illustrated in Table 6. The score of S12 stands out significantly when compared to the other alternatives, and there is a considerable distance between the subsequent alternatives. S1 and S7 are tied in the ranking with identical scores. Both S8 and S24 exhibit identical scores as well. S4 is positioned at the bottom of the ranking.

Figure 5 illustrates the outcomes of the PROMETHEE-II evaluation performance within the GAIA plane. In the GAIA plane, the red decision stick functions as a constituent of all criteria. In other words, alternatives proximate to the red decision stick and aligned in the same direction possess favorable values. All alternatives corresponding to the experimental data are depicted as points on the GAIA plane, whereas the criteria are represented as vectors. The alternatives with the highest performance ratings are those most distant from the direction specified by this red decision stick. Based on the red decision stick, S12, S19, and S16 are the

most optimally positioned alternatives, while S4, S15, and S5 have the worst positions in the GAIA plane, respectively. The *S. aureus*, *S. mitis*, and *E. coli* axes are the same oriented with a red decision stick, resulting in S12 being better at meeting *S. aureus*, *S. mitis*, and *E. coli* criteria. According to the orientation of the *L. monocytogenes* and *Salmonella* criteria axes, the optimal possibilities for satisfying these criteria were S19 and S22, respectively.

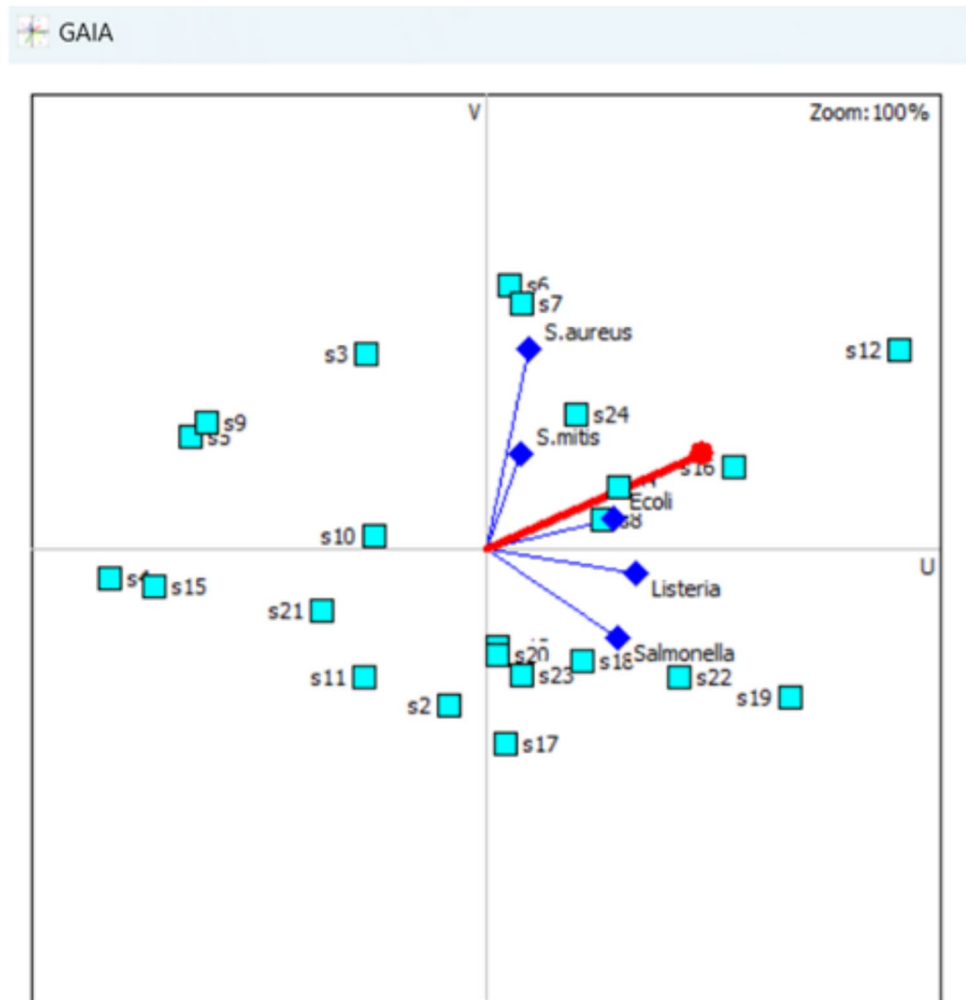
The Visual PROMETHEE software is employed to deliver advantageous graphical representations. This includes the Rainbow diagram (Fig. 6). This chart illustrates the advantages and disadvantages of each alternative. The positive characteristics are located above the histograms, on the + 1 axis, and the negative aspects are located below, on the – 1 axis. S12 does not have disadvantage indication. This clarifies the reason for the elevated position of this alternative on the list (see Table 5). Conversely, S4 and S15 serve as two alternatives that lack any advantages.

Figure 7 presents the action profiles for S12, S16, S19, and S6. The example demonstrating the greatest advantages is S12. S19 exhibits a disadvantage regarding *S. aureus* and *S. mitis*, whereas S16 is disadvantaged solely in relation to *S. mitis*. S6 exhibits a disadvantage regarding *E. coli* and *Salmonella*.

Consequently, the experimental point with an extraction temperature of 60 °C and solvents of 50% water and 50% methanol (Run 12 in Table 2) exhibited the most effective antimicrobial effect, as indicated by the PROMETHEE results. Upon evaluating the identical raw data in the expert design program, it was determined that the optimal conditions were 49.65% water, 50.35% methanol, and a 60 °C extraction temperature. The data acquired from PROMETHEE, and the expert design program exhibited compatibility.

This study revealed that the extraction conditions yielding the highest inhibition effect of *P. spinosa* occurred when water and methanol were utilized in equal proportions at an extraction temperature of 60 °C. Marčetić et al. [4] clearly indicated that methanol-extracted *P. spinosa* extracts exhibited a greater inhibitory impact on microorganisms. The results align with those obtained in the current study. Another research investigation examined the antimicrobial properties of ethanolic extracts derived from *P. spinosa*, utilizing a non-conventional extraction technique (microwave-assisted extraction and ultrasound-assisted maceration) [52]. The extract demonstrated significant efficacy against *S. aureus*, while exhibiting no effect on *Salmonella*. The current study indicates that the experimental points had limited effectiveness on *S. aureus*, while demonstrating efficacy against *Salmonella* which means that ethanol-extracted *P. spinosa* is effective on *S. aureus*, while methanol-extracted one is effective on *Salmonella*.

Fig. 5 GAIA graphic



Previous researches has demonstrated that the antimicrobial activity of *P. spinosa* extracts may be attributed to flavonoids and phenolic acids [30]. Nonetheless, S24 exhibited the highest TPC value in this study, although S12 in PROMETHEE had the most effective antibacterial action. The S24 sample is positioned 8th in terms of its antibacterial efficacy according to PROMETHEE results. The inhibitory mechanism of phenolic compounds detected in the samples differs according to polyphenol structure and bacterial species [53]. Consequently, a more accurate assessment of the antimicrobial impact can be achieved by analyzing the specific phenolic present in the samples rather than the total phenolic amount.

In the present research, during the execution of the PROMETHEE analysis, equal weight was assigned to each microorganism. Thus, the evaluation identified an alternative that exerts an inhibitory effect on all microorganisms. Nonetheless, if a specialized product incorporating *P. spinosa* is to be formulated, varying outcomes will ensue when the weights are adjusted correspondingly. For this reason,

PROMETHEE offers a distinct advantage in the evaluation of data. Furthermore, PROMETHEE is not a method that is extensively recognized or utilized in the food field. Studies are being conducted to demonstrate its visibility and applicability [54].

Conclusion

The conventional extraction method utilized three solvents (water, methanol, and acetone) at concentration volumes ranging from 0 to 100% and varying temperatures between 30 and 60 °C for the extraction of TPC from *P. spinosa*. A combined design utilizing mixture design and D-optimal design was employed to model and determine the optimal conditions for the extraction process. The applied mathematical models demonstrated statistical significance and facilitated the optimization of phenolic extraction. Optimization showed that the best solvent mixture is 38.72% acetone to 61.28% water at 56.27 °C. Optimization showed

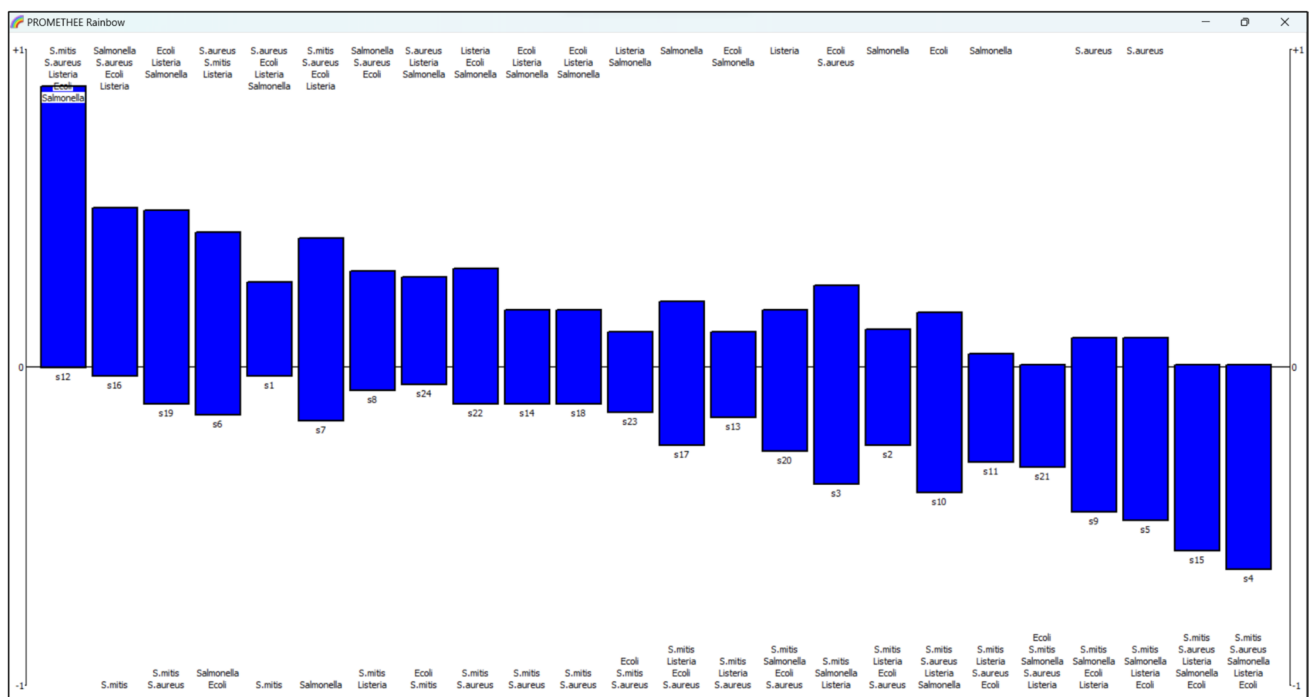


Fig. 6 Rainbow diagram

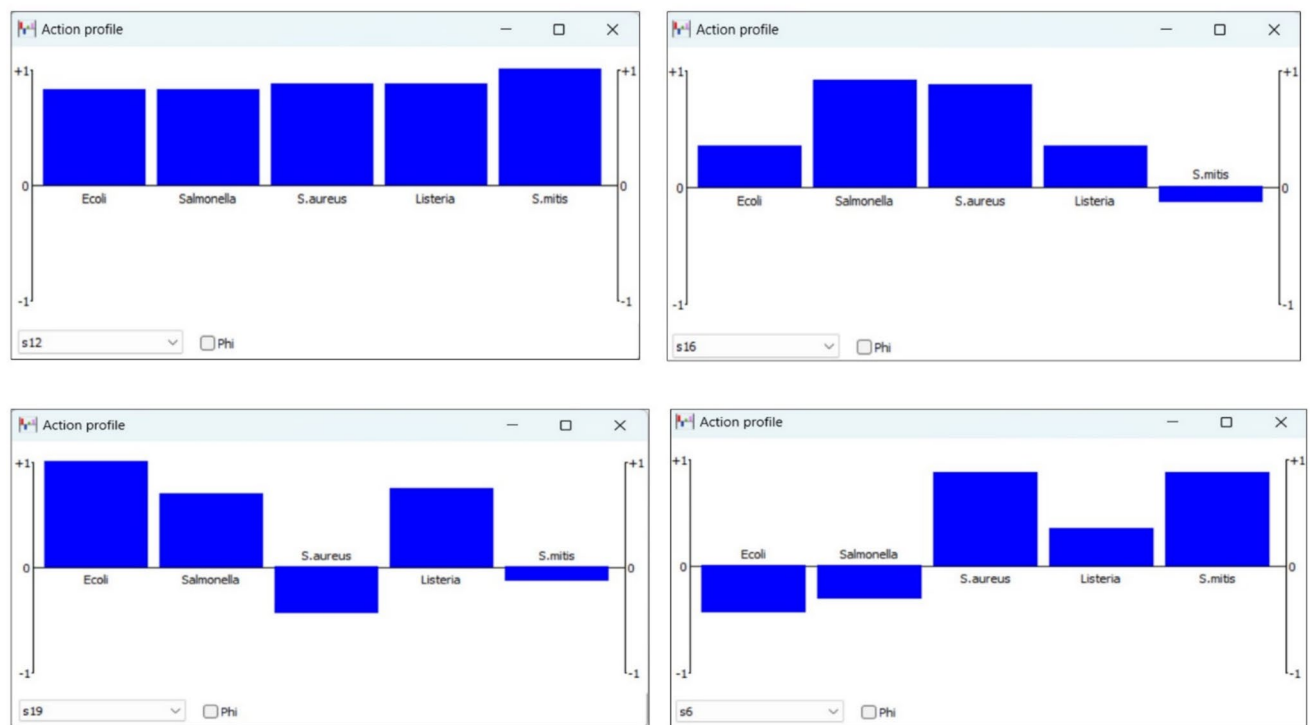


Fig. 7 Action profiles of alternatives

that the solvent mixture would extract 108.74 mg GAE/g of raw fruit. Predicted total phenolic content of the optimized solvent extract was found to be 110.977 ± 2.806 mg GAE/g raw fruit. The appropriateness of model is shown by the predicted TPC values matching the experimental results of validation test. The experimental points derived from the combined design were utilized for antimicrobial analysis, and the results were assessed using PROMETHEE. Statistical results showed that the extraction temperature of 60 °C and solvent composition of 50% water and 50% methanol had the maximum antibacterial efficacy. Nonetheless, the purity of the extract acquired in the antimicrobial parts of the investigation was not assessed. Consequently, subsequent research should assess the purity of the extract derived from the peach plum for its application in pharmacology.

This study employed the PROMETHEE approach to assess alternatives based on their inhibitory effects on various microorganisms, providing equal weights to each criterion. This method has shown PROMETHEE's capacity to discern balanced solutions in multi-criteria scenarios. Nonetheless, modifying weights to align with certain priorities, such as a product composition aimed at a particular micro-organism, may influence the outcomes and provide tailored applications. PROMETHEE demonstrates significant potential in several fields, particularly phytochemistry. This method may be employed to compare and optimize the extraction of bioactive substances from plants by evaluating variables such as yield, purity, biological activity, and environmental impact of the methods. It may also be utilized to assess the synergy of several phytochemicals about their antibacterial and antioxidant capabilities. These viewpoints indicate that PROMETHEE, despite its limited application in food and phytochemical sciences, has the potential to serve as a robust and versatile instrument for addressing intricate multi-criteria evaluation challenges. Subsequent research may concentrate on its incorporation into these domains to better illustrate its significance and application.

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Data availability The authors declare that the data supporting the findings of this study are available within the paper. Should any raw data files be needed in another format, they are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The author declares no competing interests.

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