



Ultrasound-Assisted Extraction of Bioactive Compounds from *Sesbania javanica* Flower: Phenolic Composition, Antioxidant, and Antimicrobial Applications

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Abstract

The *Sesbania javanica* Miq. flower is a rich source of bioactive phenolics and flavonoids with significant antioxidant and antimicrobial properties. This study optimized the ultrasound-assisted extraction (UAE) of bioactive compounds from *Sesbania javanica* flower extract (SFE) using response surface methodology (RSM). Optimal UAE conditions (40 °C, 20 min, and 70% ethanol concentration) significantly enhanced the extraction yield of bioactive compounds (14.89 mg GAE/g db for total phenolic compound (TPC), 8.13 mg QE/g db for total flavonoid content (TFC), 27.65 µM Trolox/g db for 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 61.56 µM Trolox/g db for Ferric reducing antioxidant power (FRAP)). High-performance liquid chromatography coupled with mass spectrometry using a quadrupole-time of flight analyzer (HPLC-MS/MS-QTOF) identified diverse phenolic compounds, including flavonols, hydroxycinnamic acids, flavanones, chalcones, and organic acids. The extract exhibited potent antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values ranging from 15 to 60 mg/mL. Thermodynamic analysis revealed the process's energy efficiency, consuming only 623 kJ under optimal conditions. These findings demonstrate the potential of SFE as a natural antioxidant and antimicrobial agent for food and pharmaceutical applications.

Keywords: *Sesbania javanica*; Phenolic compounds; Antioxidant activity; Antimicrobial activity; Natural bioactives; Functional ingredients.

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1. Introduction

It is well known that nutrition and health advantages are related, driving consumer preferences toward raw materials enriched with bioactive compounds beneficial to human health Lobo, Patil.^[1] Bioactive compounds, found in various parts of plants, are crucial for their antioxidant, anti-inflammatory, and antimicrobial properties, which are utilized in pharmaceutical,

food, and chemical industries.^[2,3] *Sesbania* (*Sesbania javanica* Miq.), a seasonal plant native to Southeast Asia, particularly Thailand, is notable for its medicinal properties. Its flowers are widely consumed in several Asian countries including Cambodia, are known for their rich content of phenolics, flavonoids, and carotenoids, which contribute to its antioxidant capabilities. A major flavonoid present in *Sesbania* flowers is quercetin 3-2G-rhamnosyl rutinoside, which has demonstrated significant inhibitory effects against mutagens such as aflatoxin B1 (AFB1) and benzo[a]pyrene (B[a]P).^[4]

Ultrasound-assisted extraction (UAE) has emerged as an efficient technique for extracting bioactive compounds, particularly phenolic compounds, due to its ability to disrupt cell walls, which enhances solvent penetration and accelerates mass transfer. This method is advantageous over conventional extraction techniques because it is faster, uses less solvent, and improves extraction yields, making it a preferred choice for

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extracting bioactive compounds from various plant materials.^[5] While UAE is widely used, the effects of extraction parameters such as temperature, extraction time, and solvent concentration on the efficiency of bioactive compound extraction from *Sesbania* flowers have not been thoroughly explored, necessitating further study on the optimization of these parameters to maximize the yield and quality of bioactive extracts.^[6,7]

Response surface methodology (RSM) is an advanced statistical technique that employs a second-order polynomial model to examine the interactions between multiple independent variables and the response variables.^[8] Unlike traditional single-variable optimization methods, RSM efficiently identifies optimal conditions by accounting for interactions between variables, thereby saving time and improving the accuracy of the results. This approach is particularly useful in processes involving multiple parameters, such as the extraction of bioactive compounds, where it helps in achieving higher yields and better quality of extracts.^[9,10]

In recent years, the thermodynamic aspects of extraction processes have gained considerable attention, as they provide insight into the energy requirements and efficiency of these processes. Understanding the thermodynamics of UAE, including the energy changes associated with the extraction of phenolic compounds, can guide the improvement of extraction parameters and contribute to more sustainable and cost-effective production processes.

The extraction of bioactive compounds from plants using UAE has garnered significant attention due to its efficiency in extracting phenolic compounds with potent antioxidant and antimicrobial properties.^[11] Although UAE has been widely used for extracting bioactive compounds from various plant materials, the application of UAE for *Sesbania javanica* remains underexplored. Most studies have focused on optimizing UAE conditions for other plant species like citrus peel or medicinal herbs.^[12,13] However, the optimization of UAE for *Sesbania* flower, a rich source of phenolics and flavonoids, and the subsequent analysis of its phenolic profile and antimicrobial properties have not been extensively investigated. Additionally, the incorporation of thermodynamic analysis to evaluate the extraction process's efficiency and energy consumption is a novel aspect of our research. This study not only optimizes UAE conditions but also provides insights into the phenolic profile and demonstrates the potential applications of *Sesbania javanica* flower extract as a natural additive and functional ingredient for pharmaceuticals, foods, and cosmetics.

2. Materials and methods

2.1 Chemicals and reagents

All the reagents, chemicals, and solvents used in this research were of analytical quality. The chemicals and materials used included; 2,2-diphenyl-1-picrylhydrazyl (DPPH) sourced from the USA, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) obtained from Fluka (USA),

aluminum chloride, ethanol, ferric chloride, gallic acid, Folin-Ciocalteu reagent, glacial acetic acid, hydrochloric acid, methanol, quercetin, and sodium carbonate (Na_2CO_3) (A.R. grade, Ajax Finechem, Australia), Tripyridyltriazine (TPTZ) (Merck, Germany), and Sodium acetate tetra-hydrate ($\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$) (A.R. grade, Ajax Finechem, Australia).

2.2 Sesbania flower sample preparation

The *Sesbania* flowers (SF) were purchased from a local market in Bangkok, Thailand. In the laboratory, the flowers were thoroughly cleaned to remove any undesirable materials and were dried in a Memmert hot air oven (Germany) at 50 °C until a moisture content of less than 5% (dry basis) was achieved. The dried flower was ground into powder form using a 50-mesh sieve, vacuum-sealed in vacuum in an aluminum-laminated foil pouches and stored in a freezer at -20 °C for utilization in the UAE experiment.

2.3 UAE and its thermodynamics

A sample of SF powder, weighing 1.2 g, was added to 40 mL of solvent (ethanol at 50, 70, and 90%) in a 50 mL tube. Ultrasound-assisted extraction (UAE) was conducted using ultrasound device (Elmasonic E 70 H, Germany) operated at a frequency and output power of 50/60 Hz and 520 W, respectively. Following extraction, the samples were centrifuged (Kubota, 6000, Japan) at 4,500 rpm for 20 min at 4 °C and then the supernatant was collected. Subsequently, the collected samples were subjected to evaporation process in vacuum state at 40 °C, concentrated to dryness using a rotary evaporator (BÜCHI, Switzerland). Finally, the dried extract was re-dissolved in distilled water and stored at -28 °C for further analysis.

2.3.1 Thermodynamics of UAE process

A thermodynamic study on the extraction of solutes from solids was conducted by evaluating the values of specific enthalpy, specific entropy, and Gibbs free energy. According to Fikry *et al.*,^[14] Eq. (1) can be used to determine ΔS and ΔH , while Eq. (2) was utilized to calculate ΔG .^[15]

$$\ln K_e = -\frac{\Delta H}{AT} + \frac{\Delta S}{R} \quad (1)$$

$$\Delta G = \Delta H - T\Delta S \quad (2)$$

where K_e refer to the proportion of solute that has been extracted compared to the solute that remains unextracted, known as the equilibrium constant for leaching. In the equation, ΔH is the enthalpy change (J per mol), ΔS signifies the entropy change (J/mol·K), ΔG denotes the change in Gibbs free energy (J/mol), T is the absolute temperature (K), and R is the constant value of gas (8.314 J per mol.K). Specifically, this ratio pertains to the contents of total flavonoid content (TFC), total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) extracted compared to the amount of these compounds remaining unextracted, as shown in Eq. (3):

$$K_e = \frac{Y_s}{Y_{max} - Y_s} \quad (3)$$

2.3.2 Calculations of energy consumption in ultrasonication

In the method of UAE, electrical energy (Ee) is converted into several forms during the process. Essentially, the ultrasonicator converts electrical energy into mechanical energy through the vibration of piezoelectric crystals. This energy subsequently becomes acoustic energy, generating ultrasonic waves within the liquid medium. As these waves propagate, they cause molecular oscillation, and once a critical distance is exceeded, the acoustic energy (E_a) is further converted to sonication or cavitation energy (EC), leading to bubble formation and heat generation. Thus, the ultrasound technique evolves three main stages of energy transformation: from electrical to acoustic, and then to cavitation (sonication), which can be quantitatively described using Eqs. (4-6), as previously discussed.^[14]

$$E_e = P \cdot t \quad (4)$$

$$E_a = m \cdot C \cdot \Delta T \quad (5)$$

$$EC = E_e - E_a \quad (6)$$

2.3.3 Experimental design and optimization process

The experiment employed the RSM technique using a Box-Behnken design to investigate the effects of ultrasound-assisted extraction conditions on the properties of Sesbania flower extract (SFE). The independent variables included extraction temperature (A1: 30–50 °C), extraction time (A2: 10–30 min), and ethanol concentration (A3: 50–90% v/v). The levels of these variables and their corresponding coded values are presented in (Table 1). Based on this design, a total of seventeen experimental runs, including five replicates at the center point, were performed as shown in Table 2. The measured responses were total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity determined by DPPH radical scavenging assay, and antioxidant activity determined by FRAP assay. The total phenolic content (Y) was calculated using Eq. (7).

$$Y = \beta_0 + \sum_{i=1}^k \beta_i A_i + \sum_{i=1}^k \beta_{ii} A_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^k \beta_{ij} A_i A_j \quad (7)$$

where Y represents the response variables, including total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity as measured by DPPH and FRAP assays. The model coefficients are defined as follows: constant term (β_0), linear coefficients (β_i), quadratic coefficients (β_{ii}), and interaction terms (β_{ij}). The variable k represents the number of predictors, which in this study is three.

2.3.4 Model validation

Triplicate experiments were conducted under optimized

conditions to compare the experimental outcomes with the model predictions, thereby evaluating the model's accuracy. Results were rounded to two decimal places, with values below 0.05 being rounded down, in accordance with RSM guidelines. To assess model validity, the percent difference was calculated as shown in Eq. (8).

$$\% \text{ Difference} = \frac{|a-b|}{(a+b)/2} \times 100 \quad (8)$$

where a is the experimental value and b is the predicted value.

Table 1: Actual and encoded values of the independent variables involved in the extraction procedure.

Independent variables	Symbol	Encoded values		
		-1	0	1
Time (min)	A ₁	10	20	30
Temperature (°C)	A ₂	30	40	50
Ethanol concentration (%)	A ₃	50	70	90

2.4 Determination of total phenolic content

The total phenolic content (TPC) was quantified by the Folin-Ciocalteu method, following the protocol previously established by Chutipajit and Sutjaritvorakul.^[16] with slight adjustments. Briefly, a 30 µL aliquot of the appropriately diluted extract was added by 1.2 mL distilled water and 1.8 mL of Folin-Ciocalteu reagent, which had been pre-diluted tenfold with distilled water. After a 5-minute incubation at ambient temperature, 1.5 mL of 7.5% (w/v) sodium carbonate solution was added. The mixture was then left for 1 hour at room temperature. Absorbance was recorded at 765 nm by spectrophotometer (GENE-SYSTEM 20 Visible, T.F.S., USA) using distilled water as a blank. A calibration curve was created using standard solutions of gallic acid in concentrations ranging from 0 to 150 mg/L, and the findings were presented in terms of milligrams of gallic acid equivalents per gram of dry basis (mg GAE/g db), based on the equation of the standard curve ($y = 0.0034x - 0.0124$, $R^2 = 0.99$).

2.5 Determination of total flavonoid content

The TFC value was analyzed using the method of aluminum chloride colorimetric following to the procedure outlined by Ennaifer and Bouzaiene with slight adjustments.^[17] Briefly, 1 mL of each diluted extract was mixed with 1 mL of 2% AlCl₃ dissolved with methanol. Following a 30-minute incubation period, the spectrophotometer (Thermo Fisher Scientific, GENESYSTEM 20 Visible, U.S.A) was utilized to measure the absorbance at 430 nm using distilled water as a blank. Flavonoid contents was calculated from a calibration curve ($y = 0.0069x + 0.032$, $R^2 = 0.99$) of quercetin (0-160 mg/L) and reported in terms of milligrams of quercetin equivalent per gram of dry basis (mg QE/g db).

2.6 DPPH radical scavenging activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method was

Table 2: The experimental trial based on the Box-Behnken Design (BBD).

Run	Extraction parameters		
	A ₁ -Temperature (°C)	A ₂ -Time (min)	A ₃ -Ethanol concentration (%)
1	40	10	90
2	30	10	70
3	50	30	70
4	30	30	70
5	40	20	70
6	40	20	70
7	50	10	70
8	40	20	70
9	40	30	90
10	50	20	90
11	30	20	50
12	40	20	70
13	30	20	90
14	50	20	50
15	40	10	50
16	40	30	50
17	40	20	70

performed following the procedure summarized by Thaipong, Boonprakob and others with a slight modification.^[18-20] Briefly, 0.2 mL of the supernatant (SFE) was added with 2 mL of DPPH prepared in methanol, vortexed, and incubated in the dark for 30 min. The absorbance of DPPH and the samples (A_{final}) were determined with a spectrophotometer (Thermo Fisher Scientific, GENESYSTM 20 Visible, U.S.A) at 515 nm using methanol as a blank. A_{diff} is the absorbance difference between sample and DPPH calculated using Eq. (9).

$$A_{diff} = A_{final} - A_{initial} \quad (9)$$

where $A_{initial}$ is the DPPH solution. A_{diff} value was used to calculate the antioxidant activity based on the standard curve ($y = 0.0016x + 0.0312$, $R^2 = 1$) of Trolox (0-600 μ M) and expressed as μ M Trolox equivalents per gram dry basis.

2.7 Ferric reducing antioxidant power (FRAP)

Antioxidant activity using the FRAP assay followed the procedure outlined by Thaipong, Boonprakob, and additional studies^[18-20] with a slight modifications. The FRAP mixture was incubated at 37 °C for 6 minutes until it changed to an orange-brown color. Subsequently, 2.85 mL of FRAP mixture and 0.15 mL of the supernatant (SFE) were vortex-mixed and left to incubate in darkness at ambient temperature for 30 minutes. The absorbance of the FRAP mixture (A_{final}) and the supernatant was determined using a spectrophotometer (Thermo Fisher Scientific, GENESYSTM 20 Visible, U.S.A.) at 593 nm, with distilled water as a blank. Antioxidant activity was calculated according to Eq. (10), and the results were expressed in μ M Trolox equivalents per gram of dry basis.

$$A_{diff} = A_{final} - A_{initial} \quad (10)$$

where $A_{initial}$ is the absorbance of the FRAP solution, and A_{diff} is the absorbance difference between the FRAP solution and

sample solution. A_{diff} was used to calculate antioxidant capacity from the standard curve ($y = 0.0009x - 0.0203$, $R^2 = 0.9945$) of Trolox (0-700 μ M), and result expressed as μ M Trolox equivalents per gram dry basis. The optimum UAE conditions for SFE was selected based on the maximum antioxidant activity.

2.8 High-performance liquid chromatography-tandem mass spectrometry/quadrupole time-of-flight (HPLC-MS/MS-QTOF) analysis

The optimized SFE was analyzed using high-performance liquid chromatography coupled with mass spectrometry using a quadrupole-time-of-flight analyzer (LC-MS/MS-QTOF, X500R QTOF System, USA). Compound identification was performed by comparing the detected compounds with standard reference compounds available in the system's library.

2.8.1 Sample preparation

The optimized SFE was dissolved in distilled water and diluted to a concentration of 500 ppm. Chromatograph separation was performed using a 5 μ L injection volume, and the analysis was performed in triplicate.

2.8.2 HPLC-MS/MS-QTOF analysis condition

Chromatographic separations were conducted on Kinetex EVO C18 columns, 100 A (100 $\times$ 2.1 mm², 2.6 μ m). The mobile phase consisted of two solvents: water (solvent A) and acetonitrile (solvent B), each containing 0.10% formic acid (v/v). The flow rate was maintained at 0.5 mL per minute and split to 0.2 mL per minute before detection of MS, utilizing a gradient elution method: starting from 0-15 minutes, the solvent B increased from 0 to 15%; at 30 minutes, it reached 20% B; at 60 minutes, 50% B; and it peaked at 100% B from 70 to 79 minutes. For the Time-of-Flight (TOF) MS settings,

the scan speed was set to cover a mass-to-charge (m/z) range from 100 to 1000 and 2500 atomic mass units per second (amu/s), with an accumulation time of 0.25 seconds. The ion source gases 1 and 2 were maintained at 50 and 60 psi, respectively, at a temperature of 500 °C; spray voltage was -4500 V; delustering potential was -60 V; collision energy was -10 V.

2.9 Antimicrobial activity experiment

Microbial cultures, including *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, were obtained from the Faculty of Science, Chulalongkorn University. Inocula of each microorganism was inoculated using 10 milliliters of aseptic growth medium, specifically, Nutrient Broth (NB) for bacteria and Yeast Malt Broth (YMB) for yeast, respectively). Incubation was carried out for 18-24 hours at 35 °C for bacterial cultures and 30 °C for yeast cultures. The initial concentration of the test microorganisms was adjusted to approximately 10^6 CFU/mL, confirmed by measuring the optical density (OD) at 600 nm.

The optimized SFE was assessed for antimicrobial activity using the disk diffusion method, as well as for determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), following the protocols described by Dahiru and Abaka with minor modifications.^[21]

For the disk diffusion method, Mueller-Hinton Agar was used for bacterial cultures and Yeast Malt Agar for yeast. Broth suspensions of each microorganism were evenly streaked onto the agar surface using a cotton swab. After allowing the inoculum to dry for 2-5 minutes, sterile discs were placed on the agar and pressed gently to ensure contact. The extract, solvent (distilled water), and antibiotic (chloramphenicol) were then applied to the discs. Chloramphenicol served as the positive control, while distilled water was utilized as the negative control. Plates were incubated at 37°C for bacterial cultures and 30°C for yeast cultures for 24 hours, after which the inhibition zones were measured in millimeters.

The MIC was determined by a two-fold serial dilution technique, following the method of Turganbay and Atageldiyeva with slight modifications.^[22] The initial concentration of SFE was 120 mg/mL, subsequently diluted in a 96-well culture plate to final concentrations of 60, 30, 15, 7.5, 3.75, 1.87, 0.94, 0.47, 0.23, and 0.12 mg/mL. Each dilution was inoculated with 100 μL of microbail suspension. Plates were incubated for 24 hours at 37 °C for bacteria and 30 °C for yeast. Negative controls consisted of wells containing extract and medium without microorganisms, while positive controls contained microorganisms without extract. The MIC was recorded as the lowest concentration showing no visible microbial growth.

For MBC determination, aliquots from MIC wells showing no turbidity were streaked onto appropriate media and incubated for 24 hours at 35 °C for bacteria and 30 °C for yeast. The MBC was defined as the lowest concentration at which no visible growth occurred on the culture medium.

2.10 Data analysis

The optimization of the UAE experiment employed the RSM using a Box-Behnken design, with three-dimensional (3D) response surface plots generated in Design Expert 13 software. The significance of the models was evaluated through analysis of variance (ANOVA) using the F-test. All results were reported as mean $\pm$ standard deviation (SD).

3. Result and discussion

3.1 Effect of UAE process conditions on total phenolic contents in SFE

The RSM employing the Box-Behnken design was utilized to investigate the impact of UAE parameters on the TPC, TFC, and antioxidant activity assessed by DPPH and FRAP assays in *Sesbania* flower extract (SFE). As shown in Table 3, the range of total phenolic content (TPC) ranged from 6.90 ± 0.59 to 20.13 ± 0.08 mg GAE/g db. TPC varied significantly across different runs, indicating that the extraction parameters substantially influence the phenolic yield. The highest TPC was observed under the condition of run 17 (40 °C, 20 min, 70% solvent concentration) with 20.13 mg GAE/g db. This suggests that a moderate temperature, combined with sufficient extraction time and solvent concentration, is optimal for maximizing phenolic extraction. High temperatures and concentrations (Run 7, 50 °C, 70%) also produced in high TPC (18.99 mg GAE/g db), confirming the effectiveness of these conditions. These findings align with studies indicating that moderate temperatures and solvent concentrations optimize the extraction of phenolic compounds due to improved solubility and extraction efficiency.^[23,24]

The analysis of TPC showed that neither the linear nor the quadratic terms, nor the interaction effects had significant impacts, as indicated in Table 4. The F-value for lack of fit was 0.58, indicating that the lack of fit was not significant relative to the pure error and suggesting a 65.98% probability that an F-value of this magnitude could arise by random chance. A non-significant lack of fit is favorable, conforming that the model is suitable for fitting and predicting the response.^[25] Moreover, the ratio of adequate precision of this model was greater than 4 which indicates an adequate signal. Predicting TPC using coded factors was successfully modeled through a linear equation (Eq. (11)), demonstrating high accuracy with an R^2 value of 0.82. A comparable predictive model was developed and applied by Sai-Ut, Kingwascharapong.^[26] and Fikry *et al.* for estimating TPC in extract from longan seeds under various extraction conditions including ethanol concentration, ultrasonic bath temperature, and extraction duration.^[14]

$$\text{TPC} = 11.42 - 1.23A_1 - 1.57A_2 + 4.50A_3 + 1.02A_1^2 - 0.0190A_2^2 - 1.05A_3^2 + 0.5350A_1A_2 + 1.22A_1A_3 + 1.09A_2A_3 \quad (11)$$

Fig. 1 illustrates a 3D surface plot of the predicted TPC of SFE sample. In Fig. 1a, it is evident that the TPC values increased with rising temperatures, likely due to the

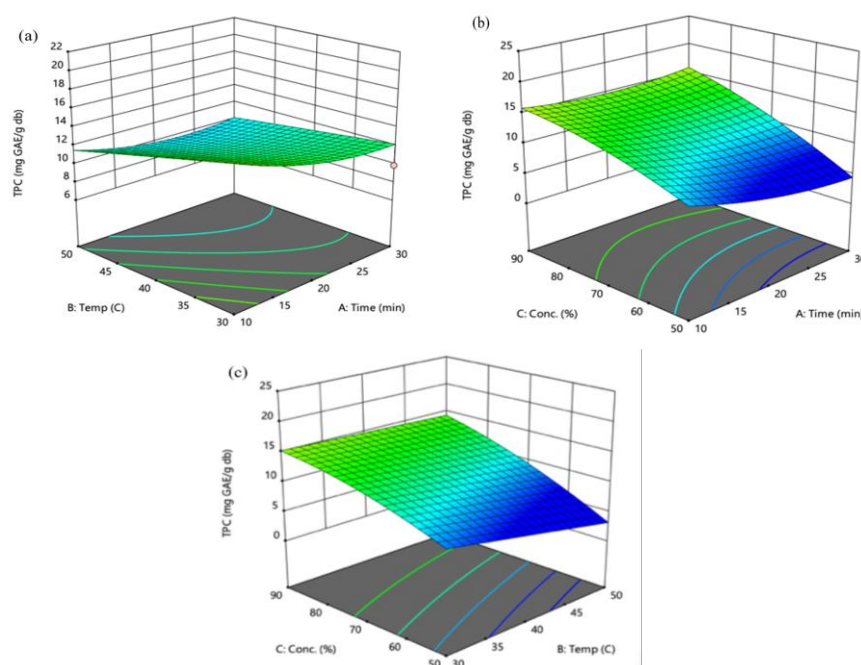


Fig. 1: 3D response surface graphs illustrating the TPC showing the interaction effects between factors: (a) temperature and duration; (b) ethanol level and duration; (c) ethanol level and temperature for *Sesbania* flower extract.

temperature enhancing the diffusion coefficient and solubility of phenolic compounds in the extraction solvent. A similar trend was observed with the effect of extraction time, as higher TPC values were recorded with longer extraction duration. This may be attributed to extended sonification period in UAE, which promotes greater release of phenolic compounds. These findings align well with the TPC results reported for mulberry extract by UAE, as reported by Insang and Kijpatanasilp.^[27] Therefore, increasing the temperature enhances both the

diffusion coefficient and diffusion rate leading to higher TPC yields. This result aligns with findings from an earlier study by Sirichan, Kijpatanasilp which reported a positive effect of extraction temperature on the yield of total phenolic compound.^[28] In Fig. 1b, the impact of both duration and ethanol level on TPC values of SFE are depicted. The surface plot demonstrates that the TPC value tended to increase as the ethanol concentration rose from 50 to 90%, with the highest TPC observed at 70% ethanol (Table 3). Similarly, Fig. 1c

Table 3: Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (measured by DPPH and FRAP assays) of *Sesbania* flower extract (SFE) under varying conditions of UAE based on BBD for response surface methodology.

Run	Dependent variables			Responses			
	A ₁ -Temperature (°C)	A ₂ -Time (min)	A ₃ -Concentration (%)	TPC (mg GAE/g db.)	TFC (mg QE/g db.)	DPPH (μM Trolox/g db.)	FRAP (μM Trolox/g db.)
1	40	10	90	16.68 ± 0.43	4.49 ± 0.07	12.05 ± 0.33	34.65 ± 1.86
2	30	10	70	11.93 ± 0.23	7.63 ± 0.03	30.93 ± 0.77	61.43 ± 1.26
3	50	30	70	19.06 ± 0.41	8.18 ± 0.07	29.38 ± 1.20	60.71 ± 1.97
4	30	30	70	9.86 ± 0.61	8.18 ± 0.03	30.56 ± 1.62	55.41 ± 0.00
5	40	20	70	10.78 ± 0.15	8.08 ± 0.09	28.74 ± 1.32	59.17 ± 1.46
6	40	20	70	10.07 ± 0.81	7.60 ± 0.07	29.44 ± 1.02	59.48 ± 2.99
7	50	10	70	18.99 ± 0.92	8.08 ± 0.07	29.76 ± 1.58	71.91 ± 1.25
8	40	20	70	10.31 ± 1.16	7.75 ± 0.00	30.03 ± 1.09	68.48 ± 0.52
9	40	30	90	18.18 ± 0.91	6.93 ± 0.05	18.68 ± 0.02	53.12 ± 0.52
10	50	20	90	19.59 ± 0.82	8.03 ± 0.07	29.24 ± 1.47	67.24 ± 3.55
11	30	20	50	10.70 ± 1.24	7.31 ± 0.03	30.96 ± 1.09	56.50 ± 1.55
12	40	20	70	18.50 ± 0.08	7.21 ± 0.07	30.11 ± 0.95	55.36 ± 2.07
13	30	20	90	18.16 ± 0.33	6.47 ± 0.07	19.07 ± 0.53	59.04 ± 0.73
14	50	20	50	7.25 ± 0.77	7.47 ± 0.00	33.84 ± 2.80	63.34 ± 1.57
15	40	10	50	9.78 ± 0.64	7.18 ± 0.07	30.51 ± 1.32	62.23 ± 2.41
16	40	30	50	6.90 ± 0.59	7.43 ± 0.05	30.98 ± 0.55	66.95 ± 1.15
17	40	20	70	20.13 ± 0.08	8.15 ± 0.03	30.82 ± 0.44	65.12 ± 2.30

Table 4: The analysis of variance (ANOVA) results for the responses concerning total phenolic content, total flavonoid contents, and antioxidant activities as determined by DPPH and FRAP assays

Terms		TPC		TFC		DPPH		FRAP	
		F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Linear	A ₁	0.016	>0.05	2.65	>0.05	2.08	>0.05	0.53	>0.05
	A ₂	0.025	>0.05	0.13	>0.05	1.76	>0.05	0.22	>0.05
	A ₃	0.48	>0.05	0.002	>0.05	2.11	>0.05	0.02	>0.05
Quadratic	A ₁ ²	0.22	>0.05	3.58	>0.05	3.48	>0.05	0.81	>0.05
	A ₂ ²	0.001	>0.05	0.95	>0.05	2.70	>0.05	0.51	>0.05
	A ₃ ²	0.23	>0.05	11.83	<0.05*	8.46	<0.05*	0.81	>0.05
Cross-product	A ₁ *A ₂	0.06	>0.05	0.15	>0.05	2.682	>0.05	0.08	>0.05
	A ₁ *A ₃	0.30	>0.05	1.45	>0.05	1.43	>0.05	0.01	>0.05
	A ₂ *A ₃	0.24	>0.05	11.83	<0.05*	1.02	>0.05	0.55	>0.05
Lack-of-fit		0.58	0.659	4.07	0.104	34.34	0.002	6.00	0.058
Adequate precision		4.40		7.82		8.48		3.34	

* significant at the 0.05 level of significance.

shows that TPC values decreased at lower temperatures, with the maximum TPC recorded at 40 °C. The use of ultrasound-assisted extraction has been shown to facilitate faster and more efficient release of intracellular compounds, leading to improved extraction of phenolic compounds from plant material.^[27,29]

3.2 Effect of UAE process conditions on total flavonoid contents in SFE

Flavonoids, a class of polyphenolic secondary metabolites found in plants, have been extensively studied for their diverse medicinal properties, including anticancer,^[30] antimicrobial, antiviral, antiangiogenic,^[31] antimalarial, antioxidant, neuroprotective, antitumor, and antiproliferative activities.^[32] With the growing interest in flavonoids derived from dietary sources, there is an increasing importance placed on examining the origins of flavonoids in food and beverages. Table 3 shows the relatively stable TFC across different runs, with slight variations, indicates a more uniform extraction efficiency for flavonoids. However, the highest TFC values were observed in Runs 7 and 3 (8.18 mg QE/g db) at higher temperatures (50°C), indicating that increased temperature may facilitate the release of flavonoids from the plant matrix. This finding is consistent with research demonstrating that higher temperatures can disrupt plant cell walls, hereby enhancing the release of bound flavonoid compounds.^[33,34]

The analysis of TFC revealed significant effects in both linear and quadratic components, as well as interaction terms (Table 4). The linear terms corresponding to ultrasonic temperature, extraction time, and ethanol concentration demonstrated substantial effects on TFC. The quadratic effects of ethanol concentration emerged as a key predictors of TFC, supported by *p*-values less than 0.05. Furthermore, the interaction effects between ultrasonic time and ethanol concentration also significantly effected TFC, as indicated by *p*-values below 0.05. In addition, the ratio of adequate

precision was 7.82, indicating a sufficient signal and confirming that the model is suitable for exploring the design space. The estimation of TFC using coded factors was established via a linear regression equation in Eq. (12), demonstrating a strong alignment with an R-squared value of 0.92. A comparable predictive model was developed by Sai-Ut, Kingwascharapong for TFC in extract of logan seed, using different extraction conditions (ethanol level and water, temperature, and time) compared to this study.^[26]

$$\text{TFC} = 9.54 - 2.10A_1 - 0.4635A_2 - 0.0388A_3 + 0.5360A_1^2 - 0.2765A_2^2 - 0.9740A_3^2 - 0.1125A_1A_2 + 0.35A_1A_3 + 0.5475A_2A_3 \quad (12)$$

A 3D plot illustrating the predicted TFC in SFE is presented in Fig. 2. In Fig. 2a, the impact of both temperature and duration on TFC values indicated that TFC tends to decrease with lower temperatures, peaking around 20 minutes. Additionally, Fig. 2b shows that increasing the ethanol concentration from 50% to 70% result in higher TFC values, while concentrations above 70% lead to a decrease in TFC. This trend is further emphasized in Fig. 2c, where the TFC value is shown to be highest at an ethanol concentration of 70%. This outcome is similar to findings reported by Hikmawanti, Wiyati.^[35] The factors influencing the efficiency of phenolic compound extraction similarly affect the ability to extract flavonoids. This suggested that using a solvent blend of water and ethanol can also enhance the efficiency of flavonoid extraction.^[36] Flavonoids, a prominent subgroup within the phenolic compound family, play a significant role in food, cosmetics, and pharmaceuticals. Extensive research has been conducted on the anti-bacterial, anti-allergic, anti-cancer, and anti-inflammatory properties of flavonoids, revealing their potential health benefits for humans. Notably, their primary biological role is as antioxidants, that have been shown through experiments to be even more potent than vitamin C. Additionally, flavonoids have been linked to

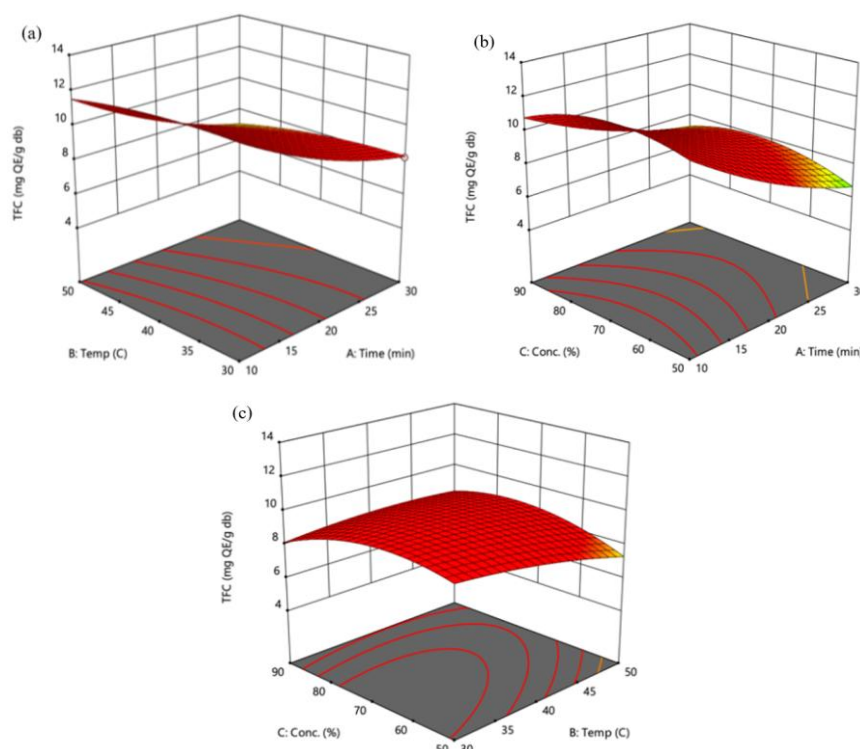


Fig. 2: 3D response surface graphs showcasing the TFC showing the interaction effects between factors: (a) temperature and duration; (b) ethanol level and duration; (c) ethanol level and temperature for *Sesbania* flower extract.

reducing the risk of coronary artery thrombosis and heart disease, mitigating nervous system disturbances, and improving cognitive performance.^[37]

3.3 Effect of UAE process conditions on antioxidant activity in SFE

The focus on antioxidant activity in food and beverages has garnered significant attention within the scientific community. Antioxidants provide protection against the deteriorative effects induced by free radicals, which play a role in the onset of chronic conditions including cardiovascular ailments, heart disease, aging, cancer, anemia, and inflammation.^[38] Table 3 shows the DPPH value ranged from a low $12.05 \pm 0.33 \mu\text{M}$ Trolox/g db to a high of $33.84 \mu\text{M}$ Trolox/g db, indicating that moderate temperature and solvent concentration are optimal for extracting compounds with strong free radical scavenging abilities. This observation is supported by literature that highlights the role of phenolic compounds in scavenging free radicals, with their extraction efficiency being highly dependent on solvent polarity and temperature.^[39,40]

The evaluation of DPPH activity showed that linear and quadratic components, along with interaction effects, are included, had negligible impacts overall. Nonetheless, the quadratic term for ethanol concentration demonstrated a substantial effect on DPPH activity, with p-values less than 0.05, denoting statistical significance. The F-value for lack of fit was recorded at 34.34, suggesting a significant lack of fit. This implies there's only a 0.26% chance that such a high lack of fit F-value could occur due to random fluctuations. Moreover, adequate precision ratio of the model was 8.48,

indicating a sufficient signal, confirming that this model is suitable for exploring the design space. Similar findings on antioxidant effects have been reported in research involving extracts from orange byproducts,^[41] Ruby S apple peel,^[33] and almond by-products.^[42] The estimation of DPPH radical scavenging ability utilizing coded variables were determined using a linear regression formula in Eq. (13), showing a strong correlation and yielding an R^2 value of 0.98 (Table 5). A protective model for DPPH radical scavenging activity in lychee and longan seed extracts was formulated by Sai-Ut, Kingwascharapong,^[26] employing various extraction conditions (ethanol level, temperature, and time) compared to those used in the present study.

$$\text{DPPH} = 30.07 - 9.77A_1 - 8.99A_2 - 6.47A_3 + 2.78A_1^2 - 2.45A_2^2 - 4.33A_3^2 - 0.0025A_1A_2 + 1.82A_1A_3 + 1.54A_2A_3 \quad (13)$$

The 3D surface plot illustrating the impact of time, temperature, and ethanol level on DPPH activity in SFE are presented in Fig. 3. At a constant ethanol concentration, the DPPH value tended to increase as the temperature rose from 30 °C to 40 °C, then slightly decreased beyond 40 °C, with the highest DPPH values observed at 40 °C. Similarly, the DPPH value peaked at an extraction time of 20 minutes (Fig. 3b), consistent with the values in Table 3, which showed higher DPPH values around 20-minutes extraction time. Regarding the impact of ethanol concentration at constant temperature, the DPPH values tended to decrease at lower and higher ethanol concentrations than 70%. In alignment with a prior investigation conducted by Cepoi and Rudi,^[43] which reported

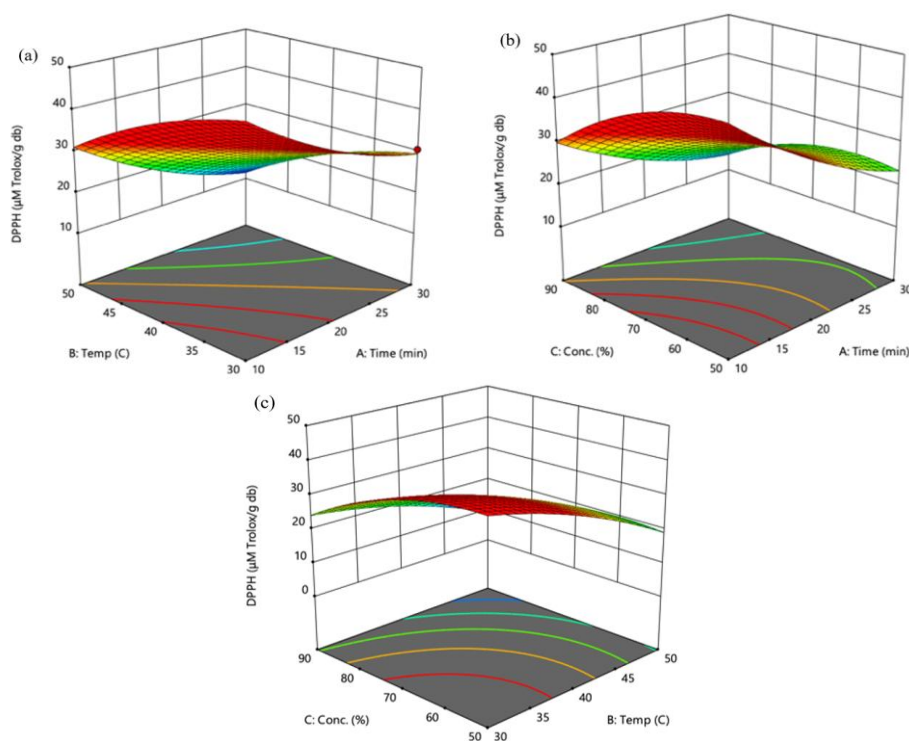


Fig. 3: 3D response surface plots of DPPH assay showing the interaction effects between factors: (a) temperature and duration; (b) ethanol level and duration; (c) ethanol level and temperature for *Sesbania* flower extract.

that extract with 70% ethanol yield the maximum value of DPPH. Additionally, Shian and Abdullah found that among ethanol concentration of 30%, 70%, and 100%, the DPPH value was highest at an ethanol concentration of 70%, consistent with the findings of this study.^[44]

On the other hand, the FRAP method is used to evaluate antioxidant capabilities, utilizing Trolox as a reference standard. It's widely applied to assess the antioxidant capabilities in products such as foods, beverages, and dietary supplements rich in polyphenols.^[45] Table 3 indicates that the highest FRAP value was observed in Run 7 (71.91 μM Trolox/g db) suggests that high temperature and ethanol concentration are effective in extracting compounds with potent reducing power. The strong correlation between FRAP and TPC/TFC in this run suggests that phenolic and flavonoid compounds play a major role in enhancing the antioxidant properties of the extract. However, the analysis of variance (ANOVA) for FRAP response was less robust, as the F-value and p-value of the model were insignificant. The adequate precision ratio was 3.34 which was lower than 4 indicated that

this model was inadequate signal. This may because of the FRAP values of each treatment were very similar to each other. However, the lack-of-fit F-value for the model stood at 6.00, and the p-value exceeded 0.05, indicating that the lack of fit was insignificant. This suggests there is only a 5.81% probability that an F-value for lack of fit this substantial could arise from random variations, meaning that the model was acceptable for fitting, with an R^2 of 0.80 (Table 5). The antioxidant activity model determined through the FRAP assay is presented in the following formula:

$$\text{FRAP} = 63.86 - 15.01A_1 - 9.56A_2 + 1.82A_3 + 4.07A_1^2 - 3.22A_2^2 - 4.06A_3^2 - 1.29A_1A_2 + 0.34A_1A_3 + 3.44A_2A_3 \quad (14)$$

The three-dimensional surface plot displays the impact of extraction duration, temperature, and ethanol concentration on antioxidant capacity as measured by the FRAP assay, as seen in Fig. 4. The surface chart (Fig. 4a) indicates that increasing the extraction temperature from 30 °C to 50 °C led to an enhancement in antioxidant capacity, as evidenced by the FRAP values. At constant temperature, the FRAP values were

Table 5: The statistical indicators for the second-order polynomial equation associated with different variables.

Property	Regression coefficients for the quadratic polynomial model										
	$Y = \beta_0 + \beta_1A_1 + \beta_2A_2 + \beta_3A_3 + \beta_{11}A_1^2 + \beta_{22}A_2^2 + \beta_{33}A_3^2 + \beta_{12}A_1A_2 + \beta_{13}A_1A_3 + \beta_{23}A_2A_3$										
	β_0	β_1	β_2	β_3	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}	R^2
TPC (mg GAE/g db.)	11.42*	-1.23	-1.57	4.50	1.02	-0.02	-1.05	0.54	1.22	1.09	0.82
TFC (mg QE/g db.)	9.54*	-2.10	-0.46	-0.04	0.53	-0.28	-0.97*	-0.11	0.35	0.55*	0.92
DPPH (μM Trolox/g db.)	30.07*	-9.77	-8.99	-6.47	2.78	-2.45	-4.33*	-0.00	1.82	1.54	0.98
FRAP (μM Trolox/g db.)	63.86*	-15.01	-9.56	1.82	4.07	-3.22	-4.06	-1.29	0.34	3.44	0.80

* Significant at the 0.05 level of significance.

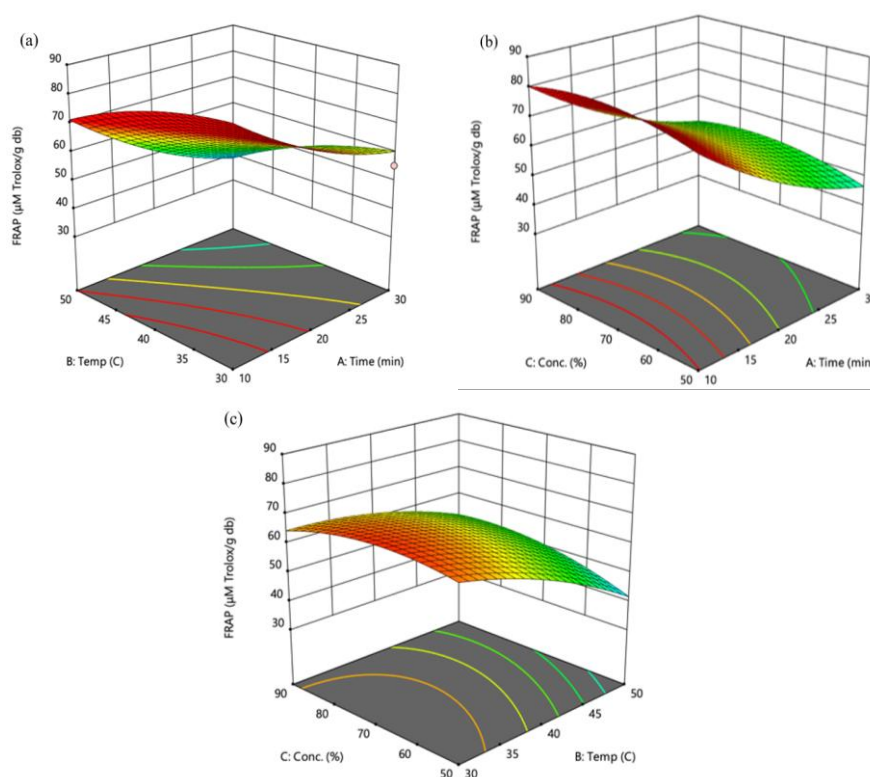


Fig. 4: 3D response surface plots of FRAP assay showing the interaction effects between factors: (a) temperature and duration; (b) ethanol level and duration; (c) ethanol level and temperature for *Sesbania* flower extract.

lower when the ethanol concentration was lower or higher than 70% (Fig. 4b), which aligns with the actual values presented in Table 3. Additionally, the FRAP values appeared to peak around the time of 20 minutes. Fig. 4c indicates that the antioxidant activity analyzed by FRAP assay was highest around the temperature of 40 $^{\circ}\text{C}$ and tended to decrease at higher temperatures. These findings align with the results of Yim and Chye, who found that DPPH scavenging ability was highest around an extraction temperature of 42 $^{\circ}\text{C}$ and decreased at higher temperatures.^[46] Another study Shang, Luo observed that among extraction time ranging from 10 to 60 minutes, found that the FRAP and TPC values were highest at the 20-minute mark, which corresponds well with the current result of this study.^[47]

The findings from the quadratic polynomial equation, paired with the response surface plots, clearly demonstrate that the quadratic polynomial equation effectively explains the three-dimensional plots and predicts TPC, TFC, and the antioxidant capacity determined by DPPH and FRAP assays for the SFE samples (as shown in Figs. 1-4). Visual inspection of the response surfaces reveals the extent to which the independent variables influence these responses. The plots in Figures 1-4 show that the factors such as temperature (A1), extraction time (A2), and solvent concentration (A3) significantly affect the levels of bioactive compounds and the antioxidant efficiency of the SFE. The various shapes of the plots indicate different level of interactions among the studied variables. An elliptical contour plot suggests significant interactions between the variables, while a circular contour

plot indicates minimal or no interactions.^[8,9]

3.4 Impact of UAE parameters on energy consumption

Table 6 presents data on the ultrasonication energy consumption (EC) for different extraction times and temperatures. The values are expressed in kilojoules (kJ), with the accompanying means and standard deviations. The EC value increased from 311.98 ± 0.002 to 935.89 ± 0.001 kJ as the extraction time and temperature rose from 10 minutes at 30 $^{\circ}\text{C}$ to 30 minutes at 50 $^{\circ}\text{C}$, respectively. The increasing trend correlates with both longer ultrasonication times and higher temperatures. This trend is consistent with the understanding that increasing the duration and temperature of ultrasonication enhances the energy required for the extraction process. This relationship aligns with thermodynamic principles governing UAE, where higher temperatures can lead to increased molecular motion, reducing the viscosity of the solvent and facilitating greater penetration of ultrasonic waves into the plant matrix.^[48,49] This condition results in more efficient cavitation, which is the formation and implosion of bubbles that disrupt cell walls, thereby enhancing the release of bioactive compounds.

Higher energy consumption during ultrasonication often improves extraction efficiency, excessive energy input can cause the breakdown of thermolabile bioactive compounds, such as phenolics and flavonoids, potentially reducing extract quality. For example, a study by Tiwari, Patras demonstrated that while ultrasonication can significantly increase the extraction yield,^[50] overly high temperatures can cause thermal

Table 6: Means and standard deviation of ultrasonication energy consumption with different times and temperatures.

Time (min)	Temperature (°C)	EC (kJ)
10	30	311.98 ± 0.002
20	40	623.94 ± 0.003
30	50	935.89 ± 0.001

degradation of antioxidant compounds. Finding a balance between energy consumption and extraction efficiency is crucial. This is supported by studies on various plant extracts, which indicate that moderate ultrasonication conditions (*e.g.*, 20-30 minutes at 40 °C-50 °C) often yield the best results in terms of both extraction efficiency and compound integrity.^[51,52]

3.5 Identification of optimal conditions of UAE process and validation

Identifying the optimal conditions for the UAE process is crucial to maintaining food properties and ensuring overall quality. Optimization was conducted to pinpoint extraction parameters that maximize yield, as well as the TPC, TFC, and DPPH value in SFE. The polynomial equations developed in this research were used to determine the optimal parameters for UAE and to predict the best outcomes for the five key variables. The optimal conditions were pinpointed through the overlay and examination of the contour plots, adhering to the specified limits. As a result, the optimal conditions were identified as an extraction temperature of 40 °C, an extraction time of 20 minutes, and an ethanol concentration of 70%. These conditions were applied to achieve the most favorable extraction outcomes within the defined parameters.

The accuracy of the predicted optimal conditions was confirmed by measuring TFC, TPC, and antioxidant capacity using both DPPH and FRAP assays. Validation results presented in Table 7 show only slight variations between experimental responses and the predicted values under these optimum conditions, confirming the reliability of the proposed UAE setting. Thus, the predictive model is shown to be effective for optimizing the extraction of TPC, TFC, and antioxidant activity as assessed by DPPH and FRAP assays. This conclusion is in consistent with previous findings by

Sirichan, Kijpatanasilp who also reported difference of less than 10% between experimental and predicted values.^[28]

3.6 Thermodynamic analysis of UAE process

The analysis of thermodynamic parameters for the UAE of bioactive compounds, including TPC, TFC, and antioxidant activities (DPPH and FRAP) from SFE at various temperatures is summarized in Table 8. Key parameters include the equilibrium constant (K_e), Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS), along with corresponding linear regression models.

The equilibrium constant (K_e) of TPC, TFC, DPPH, and FRAP increases with temperature, suggesting that higher temperatures enhance the extraction efficiency of the bioactive compounds. This trend is consistent with the principles of thermodynamics, where increased thermal energy can overcome intermolecular forces, thereby facilitating better solute dissolution and diffusion.^[53] The rise in K_e with temperature is consistent with previous findings showing that elevated temperatures boost solubility and diffusion rates of bioactive compounds, thereby increasing extraction yields.^[54]

The Gibbs free energy (ΔG) values are positive and increase with temperature, suggesting that the extraction process is non-spontaneous and requires more energy (K_e). The significant enthalpy (ΔH) values, particularly for TPC and TFC, confirm the endothermic nature of the process, indicating that considerable energy is required to disrupt solute-solvent interactions and facilitate compound release. Negative entropy (ΔS) values suggest a decrease in system randomness, likely due to more structured solute-solvent interactions or complex formation. This could be critical for the stability and bioavailability of the extracts. These results underscore the importance of optimizing UAE parameters, particularly temperature, to balance energy consumption with extraction efficiency and achieve sustainable extraction.

Table 7: Comparison between experimental values and the values estimated by the model.

Parameters	Predicted value	Experimental values	% Difference
TPC (mg GAE/g db.)	13.96 ± 0.58		
TFC (mg QE/g db.)	7.58 ± 4.41	14.89 ± 0.24	6.48
DPPH (μM)	26.87 ± 6.25	8.13 ± 0.28	7.05
Trolox/g db.)	60.01 ± 7.99	27.65 ± 0.29	2.86
FRAP (μM Trolox/g db.)		61.56 ± 1.36	2.56

Mean ± standard deviation.

3.7 Antimicrobial efficacy of SFE

Plants have historically been used as dietary supplements and remedies for various ailments, with research continually exploring their therapeutic properties. Due to concerns about microbial resistance and side effects of conventional antibiotics, much of this research focuses on the antibacterial properties of medicinal plants.^[55] Secondary metabolites such as tannins and phenolics, are believed to contribute to antibacterial activity. For instance, studies by Mahomoodally, Gurib-Fakim and Abdjan, Aminah, have demonstrated that the presence of bioactive compounds, including phenolic compounds, plays a crucial role in antimicrobial activity.^[56,57]

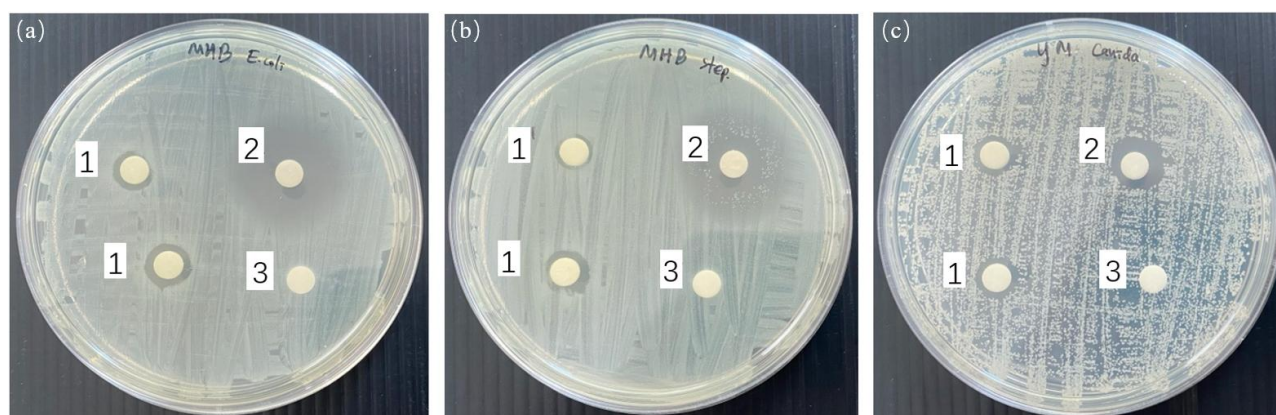


Fig. 5: Antimicrobial activity, zones of inhibition (a) Petri dish with *E. coli* culture (b) Petri dish with *S. aureus* culture (c) Petri dish with *C. albicans* culture, (1) absorbed in Sesbania flower extract (2) absorbed in antibiotics, chloramphenicol (3) absorbed in distilled water solvent.

Table 8: The thermodynamic parameters for UAE of TPC, TFC, DPPH, and FRAP assays from SFE at the optimal time, ethanol concentration and temperatures.

Property	T(K)	K_e	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/mol)	Linear model	R^2
TPC	303	5.51×10^{-5}	133535.28	81702.97	-171.06	$\ln(K_e) = -9863.6(1/T) + 22.746$	0.92
	313	1.56×10^{-4}	135246.08	81692.97	-171.10		
	323	4.13×10^{-4}	136957.20	81682.97	-171.13		
TFC	303	6.47×10^{-5}	32924.16	2409.03	-100.71	$\ln(K_e) = -326.2(1/T) - 8.5689$	1.00
	313	6.70×10^{-5}	33933.78	2399.03	-100.75		
	323	6.92×10^{-5}	34940.97	2389.03	-100.78		
DPPH	303	5.78×10^{-5}	0.109	61.13	-0.16	$\ln(K_e) = -7389.1(1/T) + 13.849$	0.80
	313	1.20×10^{-4}	0.111	61.12	-0.16		
	323	2.39×10^{-4}	0.113	61.11	-0.16		
FRAP	303	9.56×10^{-5}	53855.82	2023.51	-171.06	$\ln(K_e) = -279.83(1/T) - 8.3614$	0.83
	313	9.83×10^{-5}	55566.62	2013.51	-171.10		
	323	1.01×10^{-4}	57277.74	2003.51	-171.13		

The presence of these phytochemicals may help explain the antibacterial effects observed in various plant studies. Variations in antimicrobial effectiveness among medicinal plants are attributable to differences in types and concentration of their active compounds.

In the current study, the optimized SFE was evaluated for their antibacterial properties against bacterial and yeast. The diameter of the inhibition zones produced by SFE was measured to assess antibacterial activity. The optimized SFE demonstrated clear antimicrobial effects, with inhibition zones ranging from 9.58 mm to a maximum of 10.48, as show in Fig. 5.

The optimized SFE exhibited significant antibacterial and antifungal effectiveness against the examined microorganisms. For *Escherichia coli* and *Staphylococcus aureus*, the inhibition zones were 10.09 ± 0.18 mm and 9.58 ± 0.62 mm, respectively, at an extract concentration of 120 mg/mL. The MIC values for both bacteria were 15 mg/mL, while the MBC values were 30 mg/mL (Table 9). In the case of *Candida albicans*, the zone of inhibition was 10.48 ± 0.04 mm at the same extract concentration, with MIC and MBC values of 30 mg/mL and

60 mg/mL, correspondingly. This current result is consistent with the findings previously reported in Khan, Islam found that most of MIC values in extract were lower than their MBC value.^[58] The observed antibacterial and antifungal activity of the optimized SFE suggests its potential as a natural antimicrobial agent. This result also aligns with previous studies demonstrating that plant extracts containing phenolic compounds and flavonoids possess significant antimicrobial properties.^[59] Such bioactive compounds have been associated with antimicrobial mechanisms involving disruption of microbial cell membranes and interference with metabolic processes.^[60]

The bioactive compounds in the optimized SFE demonstrated targeted antimicrobial activity, highlighting its potential for application in food preservation. By inhibiting both spoilage and pathogenic microorganisms, SFE shows promise as a natural preservative that could enhance food safety and extend shelf life. These findings emphasize the importance of further validation in real food systems to confirm its efficacy under practical storage and processing conditions.

Table 9: The size of the inhibition zone, along with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the optimized extract of *Sesbania* flowers.

Microorganisms	Extract concentration (mg/mL)	Inhibition zone (mm)	MIC (mg/mL)	MBC (mg/mL)
<i>Escherichia coli</i>	120	10.09 ± 0.18	15	30
<i>Staphylococcus aureus</i>	120	9.58 ± 0.62	15	30
<i>Candida albicans</i>	120	10.48 ± 0.04	30	60

Table 10: Characterization of the phenolic and chemical compositions of the optimized *Sesbania javanica* Miq. flower extract by HPLC-MS/MS-QTOF.

No.	Compound group	Compound name	t _R (min)	Chemical formula	MW (g/mol)	[M-H] ⁻	MS ² (m/z)
1	Flavonol	19-Noretiocholanolone glucuronide	13.72	C ₂₄ H ₃₆ O ₈	451.33	451	451.3302
2	Flavonol	Isorhamnetin	48.88	C ₁₉ H ₃₂ N ₄	315.26	315	315.2565
3	Hydroxycinnamic acid	Caftaric acid	58.13	C ₁₃ H ₁₂ O ₉	311.20	311	311.2038
4	Flavanone	6,2'-Dihydroxyflavanone	64.86	C ₁₅ H ₁₀ O ₄	255.24	255	255.2353
5	Chalcone	2'-Hydroxy-3,3',4'-trimethoxychalcone	65.08	C ₂₁ H ₂₂ O ₅	313.24	313	313.2407
6	Organic acid	Citric acid	0.60	C ₇ H ₆ N ₄ O ₄	191.02	191	191.0212
7	Hydroxycinnamic acid	3,4-Dimethoxycinnamic acid	1.76	C ₁₁ H ₁₂ O ₄	207.08	207	207.0890
8	Flavonol	Kaempferol-7-O-neohesperidoside	15.55	C ₂₁ H ₂₆ N ₁₀ O ₉ S	593.15	593	593.1530

3.8 Identification of phenolic profile by HPLC-MS/MS-QTOF analysis

The phenolic composition of the optimized SFE was characterized using HPLC-MS/MS-QTOF analysis. Table 10 presents the identified compounds along with their retention times (t_R), chemical formulas, molecular weights (MW), and mass spectrometry data. The analysis revealed a diverse array of bioactive compounds, including flavonols, hydroxycinnamic acids, flavanones, chalcones, and organic acids. The identification of compounds such as isorhamnetin, kaempferol-7-O-neohesperidoside, and 6,2'-dihydroxyflavanone underscore the extract's rich bioactive profile.

Flavonols, including isorhamnetin and kaempferol derivatives, are known for their antioxidant and antimicrobial activities.^[61] Hydroxycinnamic acids like caftaric acid contribute to the extract's efficacy through their ability to disrupt microbial cell walls.^[62] Flavanones and chalcones enhance the extract's antimicrobial spectrum by targeting bacterial enzymes and metabolic pathways.^[63]

Organic Acid: Citric acid, a common organic acid found in plants, was detected in the extract. Citric acid enhances the antioxidant content of foods by promoting the synthesis of nutraceutical compounds, including phenolic compounds and flavonoids, which act as antioxidants. These compounds are essential for combating oxidative stress and promoting health. Additionally, organic acids like citric acid play vital roles in metabolic processes and contribute to the overall composition and bioactivity of extracts, thereby enhancing their potential

health benefits.^[64]

The characterization of these phenolic compounds provides critical insights into the extract's antimicrobial mechanisms, supporting its potential as a natural intervention in food systems. The identification of key bioactive molecules ensures the reproducibility and reliability of the antimicrobial effects, ensuring robust validation and verification of the findings to support their practical application. Future research should focus on applying these findings within food matrices to confirm their functional benefits and scalability.^[64,65]

4. Conclusion

This study systematically evaluated the influence of ultrasound-assisted extraction (UAE) conditions on the bioactive compound yield, antioxidant capacity, and antimicrobial properties of *Sesbania javanica* flower extract (SFE). Optimal UAE conditions; 40 °C temperature, 20 minutes extraction time, and 70% ethanol concentration; were identified, yielding the highest total phenolic content (TPC) of 20.13 mg GAE/g dry basis and total flavonoid content (TFC) of 8.18 mg QE/g dry basis. The significant antioxidant activity, as determined by DPPH and FRAP assays, highlighted the role of phenolic and flavonoid compounds in the extract's bioactivity.

The thermodynamic analysis confirmed the efficiency of the extraction process, indicating a favorable balance between yield and energy consumption. Furthermore, the optimized SFE exhibited strong antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida*

albicans, supporting its potential as a natural preservative. Phenolic profile analysis via HPLC-MS/MS-QTOF identified diverse bioactive compounds, including flavonols, hydroxycinnamic acids, flavanones, chalcones, and organic acids, contributing to its functional properties. These findings underscore the potential of UAE as a sustainable method for extracting high-value bioactive compounds, suitable for applications in food preservation, safety enhancement, and as a functional ingredient in pharmaceuticals and cosmetics. Future studies should focus on validating these findings within complex food systems and scaling up the process to assess its commercial viability and broader applicability.

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Conflict of Interest

There is no conflict of interest.

Supporting Information

Not applicable.

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