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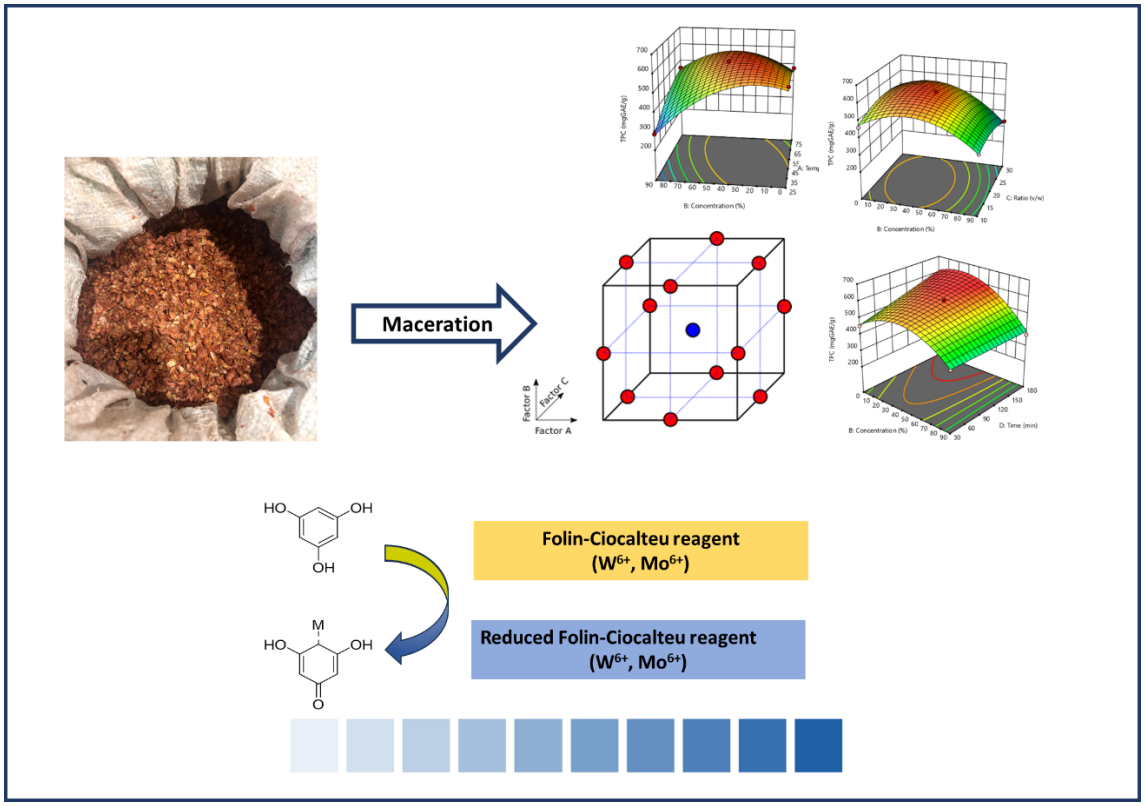
GRAPHICAL ABSTRACT



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Peanut skin, a polyphenol-rich agro-residue, constitutes a promising renewable source of natural antioxidants for food and pharmaceutical applications. In this study, response surface methodology (RSM) based on a Box–Behnken design was employed to optimize the extraction of total polyphenol content (TPC) as a function of temperature, ethanol concentration, solvent-to-solid ratio, and extraction time. The TPC was determined by the Folin–Ciocalteu method, and the developed quadratic model was statistically significant ($F = 40.74$, $R^2 = 0.98$), indicating an adequate fit. Ethanol concentration and extraction time exerted the most pronounced effects, with significant quadratic and interaction terms. The optimal extraction conditions (approximately 55 °C, 30% ethanol, 160 min) yielded extracts with high phenolic recovery and moderate antioxidant capacity (IC_{50} ranged from 120 to 150 µg/mL, DPPH assay). The results validated RSM model is a reliable tool for optimizing polyphenol recovery from peanut skin. Additionally, it also highlights the potential of peanut skin valorization as a sustainable and cost-effective strategy for producing natural antioxidants, contributing to the circular bioeconomy and industrial exploitation of agricultural residues.

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Keywords: Arachis hypogaea; Peanut skin; Total phenolic content; Optimal extracting condition; Box Behnken design; Response surface method; Quadratic model

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INTRODUCTION

Peanut (*Arachis hypogaea* L.) is one of the most widely consumed oilseed crops worldwide, serving as a naturally important source of edible oil, protein, and various nutrients (Hosseini Taheri *et al.* 2024). During the processing of peanuts for food or oil production, large quantities of byproducts, such as peanut skin, shells, and meal, are generated. Among these, peanut skin, which constitutes approximately 3 to 4% of the total kernel weight, is typically removed during blanching and discarded as low-value waste (Dean 2020). However, numerous studies have revealed that peanut skin is a valuable bioresource rich in bioactive compounds, including phenolic compounds (stilbenoids, flavonoids, condensed tannins, *etc.*), saponins, triterpenes, and indole alkaloids (Massarioli *et al.* 2022; Mingrou *et al.* 2022). Particularly, phenolic compounds were considered the major bioactive constituents with 8% by weight in peanut skin (Saenglee *et al.* 2012). This type of compound was predominantly concentrated in the peanut skin, with the total phenolic content (TPC) value in the skin being found to be 20 to 800 times higher than that

of the testa-removed kernels, depending on the genotypes (Saenglee *et al.* 2012). These phenolics are well-known for their potent antioxidant, anti-inflammatory, and antimicrobial activities, which contribute to the prevention of oxidative stress–related diseases and have potential applications in functional foods, cosmetics, and pharmaceuticals (Dean 2020). Consequently, there is a need to generate phenolic-rich fractions from peanut skin. This approach not only helps reduce agricultural waste but also leverages an abundant, inexpensive resource for producing phenolic-enriched products.

Extraction of phenolic compounds from plant matrices is a critical step influencing their recovery and functionality. Several studies have attempted to optimize extraction conditions to maximize the phenolic content in the extracts. However, the phenolic yields obtained under optimized conditions have typically remained around 500 mgGAE/g extract (Massarioli *et al.* 2022; de Matos *et al.* 2024). In addition, many of these methods involve advanced equipment or complex pretreatments, which may restrict their scalability or industrial adoption.

Response surface methodology (RSM) has been widely recognized as a useful statistical tool for designing experiments, modeling responses, and optimizing multivariable processes (Thomareis and Dimitreli 2022). It has been successfully applied to optimize extraction conditions for phenolic compounds from various plant materials and agricultural by-products (Ha *et al.* 2024; Kua *et al*. 2015; Sai-Ut *et al.* 2024; Xu *et al.* 2012). Although a few studies have systematically modeled solvent and thermal interactions in peanut skin extraction using the Box-Behnken design, the combined effects of solvent concentration, extraction temperature, and liquid-to-solid ratio during conventional maceration have not been fully investigated. Therefore, the present study applied the Box–Behnken design (BBD) coupled with RSM to optimize the maceration-based extraction of phenolics from *A. hypogaea*'s skin. The novelty of this work lies in employing a simple, scalable extraction method while systematically modeling the interactive effects of process variables to maximize phenolic recovery from this underutilized peanut by-product.

EXPERIMENTAL

Source of Material

Arachis hypogaea L.'s skin was collected in November 2024 by directed peeling at manufacturing facilities of peanut-related products throughout the Thai Binh province, Vietnam. Collected samples were preserved at -4 °C in sealed plastic bags and kept in the dark until analysis.

Analytical Methods

The maceration technique with different extracting conditions for temperature, solvent, time, and liquid/solid ratio was used to prepare phenolic-rich extracts from *A. hypogaea*'s skin. The solvent used in this study was a mixture of ethanol and water at different ratios. Then, the total phenolic content (TPC) of these samples, determined by the Folin–Ciocalteu method (Lee *et al.* 2004), was used to evaluate the influence of different factors on the extraction efficiency. RSM based on BBD was applied to determine the optimal extraction conditions for obtaining a phenolic-rich extract from the peanut skin. Subsequently, the optimized extract was evaluated for its antioxidant activity using the DPPH free radical scavenging assay, following the method described by Lee *et al.* (2004).

Folin-Ciocalteu methods

The total polyphenol content (TPC) of the extracts was determined using the Folin–Ciocalteu colorimetric method. Briefly, 0.2 mL of the appropriately diluted extract was mixed with 2.4 mL of 10% (*v/v*) Folin–Ciocalteu reagent and allowed to react for 5 min at room temperature. Then, 2.4 mL of 6% (*w/v*) Na_2CO_3 solution was added, and the mixture was incubated in the dark for 15 min. The absorbance was measured at 765 nm using a UV–Vis spectrophotometer. A calibration curve was constructed using gallic acid standard solutions (0 to 200 µg/mL), yielding the linear equation $y = 0.0023x - 0.0059$ ($R^2 = 0.9985$). TPC was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

DPPH radical scavenging assay

The antioxidant activity was evaluated using the DPPH free radical scavenging assay. The 10% DPPH methanolic solution was mixed with the extract, and the decrease in absorbance was measured at 517 nm after incubation in the dark for 20 min. Ascorbic acid was used as a positive control. The antioxidant activity was calculated using the following equation,

$$Antioxidant\ activity\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%. \quad (1)$$

where $A_{control}$ and A_{sample} are the absorbance of control and extract solution, respectively.

Single-factor experiments

The ranges of extraction factors, including temperature, ethanol concentration, extraction time, and liquid-solid ratio, were assessed through a series of experiments. First, the impact of temperature on the phenolic content in *A. hypogaea* skin extract was estimated by extracting dried powdered samples at temperatures ranging from 25 to 75 °C in 50% ethanol for 100 min, with a liquid-solid ratio of 20:1. The effect of ethanol concentration on phenolic content was examined by extracting *A. hypogaea* skin in ethanol at concentrations ranging from 0% to 90% for 100 min, using a liquid-to-solid ratio of 20/1 at 50 °C. Next, the influence of extraction time was evaluated by extracting the material in 50% ethanol with a liquid-to-solid ratio of 20/1 for durations between 30 and 180 min at 50 °C. Finally, the impact of the liquid-to-solid ratio on phenolic content was investigated in the range of 10/1 and 30/1 by extracting in 50% ethanol for 100 min at 50 °C. In all experiments, a fixed weight of *A. hypogaea* skin powder (2 ± 0.1g) was subjected to maceration extraction.

Response surface method

The response surface method (RSM) applying the Box-Behnken Design (BBD) was utilized to design experiments to optimize the conditions to enrich phenolic into the extract of *A. hypogaea* skin. The design was conducted on the Design-Expert 12.0 software (Stat-Ease, Inc., Minneapolis, MN, USA). Temperature (°C, A), ethanol concentration (%, B), liquid/solid ratio (mL/g, C), and extraction time (min, D) were selected as independent factors, while the total phenolic content (TPC, mgGAE/g) was selected as the response factor. The levels of the variables in the experimental design are detailed in Table 1. Based on the Box–Behnken Design (BBD), a total of 29 experiments were proposed, including five replicated center points. Detailed information on the experimental design is presented

in Table S1. Each sample was analyzed in triplicate, and the mean values were used for further analysis.

Table 1. Levels of the Variables in the Box-Behnken Design

Variables	Unit	Code levels		
		-1	0	1
Temperature (A)	°C	25	50	75
Ethanol concentration (B)	%	0	45	90
Liquid-solid ratio (C)	mL/g	10/1	20/1	30/1
Time (D)	min	30	105	180

RESULTS AND DISCUSSION

The Process Range Condition for the Extraction

The effects of extraction temperature, ethanol concentration, liquid-to-solid ratio, and extraction time on the total phenolic content (TPC) of *Arachis hypogaea* skin extracts are shown in Fig. 1. As the temperature increased, TPC increased progressively with temperature and reached the maximum value (approximately 620 mgGAE/g) at 60 to 70 °C, followed by a gradual decline at higher temperatures. The initial increase can be attributed to enhanced solvent penetration, reduced solvent viscosity, cell wall disruption, improved diffusion rates, and greater solubility of phenolics as temperature increases (Dai *et al.* 2010). However, prolonged exposure to higher temperatures (higher than 80 °C) may promote thermal degradation and oxidation of polyphenols, leading to a decrease in TPC (Spigno *et al.* 2007). Comparable trends in phenolic solubility and extraction efficiency under similar solvent–thermal conditions have also been documented in earlier studies (Ha *et al.* 2024, Kua *et al.* 2015).

Similarly, TPC increased with ethanol concentration up to the range 30 to 40%, after which it decreased, suggesting that aqueous ethanol mixtures provide the optimal polarity for phenolic solubilization (Pinelo *et al.* 2005, Sakanaka *et al.* 2008). At low ethanol concentrations, reduced solubility of less polar phenolics limits extraction, whereas at high ethanol concentrations, insufficient matrix swelling and limited hydrogen bonding reduce solvent penetration. Meanwhile, an increase in the liquid-to-solid ratio from 10:1 to 30:1 (mL/g) enhanced TPC to about 660 mg GAE/g, indicating improved concentration gradients and diffusion rates. This trend can be explained by mass transfer principles (Saien *et al.* 2019). A higher solvent-to-solid ratio increases the concentration gradient between the solid and liquid phases, enhancing the driving force for solute diffusion and promoting desorption of phenolic compounds into the solvent. However, once equilibrium is established between both phases, further increases in solvent volume have little effect on diffusivity and only dilute the extract. This is a reason why beyond this point, no significant improvement was observed, suggesting that mass transfer equilibrium had been reached and further dilution only lowered extract concentration (Balasundram *et al.* 2006; Wang and Weller 2006). Extraction time had a similar effect: TPC increased up to 150 min and slightly decreased afterward, possibly due to oxidation or co-extraction of impurities, thus reducing apparent TPC (Mokbel and Hashinaga 2005).

Overall, these results indicated that the extraction efficiency of phenolics from *A. hypogaea* skin was dependent on process parameters, with each exhibiting an optimum

level, beyond which the yield decreased. As can be seen, the optimum conditions for maximizing TPC were found in the range of experimental factors. The results were in good agreement with earlier studies on phenolic extraction from peanut skin and related by-products (de Matos *et al.* 2024; Massarioli *et al.* 2022). Thus, the chosen ranges of temperature, time, liquid-to-solid ratio, and ethanol concentration were suitable for optimizing the extraction of phenolics from peanut skin.

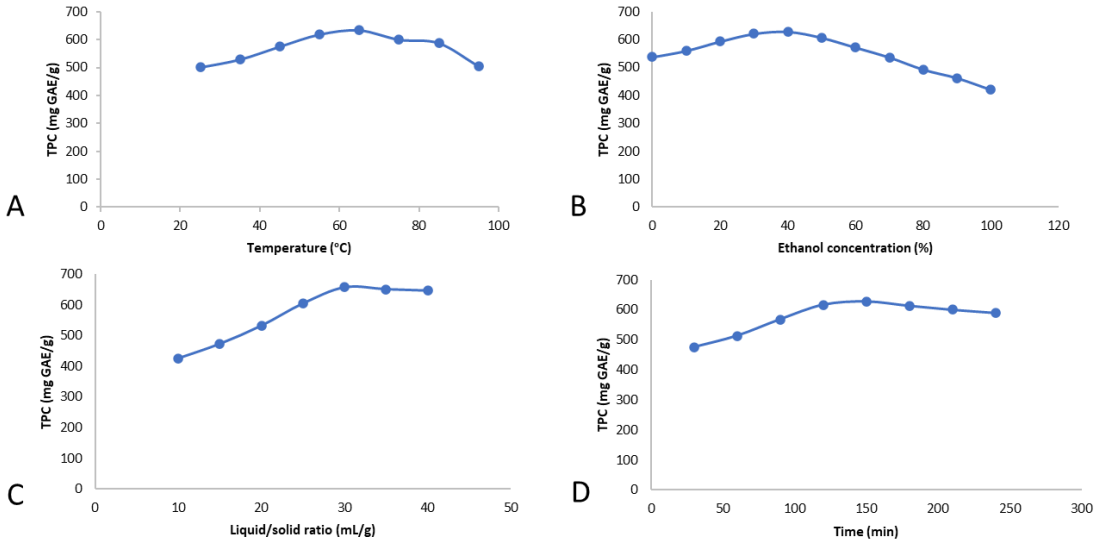

Fig. 1. Effect of independent factors on TPC of extract from *A. hypogaea*'s skin

Optimize the Extraction Conditions to Prepare the Phenolic-rich Extract from *Arachis hypogaea*'s Skin

The RSM based on the BBD design was employed to identify the optimal conditions for producing a phenolic-rich extract from the skin of *A. hypogaea*. The effects of the extraction variables on the phenolic content are presented in Table S1 in the Appendix.

Among the suggested models (linear, two-factor interaction (2FI), quadratic, and cubic), the quadratic model was identified as the most appropriate. It exhibited a very small sequential *p*-value (< 0.0001) and a high F value (F = 40.74), indicating high statistical significance. In addition, the lack-of-fit *p*-value of 0.240 (> 0.05) confirmed the absence of systematic error and thus ensured model adequacy. Moreover, the adjusted R^2 (0.952) and predicted R^2 (0.878) were both very high and close in value, demonstrating that the quadratic model not only fit the experimental data well but also possessed strong predictive capability.

ANOVA results of the chosen model have been detailed in Table S2 in the Appendix A section. Among the main factors, solvent concentration (B) exerted the strongest effect (F = 152.74, p < 0.0001), followed by extraction time (D) and temperature (A), while ratio (C) showed no significant influence with a *p*-value higher than 0.05 Moreover, several interaction terms (AB, AD, BC, and BD) were significant, highlighting the importance of combined effects, whereas the effect of CD on the response factor was negligible. For quadratic effects, B^2 contributed most strongly (F = 249.08, $p < 0.0001$), supported by significant curvature from A^2 and C^2, whereas D^2 was not significant with a *p*-value higher than 0.05 ($p = 0.9199$). Therefore, the interaction effect of CD and D^2 on TPC value can be considered negligible and may be excluded from the calculation process.

These findings indicate that TPC recovery was primarily governed by solvent concentration, with time and temperature as secondary contributors, while the liquid-to-solid ratio would be ignored in research. Moreover, nonlinear responses and factor interactions – particularly between temperature–concentration (AB) and concentration-time (BD) – play a critical role, suggesting that optimal polyphenol extraction requires careful balancing of multiple parameters rather than relying on single factors alone.

The ANOVA results were in good agreement with the extraction mechanism of polyphenols. Solvent concentration emerged as the most influential factor, as polarity governs the solubility and release of phenolic compounds, with a clear optimal level reflected by the strong quadratic effect. Extraction time also contributed significantly by allowing diffusion equilibrium, although prolonged exposure may cause oxidation or degradation, resulting in a nonlinear trend. Temperature enhanced solubility and diffusivity, but it showed a weaker effect compared with concentration and time due to the risk of thermal degradation.

The significance of interaction terms (particularly AB and BD) highlights the multivariate nature of the extraction process, where the efficiency of TPC recovery depends not only on single factors but also on their optimal combination. Overall, these findings confirmed the adequacy of the quadratic model and underline the chemical rationale for optimizing extraction through the joint adjustment of key parameters.

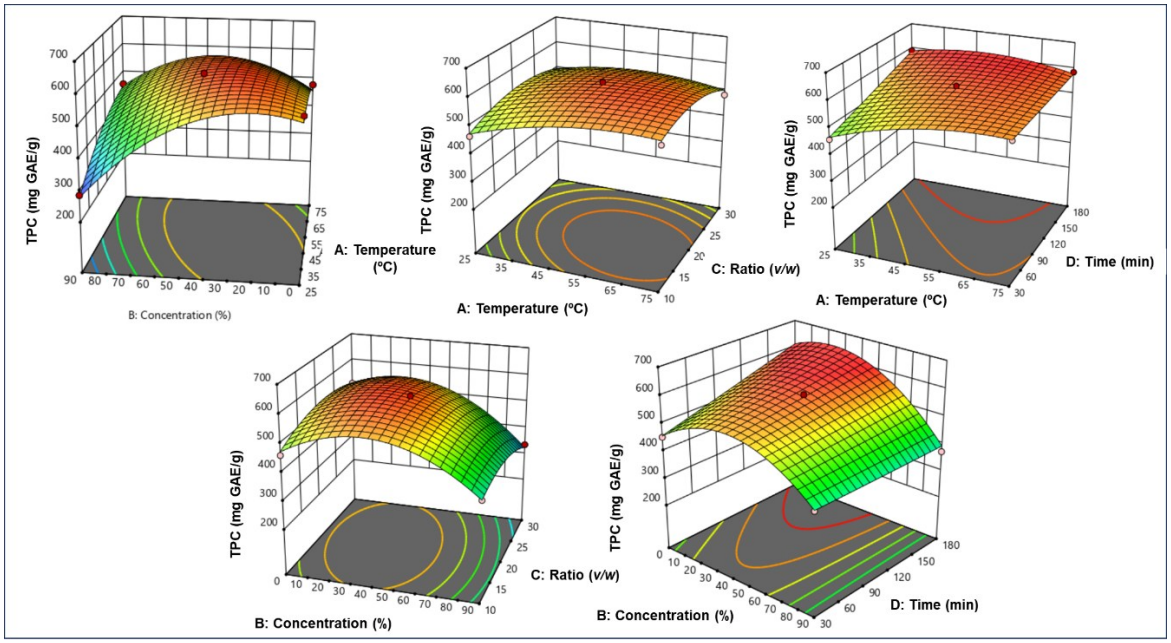

Fig. 2. The 3D plot showing the combined effects of the extraction conditions on TPC

Figure 2 visualizes the influences of factor pairs on TPC value in the form of 3D graphs. Among these factors, ethanol concentration (B) showed the strongest influence: TPC increased rapidly as concentration rose to 60 to 70%, reached a maximum, and slightly decreased at higher levels, consistent with the solubility of polyphenols in ethanol–water mixtures. Temperature (A) also had a positive effect, with TPC increasing up to 55 to 65 °C, after which a slight decline was observed, which is likely due to thermal degradation or oxidation of phenolics. Meanwhile, extraction time (D) significantly enhanced TPC, with a sharp increase between 60 and 120 min before reaching equilibrium at longer

durations, reflecting diffusion and solvent saturation. In contrast, the liquid/solid ratio (C) exhibited only a minor effect, as shown by relatively flat surfaces, indicating that once a sufficient solvent amount is provided, further increases contribute little to extraction efficiency.

Notably, the interaction plots (AB, AD, BC, and BD) revealed synergistic effects, where combining optimal ethanol concentration with appropriate temperature or extraction time further improved TPC yield compared with individual factor changes. Overall, the results suggest that optimal extraction conditions were achieved at moderate - high ethanol concentration, temperature, and extraction time, while the liquid-to-solid ratio played a less critical role

Optimal Extracting Conditions

The equation below illustrates the interaction between the dependent factor (TPC level) and the independent ones, including the concentration of ethanol (%), extraction time (min), and liquid-to-solid ratio.

TPC (mgGAE/g) = $17.4593 + 6.88905 \times A + 3.29604 \times B + 22.3922 \times C + 1.7327 \times D + 0.0566719 \times AB - 0.079216 \times AC - 0.0142335 \times AD - 0.0568832 \times BC - 0.0124172 \times BD - 0.0549718 \times A^2 - 0.0575163 \times B^2 - 0.416513 \times C^2$

A: Temperature (°C) B: Ethanol concentration (%)

C: Liquid-to-solid ratio (mL/g) D: Time (min)

This equation was also used to determine the optimal operating conditions for extracting phenolic-rich extract from *A. hypogaea* skins, aiming for the highest phenolic content while keeping all independent factors within the investigated range. Based on the computational results and proposed conditions, the optimal extraction parameters were identified and subsequently validated experimentally. Analysis using the quadratic model indicated that the liquid-to-solid ratio within the investigated range had no statistically significant effect on the phenolic content of peanut skin extracts. Consequently, all experiments under the optimized conditions were conducted at a liquid-to-solid ratio of 20:1 mL/g. The optimal extraction conditions were established at 50 to 60 °C, 25 to 40% ethanol in water, and 150 to 170 min, under which the extracts consistently exhibited TPC values exceeding 600 mg GAE/g.

In addition, the optimized extracts of peanut skin were evaluated for their antioxidant activity using the DPPH free radical scavenging assay, following the method described by Lee *et al*. (2004), with ascorbic acid serving as the positive control. The results indicated that the extracts exhibited moderate antioxidant activity, with IC_{50} values ranging from 120 to 150 µg/mL. This level of activity suggests that, although the extracts contained a considerable amount of phenolic compounds, their radical scavenging capacity was lower than that of pure ascorbic acid, which was likely due to the complex mixture of phenolics present. The presence of both highly active and less active phenolic constituents may lead to synergistic or antagonistic interactions, resulting in the observed moderate overall antioxidant effect.

Moreover, these findings suggest that *A. hypogeae* skin, a widely available agricultural by-product, could be developed as a cost-effective source of natural antioxidants for use in food, nutraceutical, and other industries. Further studies on scale-up extraction and formulation will facilitate its practical application in industrial settings

CONCLUSIONS

1. This study demonstrated that total polyphenol content (TPC) extraction from peanut skin was primarily affected by solvent concentration, extraction time, and temperature, with solvent concentration showing the strongest quadratic effect. Although the liquid-to-solid ratio had only a minor influence, significant interaction effects among variables highlight the need for multi-factor optimization. The quadratic RSM model was robust and indicated the presence of true optimal conditions rather than simple linear trends.
2. Optimal extraction at 50 to 60 °C, 25 to 40% ethanol, and 150 to 170 min produced extracts with over 60% phenolics (by mass). These optimized extracts also exhibited moderate antioxidant activity in the DPPH assay, with IC_{50} values of 120 to 150 µg/mL.
3. These findings provide a scientific basis for improving polyphenol recovery and support the valorization of peanut skin as a sustainable natural antioxidant source.

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Conflict of Interest

The authors declare no conflict of interest. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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APPENDIX

Table S1. Box Behnken Design for the Extraction of Phenolic Compounds from *A. hypogaea* Skin

Run	Temperature (ºC)	Ethanol concentration (%)	Liquid-to-solid ratio (mL/g)	Time (min)	TPC (mg GAE/g)	
					Actual	Predicted
1	75	0	20	105	478.15 ± 35.83	463.31
2	50	48	30	180	614.89 ± 60.06	595.00
3	75	48	10	105	511.49 ± 43.89	510.60
4	75	96	20	105	457.58 ± 46.79	458.20
5	50	96	30	105	335.66 ± 35.58	330.42
6	50	48	20	105	579.46 ± 39.01	588.72
7	50	0	20	30	382.73 ± 31.66	384.54
8	25	48	20	180	590.08 ± 55.02	598.38
9	25	48	30	105	435.63 ± 28.76	428.33
10	25	48	20	30	457.90 ± 46.79	452.65
11	75	48	20	30	560.27 ± 43.97	560.86
12	50	0	30	105	508.68 ± 32.88	499.24
13	25	96	20	105	306.71 ± 24.53	300.87
14	25	0	20	105	562.3 ± 41.98	511.00
15	50	48	20	105	559.74 ± 50.97	588.72
16	50	96	10	105	390.75 ± 38.68	419.06
17	25	48	10	105	664.75 ± 42.15	656.17
18	50	48	20	105	589.98 ± 64.02	588.72
19	50	48	10	180	605.77 ± 53.05	593.61
20	50	48	10	30	530.88 ± 58.92	530.10
21	50	48	20	105	593.18 ± 56.03	588.72
22	50	96	20	30	344.61 ± 23.59	346.74
23	50	0	10	105	461.38 ± 31.80	465.48
24	50	0	20	180	527.03 ± 36.91	546.71
25	75	48	30	105	683.16 ± 48.18	683.55
26	50	48	30	30	502.34 ± 34.87	473.82
27	75	48	20	180	585.71 ± 46.01	599.83
28	50	96	20	180	371.27 ± 34.64	369.27
29	50	48	20	105	591.24 ± 51.02	588.72

Table S2. ANOVA Results for the Reduced Quadratic Model

Source	Sum of Squares	df	Mean Square	F-value	*p*-value	
Model	2.010E+05	12	16748.57	54.11	< 0.0001	significant
A-Temperature	5589.21	1	5589.21	18.06	0.0006	
B-Concentration	53826.12	1	53826.12	173.91	< 0.0001	
C-Ratio (v/w)	746.81	1	746.81	2.41	0.1399	
D-Time	14423.00	1	14423.00	46.60	< 0.0001	
AB	16259.25	1	16259.25	52.53	< 0.0001	
AC	1568.79	1	1568.79	5.07	0.0388	
AD	2848.96	1	2848.96	9.20	0.0079	
BC	2620.92	1	2620.92	8.47	0.0102	
BD	7025.18	1	7025.18	22.70	0.0002	
A^2	7941.07	1	7941.07	25.66	0.0001	
B^2	91258.16	1	91258.16	294.85	< 0.0001	
C^2	11670.70	1	11670.70	37.71	< 0.0001	
Residual	4952.09	16	309.51			
Lack of Fit	4178.50	12	348.21	1.80	0.3008	not significant
Pure Error	773.59	4	193.40			
Cor Total	2.059E+05	28				