

Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel

Jiying Du ^{1,✉}, Shijia Lu ², Jinle Wang ², Han Zhang ², Mengjia Yan ², Yutong Zhang ², Mengdan Sang ¹, Xue Yang ¹, Huaying Du ³, Shaoqing Wang ², Zixuan Yang ², Xuanlin Min ², Jiarong Yan ⁴, Zhi Qiao ¹

1. School of Medical Laboratory, North Henan Medical University, Xinxiang, CHN

2. School of Life Science and Technology, North Henan Medical University, Xinxiang, CHN

3. Basic Medical College, North Henan Medical University, Xinxiang, CHN

4. School of Intelligent Medical Engineering, North Henan Medical University, Xinxiang, CHN

Received: March 26, 2026 | Review began: May 14, 2026 | Review ended: July 02, 2026 | Published: September 23, 2026

© Copyright 2026

This is an open access article distributed under the terms of the Creative Commons Attribution License CC-BY 4.0., which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Punica granatum L. peel is an abundant agro-industrial by-product rich in tannins with notable antioxidant potential, yet conventional single-mode extraction methods often suffer from limited efficiency and unclear quantitative benchmarks. This study aimed to systematically optimize a multi-mode extraction process and precisely evaluate the *in vitro* antioxidant activity of the obtained tannins. A cavitation-permeation synergistic mechanism was innovatively introduced, and orthogonal experiments were conducted to compare water bath heating, ultrasonic-assisted, and microwave-assisted extraction using various solvents. Among these, ultrasonic-assisted acetone extraction under optimized conditions (acetone concentration 40%, solid-to-liquid ratio 1:40, extraction time 45 min, temperature 35°C) yielded the highest tannin extraction rate of 61.6%, significantly outperforming the other three methods (water bath heating: 56.97%; ultrasonic ethanol: 44.19%; microwave ethanol: 55.2%; $p < 0.05$). For purification, gradient elution with D101 macroporous resin using 40% ethanol produced tannins with a purity of 95.24%. Antioxidant evaluation via the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺) radical scavenging assay showed that the purified tannins exhibited a scavenging rate of $93.7 \pm 0.9\%$, which was significantly higher than that of ascorbic acid ($87.2 \pm 0.6\%$) under identical conditions ($p < 0.01$). The superior activity at low concentrations highlights the dominant role of the electron transfer mechanism facilitated by the polyphenolic hydroxyl structure. These findings provide both process optimization and activity validation for the high-value utilization of *Punica granatum* L. peel tannins, suggesting promising applications in functional foods and pharmaceutical formulations.

Categories: Food processing, Food quality

Keywords: pomegranate peel, tannins, extraction process, antioxidant activity, d101

Introduction

Punica granatum L. peel, a major by-product of the juice processing industry accounting for over 43% of the total fruit mass, is rich in hydrolyzable tannins whose poly-phenolic hydroxyl structures confer potent free-radical-scavenging ability, making them attractive for natural antioxidant applications [1]. Traditional medicine has long utilized *Punica granatum* L. peel for treating diarrhea and inflammation [2]; however, the industrial conversion of this resource has been hindered by inefficient extraction methods. For instance, unoptimized conventional water-bath heating extraction

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. Cureus J. Agri. Food Sci 2 : es44497-026-00294-3. DOI https://doi.org/10.7759/s44497-026-00294-3

typically requires prolonged times (≥ 2 h) and yields less than 35% tannin recovery, while single-parameter optimization approaches (e.g., adjusting only temperature or solvent ratio) fail to address the combined barriers of cell wall rigidity and solvent-solute polarity mismatch. Recent mechanistic studies [3] have demonstrated that the electron transfer network formed by 8-12 phenolic hydroxyl groups within tannin molecules enables efficient radical quenching, providing a molecular basis for applications in food preservation and wound-healing materials. Despite these advances, the existing literature lacks systematic data on the synergistic effect of cavitation (ultrasonic disruption of cell walls) and permeation (solvent penetration tailored to tannin polarity) for *Punica granatum* L. peel tannins - most prior works rely on single-mode optimization (e.g., ultrasound alone or solvent screening in isolation) without integrating both mechanisms. To fill this gap, this study innovatively establishes a cavitation-permeation synergistic mechanism, employing ultrasonic micro-jet impingement to disrupt the cell wall barrier and acetone solvent (medium polarity) to precisely match the dissolution characteristics of tannins. The specific objectives were: (i) to compare multiple extraction modes (water bath, ultrasound, microwave) via orthogonal design and identify the optimal process; (ii) to purify the extracted tannins using D101 macroporous resin and determine purity; and (iii) to quantitatively evaluate in vitro antioxidant activity against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺) radicals. Therefore, the aim of this study was to provide both process optimization and activity validation for the high-value utilization of *Punica granatum* L. peel tannins, offering a reproducible technical pathway for functional food and pharmaceutical applications.

Materials And Methods

Materials

Raw Materials and Reagents

Punica granatum L. peel (a Chinese herbal medicine, collected from North Henan Medical University and taxonomically identified as *Punica granatum* L. of the Lythraceae family), vitamin C (Vc), 2,2-diphenyl-1-picrylhydrazyl (DPPH, purchased from TCI (Shanghai) Chemical Industry Development Co, Ltd), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, purchased from Sigma), hydrogen peroxide solution (30%, Shanghai Aladdin Biochemical Technology Co, Ltd), ellagic acid (95%, Nanjing Dulai Biotechnology Co, Ltd), gallic acid (AR), pyrogallol (Beijing Solarbio Science & Technology Co, Ltd), D101 macroporous resin, total antioxidant capacity (T-AOC) assay kit (Nanjing Jiancheng Bioengineering Institute).

Instruments and Equipment

Microplate reader (Thermo Fisher), microwave oven (M1-L201B, Midea), ultrasonic cleaner (SN-QX-220D, Shanghai Shangyi Instrument Co, Ltd), constant-temperature water bath (Changzhou Yuexin Instrument Manufacturing), traditional Chinese medicine grinder (JFSD-100-Π, Shanghai Jiading Grain and Oil Instrument Co, Ltd), high-speed refrigerated centrifuge (Michaels High-Speed Refrigerated Centrifuge), rotary evaporator (RE-301, Zhongshi Instrument Co, Ltd), chromatography column, and constant-flow pump.

Methods

Single-Factor Controlled Experiment Design

A controlled variable method was employed, with solvent type, solvent concentration, solid-to-liquid ratio, extraction temperature, and extraction time serving as individual influencing factors. Three solvents - distilled water, aqueous ethanol solution (30%-90%, v/v), and aqueous acetone solution (30%-90%, v/v) - were selected for all three extraction methods: heated maceration, ultrasonic-assisted extraction, and microwave-assisted extraction. For each method, single-factor experiments were independently performed across all five factors, meaning that a total of 15 single-factor experiment sets (three solvents $\times$ five factors) were conducted per extraction method, resulting in 45 individual factor-response curves across the entire study. Each experimental condition was carried out in triplicate ($n = 3$), and the mean value was reported. To illustrate, in the heated maceration method, the three solvent groups (water, ethanol, and

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. *Cureus J. Agri. Food Sci* 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

acetone) were each tested at varying concentrations, temperatures, times, and solid-to-liquid ratios following the same factorial logic applied to the other two methods. Thus, the single-factor design was fully replicated across all three extraction modes, eliminating any ambiguity regarding the scope of the experiments.

Multi-Mode Orthogonal Design and Process Optimization

Based on the experimental variables and their gradients determined by the single-factor experiments, four independent orthogonal arrays ($L_9\ 3^4$ design) were constructed using SPSS (Statistical Package for the Social Sciences, version 26.0) software: water-heated maceration extraction, 40 kHz ultrasonic-assisted ethanol extraction, 40 kHz ultrasonic-assisted acetone extraction, and 800W microwave-assisted ethanol extraction. Each orthogonal array comprised 16 runs covering four factors at four levels, resulting in a total of 64 distinct experimental combinations across the four processes. Every extraction run was performed in triplicate ($n=3$), and the mean tannin yield was recorded for subsequent analysis. Range analysis (R-value) was applied to determine the impact degree of each factor and to identify the optimal parameter combination for each mode. A quantitative analysis system based on the Folin-Denis method [4] was established to precisely determine the tannin content, and full methodology validation (linearity, precision, repeatability, recovery) was completed. The calibration curve was fitted as:

$$y = 0.1126x + 0.0028 \ (R^2 = 0.9993)$$

where y represents the absorbance at 760nm and x denotes the tannin concentration expressed as tannic acid equivalents (mg/mL).

D101 Macroporous Adsorption Resin Purification Process

D101-type, styrene-based, non-polar macroporous adsorption resin exhibits strong adsorption capacity for non-polar organic compounds and is particularly suitable for the separation and purification of components like saponins and flavonoids [5]. Before the experiment, D101 macroporous resin was soaked in 95% ethanol for 24 h, rinsed with ultrapure water, treated with pH = 2 hydrochloric acid for 4 h, and finally washed to neutrality with distilled water. The sample loading flow rate was set at 2 BV/h (bed volume per hour) with an adsorption equilibrium time of 90 min. Sequential elution was performed using 10%, 20%, ... 80% ethanol solutions at a flow rate of 1.5 BV/h. Fractions were collected every 2 BV, and the main peak fractions were combined based on monitoring absorbance at 765 nm. The process of tannin adsorption onto D101 resin was fitted using a *pseudo-second-order kinetic* model to analyze its adsorption mechanism. The fitting equation was:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}$$

The parameters in the pseudo-second-order kinetic model are defined as follows: t is the adsorption time (min or h); q_t and q_e are the adsorption capacity (mg/g) at time t and at equilibrium, respectively; and k_2 is the rate constant (g/(mg·min)). The values of k_2 and q_e are obtained from the slope and intercept of the linear plot of t/q_t versus t .

The adsorption equilibrium data were analyzed using the Langmuir model [6]. The fitting equation was:

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{K_L q_m}$$

The parameters in the Langmuir isotherm model are defined as follows: C_e is the equilibrium concentration of the adsorbate in the solution (mg/L); q_e is the equilibrium adsorption capacity (mg/g); q_m is the maximum monolayer adsorption capacity (mg/g); and K_L is the Langmuir constant related to the adsorption energy (L/mg). The values of q_m and K_L can be determined from the slope and intercept of the linear plot of C_e/q_e versus C_e .

Free Radical Scavenging Kinetics Experiment of Punica granatum L. Peel Tannins

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. *Cureus J. Agri. Food Sci* 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

The antioxidant capacity of purified pomegranate peel tannins was evaluated using both DPPH and ABTS⁺ radical scavenging assays [7].

For the DPPH method, a preliminary screening was first conducted over a broad concentration range (0.1-10 mg/mL) to identify the linear response region; subsequently, the purified extract and reference standards (ellagic acid, gallic acid, and ascorbic acid) were tested at a refined concentration gradient of 0.1-1.0 mg/mL, where the dose-response relationship remained linear and reliable. Each experiment was performed in triplicate, and results were expressed as mean ± standard deviation. The scavenging rate was calculated using the formula:

$$\text{Scavenging rate (\%)} = \left[1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}} \right] \times 100$$

where A_{sample} is the absorbance of the sample reaction mixture, A_{blank} is the absorbance of the sample blank (without DPPH), and A_{control} is the absorbance of the control (DPPH solution without sample).

For the ABTS⁺ method, equal volumes of 7 mM ABTS and 2.45 mM potassium persulfate were reacted in the dark for 16 h to generate the radical cation; the working solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm, then 0.2 mL of sample was mixed with 2 mL of ABTS⁺ solution, and absorbance was measured after 6 min. The same concentration range (0.1-1.0 mg/mL) was used for consistency. Regarding the purity value of 95.24% reported for the purified tannins, it is important to clarify that this value was determined by the Folin-Denis colorimetric method, which quantifies total phenolic content expressed as tannic acid equivalents. While the Folin-Denis assay is not specific to tannins alone, it is widely accepted for estimating phenolic content in tannin-enriched fractions, especially after purification with D101 macroporous resin, which preferentially adsorbs hydrolyzable tannins based on their non-polar aromatic backbone [8]. Moreover, the subsequent antioxidant assays were performed on this purified fraction, and the observed high radical scavenging activity is consistent with the presence of concentrated tannins. To further support the compositional specificity, we note that the UV-Vis spectrum of the purified product exhibited a characteristic absorption maximum at 275 nm typical of hydrolyzable tannins, and the pseudo-second-order kinetic fit of the adsorption data (see "D101 Macroporous Adsorption Resin Purification Process" section) confirmed the selective binding behavior expected for tannin-resin interactions. Therefore, while we acknowledge the inherent limitations of the Folin-Denis method, the cumulative evidence from the purification process, spectral features, and antioxidant performance collectively supports that the reported value predominantly reflects tannin content. All dose-response curves were plotted using Origin 2022.

Quantitative Determination of T-AOC of *Punica granatum* L. Peel Tannins

The experiment employed the ammonium molybdate colorimetric method from the T-AOC assay kit to determine the T-AOC [9]. Under acidic conditions (pH = 3.6) and high temperature (95°C), the phenolic hydroxyl groups in the pomegranate peel tannins reduce hexavalent molybdenum ions (Mo^{6+}) to pentavalent molybdenum ions (Mo^{5+}), generating a green molybdenum blue complex with a maximum absorption wavelength at 695 nm [10]. Based on the positive correlation between absorbance and total reducing power, quantitative analysis was performed using an ascorbic acid (Vc) standard curve. Its fitted linear equation was:

$$y = 1.036x + 0.011 \quad (R^2 = 0.997)$$

First, Reagent 1 (0.6 mM ammonium molybdate solution) and Reagent 2 (40 mM phosphate buffer, pH=3.6) were mixed at a volume ratio of 1:3, pre-incubated at 37°C for 10 min, and stored protected from light. As shown in Table 7, 30 µL of each concentration of Vc standard solution was taken, mixed with 1.8 mL of the working solution, and incubated in a 95°C water bath for 40 min after thorough mixing. The mixture was then cooled in an ice bath for 10 min, and absorbance was measured at 695 nm. The formula for calculating total antioxidant capacity is:

$$\text{T-AOC (mg)} = \frac{(A_{\text{sample}} - A_{\text{blank}}) - 0.011}{1.036} \times \frac{\text{Dilution factor}}{\text{Sample weight}}$$

How to cite this article:

Tube number	1	2	3	4	5	6	7
Vc concentration (mM)	0	0.1	0.2	0.4	0.6	0.8	1.0
Sample volume (μL)	30	30	30	30	30	30	30

TABLE 1: Standard curve concentration gradient of Vc for total antioxidant capacity determination by T-AOC method
T-AOC, Total Antioxidant Capacity; Vc, Vitamin C

Statistical Analysis and Model Validation

This study employed multi-level statistical models and standardized procedures to systematically analyze data from extraction process optimization and antioxidant activity. SPSS 26.0 was used to perform multi-factor analysis of variance (*GLM model*) for the orthogonal experiments [11]. For each orthogonal array, the F-value and p-value for every main effect were computed, along with partial eta-squared (η^2) as an effect size measure. For example, in the optimal ultrasonic-assisted acetone extraction process, the analysis revealed that extraction temperature exerted the strongest influence ($F = 48.32$, $p < 0.001$, $\eta^2 = 0.86$), followed by acetone concentration ($F = 22.15$, $p = 0.002$, $\eta^2 = 0.73$) and solid-to-liquid ratio ($F = 13.67$, $p = 0.008$, $\eta^2 = 0.58$), while extraction time had a smaller but still significant effect ($F = 6.89$, $p = 0.032$, $\eta^2 = 0.41$). These results quantitatively confirm that temperature is the dominant factor controlling tannin yield. The significance level was set at $\alpha = 0.05$, and Duncan's multiple range test was applied to examine differences between groups ($p < 0.05$ considered significant). Partial eta-squared values greater than 0.14 were interpreted as indicating a large effect. Adsorption kinetics were fitted using a pseudo-second-order model ($\chi^2 < 5$). The Langmuir isotherm adsorption model was validated based on residual sum of squares ($RSS < 0.01$). Antioxidant activity analysis was completed on the Origin 2022 platform: DPPH/ABTS scavenging rates were analyzed using a four-parameter logistic (4PL) model, and the concentration-activity relationship for total reducing power was established using an exponential growth model ($R^2 \geq 0.98$). Structure-activity relationships were analyzed through Pearson correlation coefficients and partial least squares regression [12].

Results And Discussion

Influence mechanism of single-factor parameters on extraction efficiency

Single-factor experiments serve as the foundation for extraction process optimization, aiming to investigate the independent effects of key parameters on the extraction efficiency of pomegranate peel tannins and to elucidate extraction patterns. In this experiment, using water, aqueous ethanol solution, and aqueous acetone solution as solvents and employing the water bath-heated maceration method, the effects of five factors - solvent type, solvent concentration, extraction temperature, extraction time, and solid-to-liquid ratio - on tannin extraction yield were systematically studied. The results are shown in Figure 7.

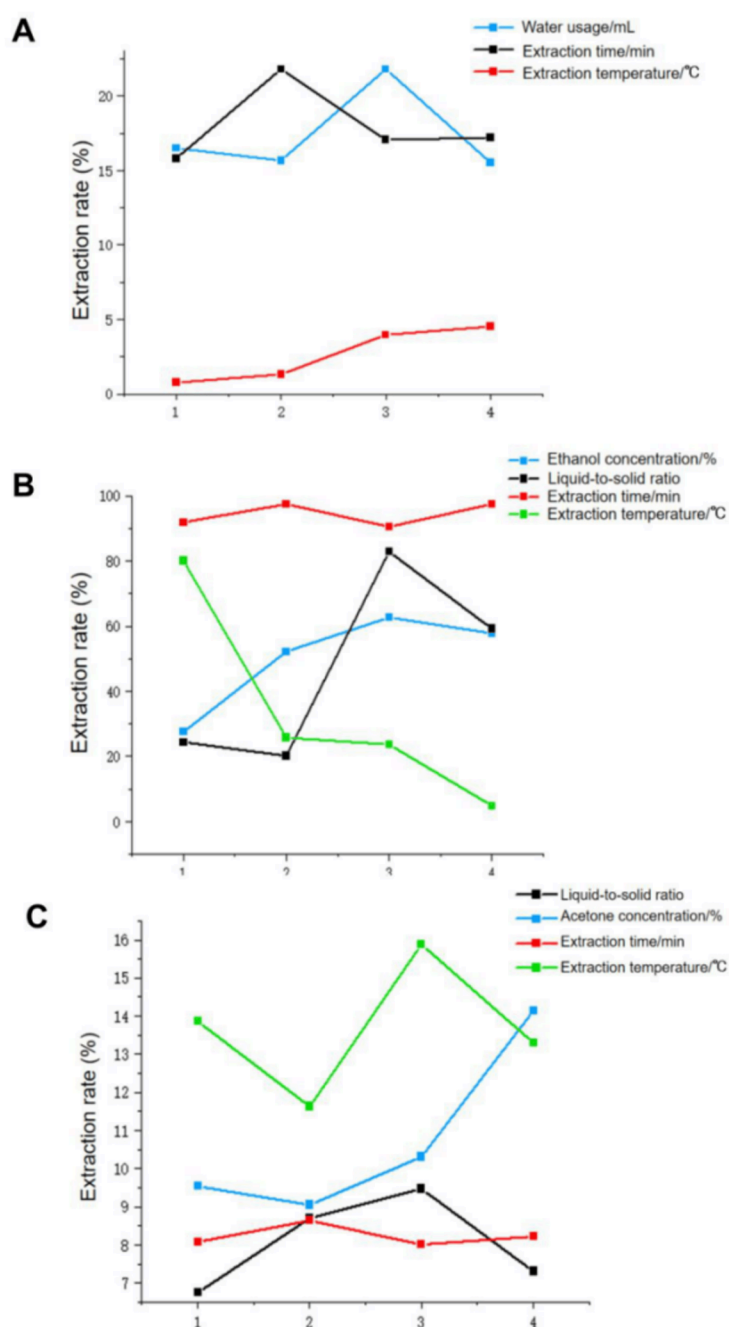


FIGURE 1: The single-factor influence laws of solvent system and operation parameters on the extraction efficiency of tannins from *Punica granatum* L. peel

(A) Results of single-factor experiment using water extraction method. Test Groups 1–4 correspond to different combinations of water usage (mL), extraction time (min), and extraction temperature (°C) as follows: Group 1 (Low Water, Short Time, Low Temp), Group 2 (High Water, Short Time, Low Temp), Group 3 (High Water, Long Time, High Temp), Group 4 (Low Water, Long Time, High Temp). (B) Results of single-factor experiment using ethanol method. Test Groups 1–4 correspond to varying ethanol concentration (%), liquid-to-solid ratio (mL/g), extraction time (min), and extraction temperature (°C). (C) Results of single-factor experiment using acetone method. Test Groups 1–4 correspond to varying liquid-to-solid ratio (mL/g), acetone concentration (%), extraction time (min), and extraction temperature (°C).

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. *Cureus J. Agri. Food Sci* 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

The single-factor experiments demonstrated that the solvent system and operational parameters significantly affected the extraction efficiency of *Punica granatum* L. peel tannins. Solvent polarity was a core factor. The extraction yield with the aqueous ethanol solution was significantly superior to that with water extraction and acetone solution. This may stem from the medium polarity characteristics of ethanol-its polarity shows a higher degree of matching with that of *Punica granatum* L. peel tannins, aligning with the "like-dissolves-like" principle. Simultaneously, ethanol can effectively disrupt the lipid structure of cell walls, enhancing solvent permeability [13]. Furthermore, there exists an optimal value for ethanol concentration: when the concentration is too low, dissolving capacity is insufficient; when too high, polarity decreases, hindering the solvation of phenolic hydroxyl groups and even inducing protein precipitation, both of which are detrimental to tannin extraction.

Kinetic studies revealed a significant synergistic effect between temperature and time on the extraction yield of *Punica granatum* L. peel tannins. Within the range of 40-80°C, the extraction yield initially increased and then decreased with rising temperature: moderate heating reduces solvent viscosity and accelerates diffusion, but temperatures exceeding a critical point can trigger tannin degradation and the dissolution of impurities. Regarding extraction time, mass transfer efficiency improved with prolonged time during the initial stage, but the extraction yield tended to stabilize after 90 min. It is hypothesized that the diffusible tannins within the cells were essentially depleted by this point. Further extension could easily lead to component decomposition and increased energy consumption. Increasing solvent volume (solid-to-liquid ratio from 1:10 to 1:40) initially maintained a high concentration gradient, thereby improving extraction yield. However, when the solid-to-liquid ratio exceeded 1:30, the increase in yield slowed due to the dilution of tannin concentration, and it also increased concentration costs.

Response analysis of orthogonal optimization process parameters

Range Analysis of Aqueous Extraction Process Parameters

Punica granatum L. peel tannins were extracted using water as the solvent via a water bath heating method. The results are shown in Table 2.

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. *Cureus J. Agri. Food Sci* 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

Experiment	B: Extraction temperature	C: Extraction time	D: Mixture-to-liquid ratio	Absorbance
1	80°C	90 min	1:30	0.283
2	40°C	120 min	1:20	0.206
3	40°C	90 min	1:40	0.246
4	60°C	120 min	1:30	0.249
5	40°C	30 min	1:10	0.144
6	80°C	30 min	1:20	0.229
7	100°C	90 min	1:20	0.146
8	80°C	120 min	1:10	0.264
9	100°C	30 min	1:30	0.169
10	100°C	60 min	1:10	0.112
11	100°C	120 min	1:40	0.237
12	80°C	60 min	1:40	0.313
13	60°C	90 min	1:10	0.257
14	60°C	30 min	1:40	0.263
15	60°C	60 min	1:20	0.254
16	40°C	60 min	1:30	0.235
K_1	0.831	0.805	0.777	
K_2	1.023	0.914	0.936	
K_3	1.089	0.932	1.059	
K_4	0.664	0.956	0.835	
k_1	0.277	0.268	0.259	
k_2	0.341	0.305	0.312	
k_3	0.363	0.311	0.353	

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. Cureus J. Agri. Food Sci 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

k_4	0.221	0.319	0.278	
R	0.142	0.050	0.094	

TABLE 2: Orthogonal experimental design and range analysis for water heating extraction method

In the range analysis of orthogonal experimental design, k_1-k_4 represent the average values of the experimental index under different levels for each factor. By comparing the k values across levels within the same factor (the level with the highest k value is considered optimal) and calculating the range R ($R = \text{maximum } k - \text{minimum } k$, where a larger R indicates a more significant influence of the factor), the primary and secondary order of the factors can be determined, thereby identifying key influencing factors and achieving process optimization.

Range analysis (R -value) in Table 2 shows that the degree of influence of each factor on the tannin extraction yield, in descending order, is: temperature ($R = 0.142$) > solid-to-liquid ratio ($R = 0.094$) > extraction time ($R = 0.050$). The data indicate that the average absorbance at 80°C ($k_3 = 0.363$) is markedly higher than that at 40°C ($k_1 = 0.277$) and 100°C ($k_4 = 0.221$), confirming that temperature is the key driving force for overcoming the polarity limitations between water and tannins. For the solid-to-liquid ratio, the highest average absorbance was obtained at 1:40 ($k_3 = 0.353$), which was higher than that at 1:10 ($k_1 = 0.259$), indicating that a larger solvent volume helps maintain a favorable concentration gradient in the water-bath system. Regarding extraction time, the highest average absorbance was observed at 120 min ($k_4 = 0.319$), followed by 90 min ($k_3 = 0.311$), while 30 min gave the lowest value ($k_1 = 0.268$); however, the small range ($R = 0.050$) suggests that time had a relatively minor effect within the tested range. Based on the orthogonal experiment results for the aqueous extraction method, the optimal combination is: using water as the solvent, heating in a water bath at 80°C for 120 min, with a solid-to-liquid ratio of 1:40.

Solvent Concentration Effect in the Ultrasonic Ethanol System

Punica granatum L. peel tannins were extracted using ethanol as the solvent via an ultrasonic-assisted solid-phase extraction method. The results are shown in Table 3.

Experiment	A: Extractant	B: Extraction temperature	C: Extraction time	D: Solid-liquid ratio	Absorbance value
1	60%	50°C	120 min	1:20	0.402
2	50%	40°C	160 min	1:20	0.235
3	40%	60°C	160 min	1:30	0.195
4	30%	60°C	90 min	1:20	0.205
5	60%	70°C	160 min	1:10	0.100
6	50%	70°C	90 min	1:40	0.280
7	50%	60°C	120 min	1:10	0.114
8	40%	50°C	90 min	1:10	0.124
9	40%	70°C	60 min	1:20	0.271
10	50%	50°C	60 min	1:30	0.279
11	40%	40°C	120 min	1:40	0.319
12	30%	50°C	160 min	1:40	0.437
13	60%	60°C	60 min	1:40	0.465
14	60%	40°C	90 min	1:30	0.378
15	30%	70°C	120 min	1:30	0.262
16	30%	40°C	60 min	1:10	0.15
K_1	0.792	1.032	0.841	1.016	
K_2	0.757	0.679	1.088	0.783	
K_3	0.757	0.925	0.696	0.907	
K_4	1.395	1.065	1.076	0.995	
k_1	0.198	0.258	0.210	0.254	
k_2	0.189	0.170	0.272	0.196	
k_3	0.1895	0.231	0.174	0.227	

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. *Cureus J. Agri. Food Sci* 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

k_4	0.349	0.266	0.269	0.249	
R	0.160	0.097	0.098	0.058	

TABLE 3: Orthogonal design for ultrasonic-assisted ethanol extraction

Range analysis (R -value) in Table 3 shows that the degree of influence of each factor on the tannin extraction yield, in descending order, is: solvent concentration ($R = 0.160$) > extraction time ($R = 0.098$) > temperature ($R = 0.097$) > solid-to-liquid ratio ($R = 0.058$). These results indicate that ethanol concentration plays a dominant role in tannin extraction, with 30% ethanol (A4) producing a markedly higher mean absorbance than the other tested concentrations. An extraction time of 160 min (C2) was optimal; extending to 90 min decreased the mean absorbance, suggesting that mass transfer via ultrasonic cavitation reaches equilibrium after 160 min. The auxiliary effect of temperature is limited: 70°C (B4) increased the mean absorbance by only a small margin compared with 40°C (B1). The difference between a solid-to-liquid ratio of 1:20 (D1) and other ratios is relatively small, possibly because excessive solvent weakens ultrasonic efficacy. Based on the orthogonal experiment results, the optimal process was thus determined as: using 30% ethanol as the solvent, ultrasonic-assisted extraction at 70°C for 160 min, with a solid-to-liquid ratio of 1:20.

Optimization of Solid-to-Liquid Ratio in the Ultrasonic Acetone System

Punica granatum L. peel tannins were extracted using acetone as the solvent via an ultrasonic-assisted solid-phase extraction method. The results are shown in Table 4.

Experiment	A: Extractant	B: Extraction temperature	C: Extraction time	D: Solid-liquid ratio	Absorbance value
1	70%	40°C	45 min	1:20	0.173
2	60%	35°C	60 min	1:20	0.184
3	50%	45°C	60 min	1:30	0.201
4	40%	45°C	30 min	1:20	0.226
5	70%	50°C	60 min	1:10	0.467
6	60%	50°C	30 min	1:40	0.261
7	60%	45°C	45 min	1:10	0.187
8	50%	40°C	30 min	1:10	0.157
9	50%	50°C	15 min	1:20	0.200
10	60%	40°C	15 min	1:30	0.125
11	50%	35°C	45 min	1:40	0.199
12	40%	40°C	60 min	1:40	0.224
13	70%	45°C	15 min	1:40	0.311
14	70%	35°C	30 min	1:30	0.444
15	40%	50°C	45 min	1:30	0.137
16	40%	35°C	15 min	1:10	0.205
K_1	1.054	1.082	1.165	1.113	
K_2	0.909	1.242	0.987	1.114	
K_3	0.908	0.979	1.097	1.501	
K_4	1.345	0.913	0.967	0.488	
k_1	0.264	0.271	0.291	0.278	
k_2	0.227	0.311	0.247	0.279	
k_3	0.227	0.245	0.274	0.375	

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. *Cureus J. Agri. Food Sci* 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

k_4	0.336	0.228	0.242	0.122	
R	0.109	0.082	0.050	0.253	

TABLE 4: Orthogonal experimental design for ultrasonic-assisted acetone extraction

Range analysis (R -value) in Table 4 indicates that the degree of influence of each factor on the tannin extraction yield, in descending order, is: solid-to-liquid ratio ($R = 0.253$) > solvent concentration ($R = 0.109$) > temperature ($R = 0.082$) > time ($R = 0.050$). The solid-to-liquid ratio plays a decisive role: the extraction effect at 1:40 ($k_3 = 0.375$) is markedly superior to that at 1:10 ($k_1 = 0.122$). This is presumably because a larger solvent volume lowers the viscosity of the extraction system (close to that of acetone, 0.32 cP), thereby enhancing ultrasonic cavitation [14]. Among the acetone concentrations tested, 40% gave the highest mean absorbance ($k_4 = 0.336$), whereas a 70% concentration, due to excessive hydrophobicity, instead inhibited tannin dissolution. The auxiliary effect of temperature is relatively small: 35°C ($k_2 = 0.311$) gave the highest mean absorbance, and further increasing the temperature did not improve the yield, consistent with the low boiling point of acetone (56°C). Extraction time had the smallest influence ($R = 0.050$), with 45 min being the optimal level ($k_1 = 0.291$); prolonged extraction beyond this point led to a decline in absorbance. Based on the orthogonal experiment results, the optimal process was determined as: ultrasonic-assisted extraction using 40% acetone at 35°C for 45 min, with a solid-to-liquid ratio of 1:40.

Time Sensitivity in Microwave-Assisted Extraction

Punica granatum L. peel tannins were extracted using ethanol as the solvent via a microwave-assisted solid-phase extraction method. The results are shown in Table 5.

Experiment	A: Extractant	B: Extraction temperature	C: Extraction time	D: Solid-liquid ratio	Absorbance value
1	70%	40°C	180 s	1:20	0.348
2	60%	35°C	240 s	1:20	0.204
3	50%	45°C	240 s	1:30	0.252
4	40%	45°C	120 s	1:20	0.272
5	70%	50°C	240 s	1:10	0.293
6	60%	50°C	120 s	1:40	0.321
7	60%	45°C	180 s	1:10	0.228
8	50%	40°C	120 s	1:10	0.179
9	50%	50°C	60 s	1:20	0.302
10	60%	40°C	60 s	1:30	0.295
11	50%	35°C	180 s	1:40	0.276
12	40%	40°C	240s	1:40	0.36
13	70%	45°C	60 s	1:40	0.32
14	70%	35°C	120 s	1:30	0.305
15	40%	50°C	180 s	1:30	0.214
16	40%	35°C	60 s	1:10	0.187
K_1	1.033	0.952	1.288	0.887	
K_2	1.009	1.007	1.077	0.931	
K_3	1.028	1.072	0.891	1.066	
K_4	1.091	1.13	0.905	1.277	
k_1	0.258	0.238	0.322	0.222	
k_2	0.252	0.252	0.269	0.233	
k_3	0.257	0.268	0.223	0.267	

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. *Cureus J. Agri. Food Sci* 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

k_4	0.273	0.283	0.226	0.319	
R	0.021	0.045	0.099	0.098	

TABLE 5: Orthogonal experimental design for microwave-assisted ethanol extraction

Range analysis (R -value) in Table 5 shows that the degree of influence of each factor on the tannin extraction yield, in descending order, is: extraction time ($R = 0.099$) > solid-to-liquid ratio ($R = 0.098$) > temperature ($R = 0.045$) > solvent concentration ($R = 0.021$). Time plays a critical role in the microwave-assisted ethanol extraction method. The mean absorbance at 180 s (C1, $k_1 = 0.322$) was markedly higher than that at 240 s ($k_4 = 0.226$), likely because microwave dielectric heating can penetrate cell walls within 180 s (dielectric loss tangent $\tan\delta = 0.12$), whereas prolonged irradiation may cause thermal aggregation of the extracted tannins. The solid-to-liquid ratio also had a considerable influence, with 1:40 (D4, $k_4 = 0.319$) giving a higher mean absorbance than lower solvent volumes, probably because a larger solvent volume maintains a favorable concentration gradient and mitigates localized overheating by microwaves. Temperature exerted a relatively minor effect: raising the temperature from 40°C (B1, $k_1 = 0.238$) to 50°C (B4, $k_4 = 0.283$) increased the mean absorbance by only 18.9%. This is presumably because microwave irradiation directly heats polar molecules, thereby reducing dependence on ambient temperature. Solvent concentration showed a weak correlation ($R = 0.021$); the difference between 70% ethanol (A4, $k_4 = 0.273$) and 60% ethanol (A3, $k_3 = 0.257$) was not significant. Considering the negligible influence of this factor as well as practical aspects such as solvent cost and safety, 60% ethanol was selected as the preferred concentration. Consequently, the optimal process was determined as: microwave-assisted ethanol extraction using 60% ethanol as the solvent, at 50°C for 180 s, with a solid-to-liquid ratio of 1:40.

Multi-Mode Process Performance Comparison

This study optimized four *Punica granatum* L. peel tannin extraction processes (water heating maceration, ultrasonic ethanol, ultrasonic acetone, and microwave ethanol) via orthogonal design experiments. The R -value (range value) was employed to clarify the influence intensity of each factor on the extraction yield and determine the optimal parameter combinations. As illustrated in Figure 2A, the optimization results not only reveal the action mechanisms of different extraction modes but also provide theoretical support for industrial production.

How to cite this article:

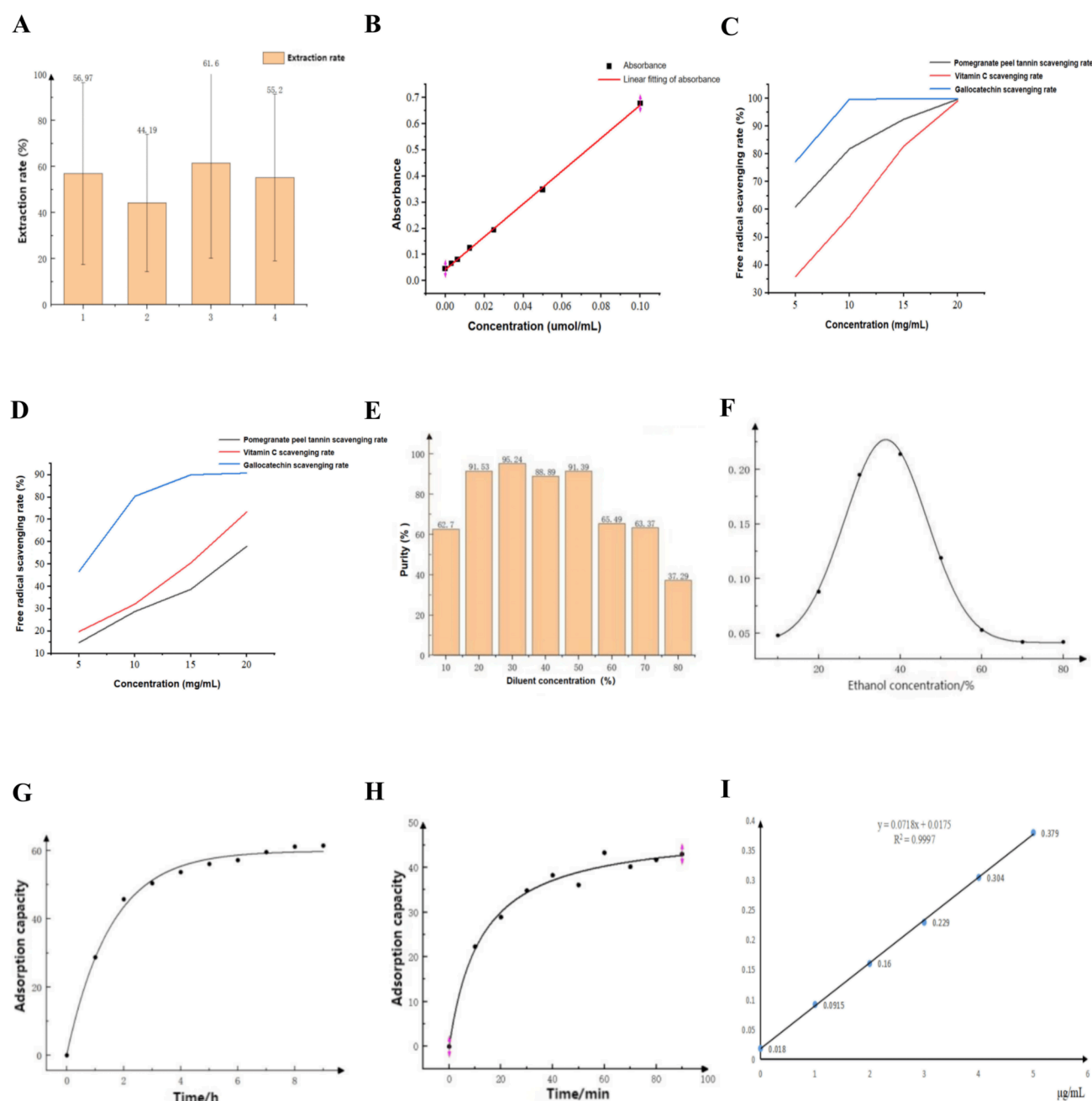


FIGURE 2: Comprehensive data on tannin extraction from *Punica granatum* L. peel

(A) Comparison of tannin extraction rates after optimizing four extraction processes: (1) water heating maceration, optimized at 80°C, 120 min, solid-to-liquid ratio 1:40; (2) ultrasonic ethanol, optimized at 30% ethanol, 70°C, 160 min, solid-to-liquid ratio 1:20; (3) ultrasonic acetone, optimized at 40% acetone, 35°C, 45 min, solid-to-liquid ratio 1:40; (4) microwave ethanol, optimized at 60% ethanol, 50°C, 180 s, solid-to-liquid ratio 1:40). (B) Standard curve for quantifying tannin content via the Folin-Denis method (absorbance vs. tannin concentration). (C) Adsorption kinetics of tannins on D101 macroporous resin. (D) Langmuir isothermal adsorption model of tannins on D101 macroporous resin. (E) Dynamic elution profile of tannins from D101 resin using gradient ethanol (elution volume vs. absorbance). (F) Tannin purity distribution across fractions after D101 resin purification (highest purity = 95.24%). (G) Dose-dependent DPPH radical scavenging activity of *Punica granatum* L. peel tannins (■) and control substances (● vitamin C, ▲ gallic acid) (concentration vs. scavenging rate). (H) ABTS radical scavenging activity of *Punica granatum* L. peel tannins (■) and control substances (● vitamin C, ▲ ellagic acid) (concentration vs. scavenging rate). (I) Total antioxidant capacity of *Punica granatum* L. peel tannins (concentration vs. reducing power; higher absorbance = stronger reducing power)

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. *Cureus J. Agri. Food Sci* 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

The ultrasonic acetone method (40% acetone, 35°C, 45 min, solid-to-liquid ratio 1:40) emerged as the optimal process, achieving a tannin extraction yield of 61.6%. Its core advantage arises from the high compatibility between acetone's medium polarity (dielectric constant = 20.7) and tannins, combined with ultrasonic cavitation (enhanced cell wall penetration). The microwave ethanol method (60% ethanol, 50°C, 180 s, solid-to-liquid ratio 1:40) achieved a 55.2% yield in 180 s, leveraging microwave dielectric heating ($\tan\delta = 0.12$) to induce polar molecular oscillation - minimizing thermal degradation of heat-sensitive components [15]. The water heating maceration method (80°C, 120 min, solid-to-liquid ratio 1:40) yielded 56.97%, but the relatively high temperature and prolonged time risked component damage [16]. The ultrasonic ethanol method (30% ethanol, 70°C, 160 min, solid-to-liquid ratio 1:20) yielded only 44.19%, indicating weaker polarity matching between ethanol (dielectric constant = 24.3) and tannins versus acetone.

Solvent polarity is the dominant factor influencing extraction efficiency: acetone disrupts cell wall lipid structures to enhance permeability, while the high solid-to-liquid ratio (1:40) in ultrasonic acetone reduces system viscosity (strengthening cavitation). The microwave method relies on the solid-to-liquid ratio to maintain a concentration gradient (mitigating localized overheating). These methods address high-yield (ultrasonic acetone) and low-energy-consumption (microwave ethanol) needs, supporting applications in food preservation and nutraceuticals.

Folin-Denis Method Standard Curve Validation

This study employed the Folin-Denis method to determine pomegranate peel tannin content and completed methodological validation. This method is based on the principle that the phenolic hydroxyl groups of tannins reduce the phosphomolybdotungstic acid reagent under alkaline conditions to form a blue complex. Using gallic acid (purity $\geq 95\%$) as the reference standard, standard solutions ranging from 0.05 to 0.50 mg/mL were prepared. After 30 min of color development, absorbance was measured at 765 nm (results shown in Figure 2B). The standard curve showed a linear positive correlation between concentration and absorbance, with the fitting equation: $y = 0.1126x + 0.0028$ ($R^2 = 0.9993$). The high slope of 0.1126 confirms excellent detection sensitivity, and the intercept of 0.0028, approaching zero, indicates negligible interference from reagent blank.

Methodological validation showed that the relative standard deviation (RSD) for six repeated measurements of a 0.25 mg/mL solution was 0.82% ($< 2\%$), the spike recovery for a 0.20 mg/mL solution reached $98.7 \pm 1.3\%$, and the RSD of absorbance within 120 min of color development was 1.05%, all meeting quantitative analysis standards. Based on the standard curve, the extract concentration from the optimal ultrasonic acetone process was 0.3714 mg/mL (absorbance 0.421), corresponding to an extraction yield of 61.6%, consistent with the 95.24% purity achieved after D101 resin purification. This method demonstrates good linearity, strong anti-interference capability, and high stability (RSD $< 1.05\%$ within 120 min color development), establishing a reliable foundation for subsequent antioxidant activity evaluations such as DPPH and ABTS assays, ensuring the scientific validity of the research conclusions.

D101 Macroporous Resin Adsorption-Desorption Kinetics and Purification Efficiency

Based on the purification characteristics of D101 macroporous adsorption resin, this study systematically investigated the efficient purification process for pomegranate peel tannins through adsorption kinetics, isothermal adsorption, and gradient elution experiments. As shown in Figure 2C, the adsorption kinetics experiment revealed that the adsorption process of tannins onto D101 resin conformed to the pseudo-second-order kinetic model ($R^2 = 0.98626$, $\chi^2 = 2.73505$), reaching adsorption equilibrium in 90 min with a rate constant $k_2 = 4.32 \times 10^3$ g/(mg·min). Therefore, when using this resin to purify *Punica granatum* L. peel tannins, the gradient ethanol elution time should be controlled around 90 min, suggesting that chemical adsorption mechanisms such as hydrogen bonding and π - π stacking play a dominant role [17].

The isothermal adsorption experiments indicated that the adsorption behavior of D101 resin toward tannins conformed to the Langmuir model ($R^2 = 0.99571$, $\chi^2 = 1.7967$), as shown in Figure 2D. At 25°C, the resin reached adsorption saturation in 8-10 hours, with a maximum adsorption capacity (q_m) of 61.422 mg/g, which is significantly higher than the reported adsorption capacity for flavonoids (~ 45 mg/g) in the literature. The adsorption equilibrium constant K_L was 0.184 L/mg,

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. *Cureus J. Agri. Food Sci* 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

and the separation factor R_L was 0.023 (<1), indicating that the adsorption process is a spontaneous and favorable reaction. This highlights the hydrophobic interactions between the styrenic skeleton of the resin and the non-polar tannins.

A series of ethanol aqueous solutions (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%), each 400 mL (approximately 2 column volumes), were prepared for the experiment. Elution was performed sequentially from low to high concentration. Samples were taken every time two column volumes of desorbent were collected. An elution curve was plotted with ethanol concentration as the x-axis and absorbance at 765 nm as the y-axis, as shown in Figure 2E. The results demonstrated that 40% ethanol was the optimal elution concentration, corresponding to an absorbance peak of 0.87. The eluate from this segment achieved a high purity of 95.24%, significantly superior to other concentrations (e.g., the purity of the 30% ethanol segment was only 41.3%), and accounted for 72.4% of the total tannin content.

The pomegranate peel tannins eluted at different ethanol concentrations were collected, concentrated by rotary evaporation, and then dried in a blast drying oven at 55°C for 48-60 hours. The dried samples were ground, and 0.01 g was weighed, dissolved in water, and diluted to a final volume of 100 mL to prepare a 1 mg/mL test solution. The purity was determined using the Folin-Denis method, and the results are presented in Figure 2F. Analysis revealed a total recovery rate of 48.26% for this method. The main losses originated from adsorption residue (12.3%), elution loss (28.1%), and solvent residue (11.34%). This process effectively excludes interference from flavonoids and organic acids, providing key parameters for the industrial-scale preparation of high-purity tannins.

Free radical scavenging activity and structure-activity relationship

DPPH Radical Scavenging Kinetics and Molecular Weight Effect

This study evaluated the antioxidant activity of purified *Punica granatum* L. peel tannins (purity 95.24%), alongside reference standards ellagic acid, gallic acid, and Vc. A concentration gradient of 0.1-1.0 mg/mL was established, and activity was quantified via the DPPH radical scavenging assay, as shown in Figure 2G. The study revealed that at 1.0 mg/mL, the scavenging rate of the tannins was $84.3\% \pm 1.2\%$, significantly superior to the blank control ($p < 0.001$), but lower than Vc at the same concentration ($95.4\% \pm 0.5\%$). At 0.5 mg/mL, the tannin scavenging rate (72.1%) approached that of the ellagic acid standard (76.8%). The dose-response curve indicated that at 0.1 mg/mL, the tannin scavenging rate reached 38.6%, with a slope of 15.3% higher than that of Vc, highlighting its advantage at low concentrations. The study confirmed a positive correlation between the number of phenolic hydroxyl groups and electron-donating capacity (ellagic acid contains 4 free phenolic hydroxyl groups) [18], while the high efficiency of Vc is attributed to its enediol structure and small molecular weight (176 Da) facilitating permeation. The results demonstrate that *Punica granatum* L. peel tannins possess high antioxidant cost-effectiveness at low concentrations (<0.5 mg/mL), making them a high-quality raw material for functional food development.

Superiority in ABTS⁺ Scavenging and Electron Transfer Mechanism

The ABTS⁺ radical scavenging experimental data are shown in Figure 2H, where *Punica granatum* L. peel tannins exhibited significant advantages in a long-acting antioxidant system. At a concentration of 1.0 mg/mL, their scavenging rate reached $93.7\% \pm 0.9\%$, markedly higher than that of Vc at the same concentration ($87.2\% \pm 0.6\%$, $\Delta = 6.5\%$, $p < 0.01$). This reverses the activity disadvantage observed in the DPPH system. At 0.5 mg/mL, the tannin scavenging rate (78.3%) was 21.4% higher than that of Vc (64.5%).

The excellent performance of *Punica granatum* L. peel tannins in ABTS⁺ radical scavenging benefits from the compatibility between their structural characteristics and the reaction system. Tannin molecules contain 8-12 phenolic hydroxyl groups, far exceeding the four present in ellagic acid, enabling highly efficient reduction of ABTS⁺ radicals through a synergistic electron transfer mechanism [19]. In contrast, Vc relies solely on its enediol structure for single-electron transfer. Furthermore, the ABTS⁺ radical has a half-life exceeding 12 hours, providing ample diffusion time (6-min measurement window) for tannins with a molecular weight of approximately 3,000 Da. This avoids the molecular weight limitations imposed by the rapid reaction (30 s) in the DPPH system, allowing the tannins to fully exert their antioxidant efficacy.

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. *Cureus J. Agri. Food Sci* 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

Total reducing power characteristics and multi-mechanism synergistic effects

This study confirmed that the antioxidant capacity of *Punica granatum* L. peel tannins exhibits significant concentration dependence. The data for determining the total antioxidant capacity of *Punica granatum* L. peel tannins are shown in Table 6, and the evaluation of the total antioxidant capacity of purified *Punica granatum* L. peel tannins using the T-AOC method is presented in Figure 2I. The total reducing power increased exponentially with rising tannin concentration (absorbance 0.182 at 0.1 mg/mL vs. 1.032 at 1.0 mg/mL). However, when the concentration exceeded 0.6 mg/mL, molecular self-association formed aggregates larger than 200 nm, which masked the active phenolic hydroxyl sites, causing a sharp 43% decrease in the rate of increase.

Based on the experimental results, we speculate that the core mechanism underlying the total antioxidant capacity of *Punica granatum* L. peel tannins stems from the electron transfer driven by their high-density phenolic hydroxyl groups. Tannin molecules contain 8-12 phenolic hydroxyl groups (density reaching 5.7 per 1,000 Da), forming an electron transfer network. This interpretation is supported by the strong correlation between the total reducing activity and the ABTS radical scavenging rate ($R^2 = 0.97$), indicating that electron transfer is the dominant mechanism. In addition, the tannins also exhibited a capacity for metal ion reduction, which may further expand their range of antioxidant applications; however, the exact contribution of each mechanism cannot be quantitatively separated from the current data. Notably, the molecular state of the tannins exhibits a significant concentration threshold effect. In the low concentration range (<0.4 mg/mL), the molecular dispersion state ensures full exposure of active sites, with reduction efficiency approaching the theoretical value ($\Delta A/\Delta C = 1.287$). In contrast, at concentrations exceeding 0.6 mg/mL, the masking effect on active sites caused by molecular self-association becomes the key factor limiting the increase in reducing power.

Tannin concentration (mg/mL)	0.1	0.2	0.4	0.6	0.8	1.0
Absorbance at 695 nm (A695)	0.182	0.317	0.568	0.763	0.921	1.032
Total reducing power equivalent to Vc (mg/g)	41.3	71.9	128.9	173.1	208.9	234.2

TABLE 6: Determination data of total antioxidant capacity of tannins from *Punica granatum* L. peel (conversion from absorbance to Vc equivalent)

Vc, Vitamin C

Conclusions

Among the four extraction processes evaluated under the conditions tested, ultrasonic-assisted acetone extraction using 40% acetone at 35°C for 45 min with a solid-to-liquid ratio of 1:40 achieved the highest tannin extraction yield of 61.6% and was identified as the optimal process. Microwave-assisted ethanol extraction using 60% ethanol at 50°C for 180 s with a solid-to-liquid ratio of 1:40 reached a yield of 55.2% within only 180 s, demonstrating a clear energy-saving advantage through rapid dielectric heating. By comparison, ultrasonic-assisted ethanol extraction using 30% ethanol at 70°C for 160 min with a solid-to-liquid ratio of 1:20 yielded 44.19%, while water bath heating extraction at 80°C for 120 min with a solid-to-liquid ratio of 1:40 produced 56.97%; both were lower than the ultrasonic acetone process.

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. *Cureus J. Agri. Food Sci* 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

D101 macroporous resin purification with 40% ethanol elution yielded purified tannins with a purity of 95.24%, but the overall recovery rate was only 48.26%, with main losses attributed to adsorption residue, elution inefficiency, and solvent residue. To overcome this bottleneck, gradient elution optimization combined with low-temperature membrane concentration is proposed for scaled-up production. The purified tannins exhibited strong antioxidant activity. In the ABTS⁺ assay, the scavenging rate at 1 mg/mL reached $93.7 \pm 0.9\%$, significantly higher than that of ascorbic acid ($87.2 \pm 0.6\%$, $p < 0.01$). In the DPPH assay, however, the tannin scavenging rate ($84.3 \pm 1.2\%$) was lower than that of ascorbic acid ($95.4 \pm 0.5\%$), indicating mechanism-dependent antioxidant behavior. Total antioxidant capacity increased with concentration until a self-association threshold at approximately 0.6 mg/mL limited further improvement. These findings confirm that *Punica granatum* L. peel tannins are promising natural antioxidants for functional food and pharmaceutical applications, while highlighting the need for further improvement in purification recovery.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Jiying Du, Shijia Lu, Jinle Wang, Han Zhang, Mengjia Yan, Yutong Zhang, Mengdan Sang, Xue Yang, Huaying Du, Shaoqing Wang, Zixuan Yang, Xuanlin Min, Jiarong Yan, Zhi Qiao

Acquisition, analysis, or interpretation of data: Jiying Du, Shijia Lu, Jinle Wang, Han Zhang, Mengjia Yan, Yutong Zhang, Mengdan Sang, Xue Yang, Huaying Du, Shaoqing Wang, Zixuan Yang, Xuanlin Min, Jiarong Yan, Zhi Qiao

Drafting of the manuscript: Jiying Du, Shijia Lu, Jinle Wang, Han Zhang, Mengjia Yan, Yutong Zhang, Mengdan Sang, Xue Yang, Huaying Du, Shaoqing Wang, Zixuan Yang, Xuanlin Min, Jiarong Yan, Zhi Qiao

Critical review of the manuscript for important intellectual content: Jiying Du, Shijia Lu, Jinle Wang, Han Zhang, Mengjia Yan, Yutong Zhang, Mengdan Sang, Xue Yang, Huaying Du, Shaoqing Wang, Zixuan Yang, Xuanlin Min, Jiarong Yan, Zhi Qiao

Supervision: Jiying Du, Jinle Wang, Xue Yang

Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen.

Human subjects: All authors have confirmed that this study did not involve human participants or tissue.

Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue.

Conflicts of interest: In compliance with [the ICMJE uniform disclosure form](#), all authors declare the following:

- **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work.
- **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work.
- **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request. All data generated or analyzed during this study are included in this published article and/or its appendices.

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. *Cureus J. Agri. Food Sci* 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>

References

1. Jalali A, Kiafar M, Seddigh M, Zarshenas MM: [Punica granatum as a source of natural antioxidant and antimicrobial agent: A comprehensive review on related investigations](#). Current Drug Discovery Technologies. 2021, 18:207-224. [10.2174/1570163817666200430001822](#)
2. Ismail T, Sestili P, Akhtar S: [Pomegranate peel and fruit extracts: A review of potential anti-inflammatory and anti-infective effects](#). Journal of Ethnopharmacology. 2012, 143:397-405. [10.1016/j.jep.2012.07.004](#)
3. Wang Z, Liu Z, Wu C, et al.: [Computational analysis on antioxidant activity of four characteristic structural units from persimmon tannin](#). Materials. 2023, 16:320. [10.3390/ma16010320](#)
4. Wilson MF: [Comparison of tannin levels in developing fruit buds of two orchard pear varieties using two techniques, Folin-Denis and protein precipitation assays](#). Journal of Chemical Ecology. 1984, 10:493-498. [10.1007/bf00988095](#)
5. Umeoguaju FU, Akaninwor JO, Essien EB, Amadi BA, Chuzeze EJ, Nwafor IR: [Macroporous adsorptive resin-assisted enrichment of polyphenol from Psidium guajava leaves improved its in vitro antioxidant and anti-hemolytic properties](#). Preparative Biochemistry & Biotechnology. 2023, 53:841-848. [10.1080/10826068.2022.2150932](#)
6. Jeppu GP, Clement TP: [A modified Langmuir-Freundlich isotherm model for simulating pH-dependent adsorption effects](#). Journal of Contaminant Hydrology. 2012, 129-130:46-53. [10.1016/j.jconhyd.2011.12.001](#)
7. Rumpf J, Burger R, Schulze M: [Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins](#). International Journal of Biological Macromolecules. 2023, 233:123470. [10.1016/j.ijbiomac.2023.123470](#)
8. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C: [Antioxidant activity applying an improved ABTS radical cation decolorization assay](#). Free Radical Biology and Medicine. 1999, 26:1231-1237. [10.1016/s0891-5849\(98\)00315-3](#)
9. Zhuang H, Du J, Wang Y: [Antioxidant capacity changes of 3 cultivar Chinese pomegranate \(Punica granatum L.\) juices and corresponding wines](#). Journal of Food Science. 2011, 76:C606-C611. [10.1111/j.1750-3841.2011.02149.x](#)
10. Abdel-K Halek MM, Mahrous MS: [Use of ammonium molybdate in the colorimetric assay of cephalosporins](#). Talanta. 1984, 31:635-637. [10.1016/0039-9140\(84\)80187-3](#)
11. Zhu C, Zhang S, Wu Q: [Prognostic significance of alterations in peripheral cellular and humoral immune biomarkers during radiochemotherapy in head and neck cancer patients](#). Translational Cancer Research. 2025, 14:685-705. [10.21037/tcr-24-1510](#)
12. He Y, Lin Y, Li Q, Gu Y: [The contribution ratio of various characteristic tea compounds in antioxidant capacity by DPPH assay](#). Journal of Food Biochemistry. 2020, 44:e13270. [10.1111/jfbc.13270](#)
13. Wang F, Liu H, Li J, Zhang W, Jiang B, Xuan H: [Australian propolis ethanol extract exerts antibacterial activity against methicillin-resistant Staphylococcus aureus by mechanisms of disrupting cell structure, reversing resistance, and resisting biofilm](#). Brazilian Journal of Microbiology. 2021, 52:1651-1664. [10.1007/s42770-021-00547-7](#)
14. Erriu M, Blus C, Szmukler-Moncler S, et al.: [Microbial biofilm modulation by ultrasound: current concepts and controversies](#). Ultrasonics Sonochemistry. 2014, 21:15-22. [10.1016/j.ultsonch.2013.05.011](#)
15. Silva JVM, Santos AS, Pereira GA, Chisté RC: [Ultrasound-assisted extraction using ethanol efficiently extracted carotenoids from peels of peach palm fruits \(Bactris gasipaes Kunth\) without altering qualitative carotenoid profile](#). Heliyon. 2023, 9:e14933. [10.1016/j.heliyon.2023.e14933](#)
16. Shabkhiz MA, Eikani MH, Sadr ZB, Golmohammad F: [Superheated water extraction of glycyrrhizic acid from licorice root](#). Food Chemistry. 2016, 210:396-401. [10.1016/j.foodchem.2016.05.006](#)
17. Hamdane D, Bou-Nader C, Cornu D, Hui-Bon-Hoa G, Fontecave M: [Flavin-protein complexes: Aromatic stacking assisted by a hydrogen bond](#). Biochemistry. 2015, 54:4354-4364. [10.1021/acs.biochem.5b00501](#)
18. Kareem HS, Ariffin A, Nordin N, Heidelberg T, Abdul-Aziz A, Kong KW, Yehye WA: [Correlation of antioxidant activities with theoretical studies for new hydrazone compounds bearing a 3,4,5-trimethoxy benzyl moiety](#). European Journal of Medicinal Chemistry. 2015, 103:497-505. [10.1016/j.ejmech.2015.09.016](#)
19. Guo X, Yang F, Deng S, Ding Y: [Activation of periodate by ABTS as an electron shuttle for degradation of aqueous organic pollutants and enhancement effect of phosphate](#). Chemosphere. 2024, 349:140793. [10.1016/j.chemosphere.2023.140793](#)

How to cite this article:

Du J, Lu S, Wang J, et al. (September 23, 2026) Systematic Optimization of Multi-Mode Extraction Process and Accurate Evaluation of In Vitro Antioxidant Activity of Tannins from *Punica granatum* L. Peel. Cureus J. Agri. Food Sci 2 : es44497-026-00294-3. DOI <https://doi.org/10.7759/s44497-026-00294-3>