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Article

Integrated isopropanol/sodium citrate aqueous two-phase extraction and AB-8 macroporous resin purification of tea saponin from Camellia oleifera seed cake

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Abstract: Tea saponin was recovered from Camellia oleifera seed cake through an integrated process coupling isopropanol/trisodium citrate aqueous two-phase extraction with AB-8 macroporous resin purification. Extraction and purification parameters were optimized using Box–Behnken response surface methodology with triplicate validation experiments. The optimal extraction conditions: 1.30 g/10 mL trisodium citrate, 40 mesh particle size, 15:1 liquid-to-solid ratio, 77.4 °C, and 32.8 min, under this condition, the yield reached up to 22.90% (RSD = 0.253%,value from predicted value = 0.02%). Purification at pH 6.1, 1.4 BV/h loading flow rate, and 3.9 BV of 75% ethanol with 92.21% purity (RSD = 0.191%, deviation from predicted value = 0.01%). Purified tea saponin scavenged DPPH radicals IC_{50} = 0.42 mg/mL and hydroxyl radicals IC_{50} = 0.56 mg/mL with a reducing power EC_{50} of 0.22 mg/mL. AB-8 resin reached adsorption equilibrium within 6 h (equilibrium capacity 42.3 mg/g), enabling efficient batch processing. Integrating extraction and purification eliminated intermediate concentration steps, reducing product loss while maintaining antioxidant bioactivity.

Keywords: Tea saponin, Aqueous two-phase extraction, Purification, Antioxidant activity

Introduction

As one of China's principal woody oil crops, *Camellia oleifera* Abel. yields substantial seed cake after oil extraction, most of which currently ends up as low-value feed or fertilizer[1]. Tea saponin, an oleanane-type pentacyclic triterpenoid saponin (Supplementary Fig. 1), exhibits antibacterial[2], anti-inflammatory[3], antioxidant[4], and immunomodulatory[5] activities, as well as nonionic surface activity[6], indicating potential applications in food, cosmetic, and pharmaceutical fields. Conventional solvent extraction methods for tea saponin are simple to operate but suffer from long extraction times, high solvent consumption, and low yield[1]. For example, the yield of ethanol reflux extraction was only 10.32%, and that of water extraction followed by alcohol precipitation was even lower[7]. Aqueous two-phase extraction achieves separation based on the differential partitioning of target compounds between two phases, without the need for volatile organic solvents throughout the process, which is beneficial for maintaining the bioactivity of target compounds[8]. Among these, alcohol/salt aqueous two-phase systems offer advantages such as low viscosity[9], rapid phase separation[10], and ease of recovery[11]. Du et al. reported a tea saponin yield of 26.21% using ultrasound-assisted aqueous two-phase extraction, with purity improved by 6.57% compared to conventional ethanol extraction[12]. Additionally, novel green solvents such as deep eutectic solvents have been used for tea saponin extraction, achieving purity up to 95.94% after macroporous resin purification[13,14]. In the purification step, macroporous adsorption resin is currently the most widely used separation medium[1], which can significantly improve the purity of tea saponin[15]. The tea saponin content in camellia seed cake can reach 12%–22%, making it a core raw material for industrial preparation of tea saponin[1]. However, most existing studies focus on either extraction or purification alone, lacking systematic integration of the entire process from extraction to purification[1,16].

The isopropanol/trisodium citrate aqueous two-phase system produces an upper phase that can be directly loaded onto AB-8 resin, eliminating the concentration step between extraction and purification. Ultrasound-assisted extraction utilizes cavitation effects to promote cell wall disruption, effectively improving extraction efficiency[1,17]. Single-factor screening coupled with response surface methodology pinpointed optimal conditions for both stages, while complementary experiments clarified the extraction synergy and resin adsorption kinetics. In vitro antioxidant assays confirmed that the purification protocol preserved tea saponin

bioactivity. Collectively, these findings establish a solvent-efficient, continuous workflow for recovering surface-active, antioxidant-rich tea saponin from camellia seed cake as a raw material for food emulsifiers and functional additives.

Methods

Materials and reagents

Camellia seed cake from Shaoyang, Hunan, China (2024) was ground and sieved. Tea saponin standard (purity 98%) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Petroleum ether, anhydrous ethanol, isopropanol, trisodium citrate, dipotassium hydrogen phosphate, polyethylene glycol 6000, vanillin, concentrated sulfuric acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, salicylic acid, ferrous sulfate, hydrogen peroxide, potassium ferricyanide, and ferric chloride were all of analytical grade and purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Deionized water was used throughout the experiments.

Standard curve of tea saponin

The vanillin-sulfuric acid colorimetric method was used to determine tea saponin content [18,19]. A 1.0 mL aliquot of tea saponin standard solution was placed in a stoppered test tube, and 0.2 mL of 5% vanillin-glacial acetic acid solution was added. Then, 0.8 mL of concentrated sulfuric acid was slowly added along the tube wall. After mixing, the tube was heated in a 60 °C water bath for 15 min, then cooled in an ice-water bath for 5 min. The absorbance was measured at 513 nm against a reagent blank. The regression equation was $y = 0.7285x - 0.0225$ （R^2=0.9992）

Determination of tea saponin yield

After measuring the absorbance of tea saponin using the method described in the standard curve section, the tea saponin yield was calculated according to Eq. (1):

$$W(\%)=\frac{c.V.N}{1000.m}\times 100\% \quad (1)$$

Where W is the tea saponin yield (%); c is the mass concentration of tea saponin in the extract(mg/mL); V is the final volume of the extract (mL); N is the dilution factor; and m is the mass of camellia seed cake powder (g).

Extraction process optimization

Single-factor experiment of extraction process

Under the conditions of particle size 40 mesh, liquid-to-solid ratio 10:1 (mL/ g), extraction temperature 60 ℃, and extraction time 30 min, the effects of solvent systems (deionized water, 95% ethanol, ethanol-dipotassium hydrogen phosphate, PEG/dipotassium hydrogen phosphate, and isopropanol/trisodium citrate) on tea saponin yield were investigated.

Using the aqueous two-phase system as the solvent, the effects of salt dosage 0.5, 0.8, 1.1, 1.4, and 1.7 g/10 mL, particle size (uncrushed, 20, 40, 60, and 80 mesh), liquid-to-solid ratio 5:1, 10:1, 15:1, 20:1, and 25:1 mL/g, ultrasonic temperature (65, 70, 75, 80, and 85 ℃), and ultrasonic time (25, 30, 35, 40, and 45 min) on tea saponin yield were sequentially examined.

Response surface optimization of extraction process

Three factors, trisodium citrate dosage (A), extraction temperature (B), and extraction time (C), were selected for a Box–Behnken design (Supplementary Table 1), with tea saponin yield as the response value. The design consisted of 17 experimental runs, including 5 center points. Three replicate validation experiments were performed under optimal conditions.

Macroporous resin purification

Resin pretreatment

AB-8 and D101 macroporous adsorption resins were activated and pretreated according to the literature[20]. The resins were sequentially soaked in anhydrous ethanol for impurity removal, rinsed with deionized water until alcohol-free, and then cyclically washed with 5% HCl and 5% NaOH solutions (2 BV each, flow rate 2 BV/h, 1 h, respectively). The resins were rinsed with deionized water until the effluent pH reached 7.0 and then stored for later use.

Static adsorption and desorption

The adsorption rate represents the proportion of tea saponin adsorbed by the macroporous resin relative to that in the crude extract, and the desorption rate represents the proportion of tea saponin in the desorption solution relative to that adsorbed on the resin. They were calculated according to Eqs. (2) and (3), respectively:

$$Adsorption\ rate = \frac{C_0 - C_e}{C_0} \times 100\% \quad (2)$$

where C_0 is the concentration of tea saponin in the crude extract (mg/mL), and C_e is the concentration of tea saponin in the supernatant after 24 h (mg/mL).

The resin saturated with adsorption was transferred to a stoppered conical flask containing 75% ethanol (50 mL) and placed on a constant-temperature shaker (25 °C) at 150 rpm/min for 24 h of desorption. After completion, the filtrate was collected, and the tea saponin

concentration was determined. The static desorption rate at room temperature was calculated according to Eq. (3):

$$Desorption\ rate=\frac{C_d.V_d}{(C_0-C_e).V}\times 100\% \quad (3)$$

where C_d is the concentration of tea saponin in the desorption solution (mg/mL), V_d is the total volume of the desorption solution (mL), and V is the volume of the crude sample solution loaded (mL).

The purity of tea saponin represents the enrichment and impurity removal effect of resin purification and was calculated according to Eq. (4):

$$Tea\ saponin\ purity=\frac{C_d.V_d}{M_{solid}}\times 100\% \quad (4)$$

where M_{solid} is the total mass of the dried solid after elution (mg).

Static adsorption kinetics

A 2.00 g portion of pretreated wet resin was weighed, surface moisture was removed, and the resin was placed in a 250 mL stoppered conical flask. Then, 50.00 mL of tea saponin crude extract diluted to 15 mg/mL was added, and the mixture was shaken at 25 °C and 150 rpm/min for adsorption. Within 7 h, 0.5 mL of supernatant was sampled every 1 h, appropriately diluted, and the tea saponin concentration was determined.

The adsorption capacity represents the mass of tea saponin adsorbed per gram of macroporous resin and was calculated according to Eq. (5):

$$Q_e=\frac{(C_0-C_e)\times V}{W} \quad (5)$$

Where Q_e is the adsorption capacity (mg/g); C_0 is the concentration of tea saponin in the crude extract (mg/mL); C_e is the concentration of tea saponin in the supernatant after adsorption equilibrium (mg/mL); V is the volume of the crude extract (mL); and W is the mass of resin (g).

The adsorption kinetics were analyzed by fitting the experimental data to the pseudo-first-order and pseudo-second-order models via nonlinear regression (Origin 2021, OriginLab Corp.). Model comparison was based on adjusted R^2, Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and residual analysis, instead of linearized R^2 values, because the linearized forms have different dependent variables and are therefore not directly comparable. The nonlinear equations are expressed as:

$$Q_t = Q_e\,(1 - e^{-k_1 t}) \quad (6)$$

$$Q_t = \frac{k_2 {Q_e}^2 t}{1+k_2 Q_e t} \quad (7)$$

where Q_e (mg/g) is the equilibrium adsorption capacity, Q_t (mg/g) is the adsorption capacity at time t, k_1 (h^{-1}) is the pseudo-first-order rate constant, k_2 g/(mg·h) is the pseudo-second-order rate constant, and t (h) is the adsorption time.

Single-factor experiments purification process

Using the selected AB-8 resin as the carrier, with a loading concentration of 15 mg/mL and 75% ethanol as the eluent, the effects of loading pH (4, 5, 6, 7, and 8), loading flow rate 0.5, 1.0, 1.5, 2.0, and 2.5 BV/h, elution flow rate 0.5, 1.0, 1.5, 2.0, and 2.5 BV/h, and elution volume (2, 3, 4, 5, and 6 BV) on purification efficiency were sequentially examined.

Response surface optimization of purification process

Three factors, loading pH (A), loading flow rate (B), and elution volume (C), were selected for a Box–Behnken design (Supplementary Table 2), with tea saponin purity as the response value. The design consisted of 17 experimental runs, including 5 center points. Three replicate validation experiments were performed under optimal conditions.

Antioxidant activity evaluation

DPPH radical scavenging activity

The DPPH radical scavenging activity was determined according to the method of Molyneux *et al.* [21]. A series of concentrations of tea saponin and vitamin C solutions were prepared. Sample solution and DPPH ethanol solution were mixed and reacted at room temperature in the dark for 30 min. The absorbance A_1 was measured at 517 nm, while the background A_2 and blank A_0 were measured simultaneously. The scavenging rate was calculated according to Eq. (8), and the IC_{50} value was fitted:

$$Scavenging\ rate=\left[1-\frac{A_1-A_2}{A_0}\right]\times 100\% \quad (8)$$

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined using the Fenton system according to the method of Choi *et al.* [22] A series of sample solutions was mixed with ferrous sulfate, hydrogen peroxide, and salicylic acid, and reacted in a 37 °C water bath for 30 min. The absorbance A_1 was measured at 510 nm, and the blank A_0 and background A_2 were measured simultaneously. The scavenging rate was calculated according to Eq. (9), and the IC_{50} value was fitted:

$$Scavenging\ rate=\left[1-\frac{A_1-A_2}{A_0-A_2}\right]\times 100\% \quad (9)$$

Total reducing power

The total reducing power was determined according to the method of Oyaizu[23] and Berker *et al*.[24]. The sample solution was mixed with buffer and potassium ferricyanide, reacted in a 50 °C water bath, and then ferric chloride was added. The absorbance was measured at 700 nm. The EC_{50} value was calculated using a four-parameter logistic model[25], as shown in Eq. (10):

$$Y(\%) = \frac{A}{1+e^{K(X-IC_{50})}} + B \quad (10)$$

When applied to the reducing power assay, IC_{50} is replaced by EC_{50}.

Statistical analysis

All experiments were performed in triplicate. Data are presented as mean ± standard deviation (SD) (n=3). One-way analysis of variance (ANOVA) was performed using SPSS 26.0 (IBM Corp., Armonk, NY, USA), and $P < 0.05$ was considered statistically significant. Box-Behnken response surface design and quadratic regression model fitting were performed using Design-Expert 8.0.6 (Stat-Ease, Inc., Minneapolis, MN, USA). Graphs were plotted using Origin 2021 (OriginLab Corp., Northampton, MA, USA). IC_{50} and EC_{50} values were calculated by fitting dose-response curves using the logistic model in the nonlinear regression module of SPSS 26.0[25,26].

Results

Full-wavelength scanning and standard curve

As shown in Fig. 1A, the tea saponin solution exhibited a maximum absorption peak at 513 nm. As shown in Fig. 1B, in the concentration range of 0.2–1.0 mg/mL, the tea saponin concentration showed a linear relationship with absorbance, with the regression equation $y = 0.7285\,x - 0.0225$, $R^2 = 0.9992$.

Optimization of Tea Saponin Extraction Process

Single-factor results for tea saponin extraction

The isopropanol/trisodium citrate aqueous two-phase system gave the highest yield (15.69%) (Fig. 2A), which was significantly higher than 95% ethanol (13.00%) and deionized water (10.80%) ($P < 0.05$). Therefore, this system was selected for subsequent extraction experiments.

Then, the effects of trisodium citrate dosage, particle size, liquid-to-solid ratio, extraction temperature, and extraction time on tea saponin yield were successively investigated under

other conditions constant. Within the range of 0.5–1.7 g/10 mL, the tea saponin yield first increased and then decreased with increasing trisodium citrate dosage, reaching a maximum of 17.54% at 1.1 g/10 mL (Fig. 2B). When the particle size was reduced from uncrushed to 40 mesh, the yield increased from 13.21% to a peak of 17.64%, and then decreased to 16.89% when further increased to 80 mesh (Fig. 2C). As the liquid-to-solid ratio increased from 5:1 to 15:1, the yield increased from 12.31% to 18.74%, and continued to increase to 19.02% at 25:1, with a slower growth rate (Fig. 2D). When the extraction temperature increased from 65 °C to 75 °C, the yield increased from 16.84% to a maximum of 20.10%, and then decreased to 19.13% and 18.02% at 80 °C and 85 °C, respectively (Fig. 2E). When the extraction time was extended from 25 min to 35 min, the yield increased from 20.33% to a maximum of 22.95%, and then decreased to 21.70% and 20.94% at 40 min and 45 min, respectively (Fig. 2F).

Response surface optimization of extraction process

A total of 17 experiments were conducted using the Box–Behnken design (Table 1). The quadratic polynomial regression model for tea saponin yield (Y) in terms of coded variables was established as:

$$Y = 23.0 + 0.247A - 0.0409B + 0.0101C - 0.103AC - 0.431A^2 - 0.330B^2 - 0.163C^2.$$

The ANOVA (Supplementary Table 3) showed that the model was highly significant ($F = 45.19$, $P < 0.0001$), and the lack of fit was not significant ($P = 0.0699 > 0.05$), indicating good model fit. The coefficient of determination ($R^2 = 0.972$), adjusted R^2 (0.951), predicted R^2 (0.846), and adequate precision (19.1) confirmed the reliability of the model. The order of influence of the factors was: A > B > C. The AC interaction was significant ($P < 0.05$), whereas the AB and BC interactions were not significant and were therefore excluded from the final model.

As shown in Fig. 3, the contour plots for AC and BC interactions were distinctly elliptical, and the response surfaces were steep, indicating significant interactions ($P<0.01$). The AB contour plot was nearly circular, and the response surface was relatively flat, indicating no significant interaction.

Through model optimization, the predicted optimal conditions were trisodium citrate dosage 1.30 g/10 mL, extraction temperature 77.4 °C, and extraction time 32.8 min, with a predicted yield of 22.9%. Three parallel validation experiments yielded an average yield of

22.90% (Supplementary Table 4), which was close to the predicted value (deviation from predicted value 0.02%, RSD = 0.253%).

Optimization of Tea Saponin Separation Process

Comparison of macroporous adsorption resin purification performance

The static adsorption-desorption performance of AB-8 and D101 resins is compared in Supplementary Table 5. As shown in Supplementary Table 5, the adsorption rate of AB-8 resin was 85.3%, and that of D101 resin was 86.2%, with no statistically significant difference ($P > 0.05$). However, the desorption rate of AB-8 resin (92.4%) was significantly higher than that of D101 (87.5%) ($P < 0.05$). Therefore, the two resins exhibited comparable adsorption capacity for tea saponin, while AB-8 resin had superior desorption performance. Accordingly, AB-8 was selected for subsequent purification experiments.

Static adsorption kinetics curve of AB-8 resin

As shown in Supplementary Fig. 2, the adsorption capacity of AB-8 resin increased rapidly within the first 2 h and then gradually leveled off, reaching an equilibrium capacity of 42.3 mg/g at approximately 6 h. During the initial 2 h, the adsorption capacity reached 30.3 mg/g, accounting for 71.6% of the equilibrium value. Between 6 and 7 h, the increase was only 0.3 mg/g, confirming that equilibrium had been essentially attained.

Nonlinear regression was employed to fit the data to the pseudo-first-order and pseudo-second-order models. The fitting parameters and comparison statistics are summarized in Supplementary Table 6. The pseudo-first-order model yielded a higher adjusted R^2 (0.9987) and lower AIC (−9.66) and BIC (−9.50) than the pseudo-second-order model (adjusted R^2 = 0.9898, AIC = 6.92, BIC = 7.08). Moreover, the equilibrium capacity predicted by the pseudo-first-order model (Qe,cal = 43.47 mg/g) was closer to the experimental value (Qe,exp = 42.3 mg/g) than that predicted by the pseudo-second-order model (Qe,cal = 54.22 mg/g). These results indicate that the adsorption behavior of tea saponin on AB-8 resin was better described by the pseudo-first-order model, suggesting that physisorption may be the dominant mechanism.

Single-factor results of purification

The effects of loading pH, loading flow rate, elution flow rate, and elution volume on adsorption rate or desorption rate were sequentially examined under fixed other conditions.

Within the pH range of 4.0–8.0, the adsorption rate first increased and then decreased with increasing pH. It was 72.5% at pH 4.0, reached a maximum of 85.0% at pH 6.0, and decreased to 79.2% at pH 8.0 (Fig. 4A). When the loading flow rate increased from 0.5 BV/h to 1.5 BV/h, the adsorption rate decreased from 88.2% to 85.5%, and further decreased to 76.2% at 2.5 BV/h (Fig. 4B). When the elution flow rate increased from 0.5 BV/h to 1.5 BV/h, the desorption rate increased from 78.5% to a maximum of 87.5%, and then decreased to 82.3% at 2.5 BV/h (Fig. 4C). When the elution volume increased from 2 BV to 4 BV, the cumulative desorption rate increased from 78.5% to 93.5%, and further increased to 94.8% and 95.0% at 5 BV and 6 BV, respectively (Fig. 4D).

Response surface optimization of purification process

A total of 17 experiments were conducted using the Box-Behnken design (Table 2). The quadratic polynomial regression model for tea saponin purity (Y) in terms of coded variables was established as:

$$Y = 92.2 + 0.245A - 0.154B - 0.109C - 0.200AC + 0.217BC - 1.17A^2 - 0.784B^2 - 0.559C^2.$$

The ANOVA showed that the model was highly significant $F = 221.14$, $P < 0.0001$ and the lack of fit was not significant $P = 0.577 > 0.05$, indicating good model fit (Supplementary Table 7). The coefficient of determination R^2=0.995, adjusted R^2 (0.991), predicted R^2 (0.972), and adequate precision (39.6) confirmed the reliability of the model (Supplementary Table 7). The order of influence of the factors was: A > B > C.

As shown in Fig. 5, the contour plots of AC and BC interactions were distinctly elliptical, and the response surfaces were steep, indicating highly significant interactions. In contrast, the AB interaction was not significant ($P > 0.05$) and was therefore excluded from the final model.

Through model optimization, the predicted optimal conditions were adjusted to practical conditions: loading pH 6.1, loading flow rate 1.4 BV/h, and elution volume 3.9 BV, with a predicted purity of 92.2%. These were adjusted to practical conditions: loading pH 6.1, loading flow rate 1.4 BV/h, and elution volume 3.9 BV. Three parallel validation experiments yielded an average purity of 92.21% (Supplementary Table 8), which was close to the predicted value (deviation from predicted value 0.01%, RSD = 0.191%).

It should be noted that the purity value reported in this study refers to the total tea saponin content determined by the vanillin-sulfuric acid colorimetric method, using a commercial

standard (purity ≥ 98%) as the reference. Although chromatographic profiling of individual saponin congeners was not performed, this colorimetric method is widely accepted for routine quantification of total saponins in plant extracts[18,19]. Therefore, the obtained purity level (92.21%) is acceptable and comparable for evaluating the integrated extraction-purification process and for subsequent antioxidant activity assessment.

Antioxidant activity of tea saponin

Tea saponin scavenged DPPH radicals in a concentration-dependent manner (Fig. 6A). DPPH scavenging by tea saponin increased from 14% to 90% over the 0.0625–2.0 mg/mL range, reaching 38% at 0.25 mg/mL and 75% at 1.0 mg/mL with progressively slower gains thereafter. Four-parameter logistic regression yielded an IC_{50} of 0.42 mg/mL for tea saponin and 0.09 mg/mL for vitamin C (Supplementary Table 9).

Hydroxyl radical scavenging by tea saponin increased from 10% to 83% over 0.0625–2.0 mg/mL, with incremental gains above 1.0 mg/mL proceeding more gradually than those for DPPH (Fig. 6B). IC_{50} values were 0.56 mg/mL for tea saponin versus 0.21 mg/mL for vitamin C (Supplementary Table 8).

Total reducing power of tea saponin, expressed as A_{700} absorbance, rose from 0.21 to 0.79 over 0.0625–2.0 mg/mL, reaching 0.42 at 0.25 mg/mL and 0.71 at 1.0 mg/mL with progressively smaller gains thereafter (Fig. 6C). EC_{50} values were 0.22 mg/mL for tea saponin versus 0.09 mg/mL for vitamin C (Supplementary Table 9).

Discussion

In this study, the isopropanol/trisodium citrate aqueous two-phase system was used to extract tea saponin, achieving a yield of 22.90% under optimal conditions. To objectively evaluate the extraction efficiency of this process, it was systematically compared with various previously reported extraction methods. Compared with methanol extraction combined with aqueous two-phase purification (yield 25.24%, initial purity 36.15%)[27], flash extraction (22.46%)[28], propanol extraction (15.31%)[29], and deep eutectic solvent extraction (20.93 ± 0.48%)[13], the yield obtained in this study showed a distinct advantage. Although the yield was slightly lower than that of the methanol extraction-aqueous two-phase purification method[27], the present process simultaneously achieved primary purification during extraction, offering superior process integration.

The high extraction efficiency of the isopropanol/trisodium citrate aqueous two-phase system can be attributed to the synergistic effects of multiple physicochemical phenomena. Isopropanol, with moderate polarity (log P ≈ 0.28), exhibits favorable polarity matching with the amphiphilic molecular structure of tea saponin (hydrophobic oleanane-type aglycone and hydrophilic sugar chains), facilitating selective dissolution of the target compound from the solid matrix into the liquid phase. Trisodium citrate, as a strong salting-out salt in the Hofmeister series, enhances the hydrogen-bonding network among water molecules through its citrate ions, reducing the solubility of isopropanol in the aqueous phase and thereby inducing liquid-liquid phase separation. Mokarizadeh *et al.*[9] systematically investigated the liquid-liquid equilibrium of the isopropanol-trisodium citrate-water ternary system over the temperature range of 288.15–318.15 K, confirming the favorable phase separation capacity and temperature-responsive characteristics of this system. Meanwhile, the ionic strength variation induced by the salting-out effect can weaken the electrostatic interactions and hydrogen bonding between tea saponin and the camellia seed cake solid matrix, promoting the mass transfer driving force of tea saponin from the solid to the liquid phase. The localized high temperatures (up to 5000 K) and high pressures (up to 1000 atm), as well as intense microjets and shock waves generated by ultrasonic cavitation, can effectively disrupt the cellulose-lignin network structure of camellia seed cake cell walls. Du *et al.*[30] observed by scanning electron microscopy that the camellia seed cake meal treated with ultrasound-assisted aqueous two-phase extraction exhibited exacerbated tissue disruption, with numerous cavities and intense surface shrinkage due to ultrasonic cavitation, effectively promoting the release of tea saponin.

The unimodal response characteristics observed in the single-factor experiments reflect the dual effects of various factors on extraction efficiency. The decrease in yield when trisodium citrate dosage exceeded 1.1 g/10 mL was attributed to excessive salting-out effects leading to increased system viscosity and mass transfer resistance[9,11]. The decrease in yield when particle size was too fine (>40 mesh) was associated with enhanced powder agglomeration and reduced effective contact area between solvent and solid particles[28]. The decrease in yield when extraction temperature exceeded 75 °C was related to the accelerated hydrolysis of glycosidic bonds in the tea saponin structure and oxidation of the aglycone at elevated temperatures[6,29]. Prolonged ultrasonication likely generated hydroxyl radicals, which attacked unsaturated or glycosidic bonds in tea saponin, reducing the yield[28]. Previous studies

have shown that ultrasonic cavitation effects can cause degradation and inactivation of bioactive compounds from plants under certain conditions, and the negative sonochemical effects should not be overlooked[28]. Reactive species such as hydroxyl radicals generated by ultrasonic cavitation can attack unsaturated bonds of target compounds, leading to structural damage and reduced bioactivity[28]. This result indicates that there is an optimal window for ultrasonic energy input in ultrasound-assisted extraction processes; beyond this window, the promoting effect of cavitation on mass transfer enhancement is counteracted by the weakening effect of degradation side reactions on yield[28]. Furthermore, the low viscosity and rapid phase separation of this system allow the clear upper phase obtained from extraction to be directly loaded onto the macroporous resin column without concentration or organic solvent replacement, simplifying the extraction–purification process and reducing target compound loss during intermediate steps[6,29].

In the resin screening stage, the adsorption rates of AB-8 and D101 resins for tea saponin were similar (85.3% vs. 86.2%), but the desorption rates differed significantly (92.4% vs. 87.5%, $P < 0.05$). This difference can be explained from the perspective of the interaction between the physicochemical properties of the resins and the molecular structure of tea saponin. AB-8 is a weakly polar resin with a polystyrene-divinylbenzene matrix containing an appropriate amount of ester groups, which confer certain weak polarity characteristics to the resin (specific surface area 500 ± 20 m^2/g, average pore size 13–14 nm). D101 is a non-polar resin with a matrix predominantly composed of pure styrene-divinylbenzene structure (specific surface area 650 ± 50 m^2/g, average pore size 20–22 nm), exhibiting stronger hydrophobicity. The molecular structure of tea saponin consists of a hydrophobic aglycone core (containing multiple methyl substituents) and hydrophilic sugar chains (usually containing 2–4 sugar residues). During adsorption, the hydrophobic aglycone of tea saponin interacts with the aromatic matrix of both resins primarily through van der Waals forces and π-π stacking interactions. Kinetic analysis using nonlinear regression revealed that the pseudo-first-order model provided a better fit than the pseudo-second-order model (adjusted R^2 = 0.9987 vs. 0.9898; AIC = −9.66 vs. 6.92; BIC = −9.50 vs. 7.08). The equilibrium capacity predicted by the pseudo-first-order model Qe,cal=43.47mg/g was also closer to the experimental value Qe,exp=42.3mg/g. These findings suggest that physisorption, rather than chemisorption, was the dominant adsorption mechanism, which is consistent with the weak polarity of the AB-8

surface and the predominant role of van der Waals forces and π–π stacking interactions. The moderately strong hydrophobic interactions provided by the weakly polar surface of AB-8 resin ensure high adsorption capacity while facilitating the synergistic disruption of hydrophobic interactions and hydrogen bonds by 75% ethanol during subsequent elution. In contrast, the strongly non-polar characteristics of D101 resin result in excessively strong resin-tea saponin hydrophobic interactions, requiring a higher energy barrier to overcome during desorption, thereby reducing elution efficiency. This explanation is consistent with the findings of Yang *et al.*[15] who reported that AB-8 exhibited the optimal separation capability for tea saponin among four resins AB-8, NKA-9, XDA-6, and D4020.

The adsorption kinetics experiments showed that AB-8 resin reached 71.6% of the equilibrium adsorption capacity within 2 h and reached equilibrium at 6 h (equilibrium adsorption capacity 42.3 mg/g), indicating that it is a fast-adsorbing resin. The adsorption process may be primarily limited by the diffusion of tea saponin molecules (molecular weight approximately 1200–2000 Da) in the external liquid film around the resin particles and surface diffusion within the macroporous structure, rather than by slow intraparticle diffusion within the pores. Yang *et al.*[15] screened AB-8 as having the optimal separation capability for tea saponin among AB-8, NKA-9, XDA-6, and D4020 resins, further validating the rationality of the resin selection in this study. The relatively short adsorption equilibrium time is beneficial for improving batch processing efficiency and has practical significance for industrial production.

In dynamic purification, the effect of loading pH on adsorption rate can be explained from the perspective of electrostatic interactions between tea saponin molecules and the resin surface charge. The glucuronic acid residues (pKa ≈ 3.2) in tea saponin molecules are partially protonated under low pH conditions, enhancing electrostatic repulsion with the AB-8 resin surface. As the pH increases to 6.0, the carboxyl groups deprotonate to carboxylate anions, reducing electrostatic repulsion and allowing hydrophobic interactions to fully take effect. When the pH further increases to 7.0~8.0, the adsorption rate decreases, which may be attributed to conformational changes caused by the ionization of other ionizable groups (such as phenolic hydroxyl groups) in the tea saponin molecule, altering the spatial matching between the molecules and the resin pores.

This pH-dependent pattern is consistent with the observations of Yang *et al.* [15] in the AB-8 resin purification system for tea saponin, further confirming the key regulatory role of electrostatic interactions in the tea saponin-resin adsorption system.

Flow rate is another key operating parameter affecting column efficiency. Increasing the loading flow rate led to a decrease in adsorption rate, attributed to the shortening of effective contact time between tea saponin molecules and resin adsorption sites, resulting in mass transfer failing to reach equilibrium and some target compounds flowing out with the mobile phase without being adsorbed. When the elution flow rate exceeded 1.5 BV/h, the desorption rate decreased, which was related to insufficient contact time between the eluent and the resin bed, failing to adequately disrupt the non-covalent interactions between the resin and tea saponin.

Yang *et al.* [15] used AB-8 resin to separate and purify tea saponin from camellia seed cake extract, achieving a high purity product of 96.0% under optimal dynamic adsorption/desorption conditions, indicating that AB-8 resin has good separation selectivity for tea saponin triterpenoid saponins.

The optimal loading flow rate in this study was 1.4 BV/h, which differed from the literature reported values, which may be related to differences in raw material pretreatment methods, crude extract composition, and loading concentration. After the elution volume exceeded 4 BV, the increase in cumulative desorption rate was less than 1.5%, indicating that tea saponin had been essentially completely eluted, and further increasing the elution volume contributed limitedly to improving recovery while increasing solvent consumption and subsequent processing burden. Through response surface optimization, the purity of tea saponin after AB-8 resin purification reached 92.21%.

Compared with previous reports, Zhu *et al.*[27] achieved a purity of 83.72% for camellia seed saponins using direct aqueous two-phase extraction; Gong *et al.*[31] achieved a purity of 82.5% for tea saponin from camellia seed shells using AB-8 macroporous resin combined with Sephadex G-15 purification; Yang *et al.*[15] achieved a purity of 96.0% for tea saponin from camellia seed cake extract using AB-8 resin; Yin *et al.*[33] screened X-5 resin from 18 macroporous adsorption resins and increased tea saponin purity from 24.18% to 80.62%; review articles reported that AB-8 resin purification of tea saponin can achieve purities ranging from 80% to 96.13%[1].

In the above reports, crude extract purities ranged from 24% to 50%, and all were significantly increased to above 80% after macroporous resin purification. In this study, the tea saponin purity after AB-8 resin purification was 92.21%, which is at the relatively high level among the above reports, further verifying the favorable purification performance of AB-8 resin for tea saponin triterpenoid saponins.

The DPPH radical scavenging activity, hydroxyl radical scavenging activity, and total reducing power of the purified tea saponin all increased with increasing concentration, exhibiting concentration dependence. The IC_{50} values were 0.42 mg/mL (DPPH) and 0.56 mg/mL (hydroxyl radical), and the EC_{50} for total reducing power was 0.22 mg/mL. These results were similar to the values of 0.38 and 0.49 mg/mL reported by Guo *et al.*[32], but slightly lower than their reported values. This difference may be related to factors such as raw material source (different compositions of tea saponin monomer saponins in camellia seed cakes from different origins) and extraction/purification processes (selectivity differences between aqueous two-phase systems and conventional organic solvent systems for specific saponin monomers).

Yin *et al.*[33] reported that the DPPH scavenging activity and total reducing power of tea saponin after macroporous resin purification were 1.61-fold and 1.57-fold higher than those before purification, respectively, suggesting that the purification process enhances the activity. The removal of non-saponin impurities such as pigments and polysaccharides during purification reduced the coexisting substances that may interfere with free radical scavenging reactions, which may be an important reason for the enhanced antioxidant activity after purification.

Although the antioxidant activity of tea saponin was weaker than that of vitamin C, tea saponin, as a natural product with both surface activity and antioxidant function, has potential application value in food emulsification and cosmetics. Previous studies have shown that tea saponin exhibits good surface activity, with a low critical micelle concentration, and its emulsifying and foaming properties are superior to some synthetic surfactants[6,34]. This is closely related to the amphiphilic characteristics of the hydrophobic aglycone core and hydrophilic sugar chains in the molecular structure of tea saponin[15]. This amphiphilic structure not only endows tea saponin with excellent surface activity but may also contribute to its antioxidant performance by affecting its distribution behavior at the oil–water interface and in

the free radical scavenging system. The high-purity tea saponin (92.21%) obtained by this process can provide a material basis for further research on the synergistic effects of its surface activity and antioxidant activity.

Conclusion

In conclusion, under the optimized conditions, the extraction yield of tea saponin reached 22.90%, and the purification purity reached 92.21%. The purified product exhibited concentration-dependent antioxidant activity, with IC_{50} values of 0.42 and 0.56 mg/mL against DPPH and hydroxyl radicals, respectively, and an EC_{50} of 0.22 mg/mL for total reducing power. This integrated approach efficiently connects extraction with purification, providing a technical basis for high-value utilization of camellia seed cake.

Data availability: The datasets generated and analyzed during the current study are included in this published article and its Supplementary Information files. No datasets were deposited in external repositories.

Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions: D.X., Z.L., and Y.Z. conceived and designed research. D.X., H.Z., M.L., D.W., and X. S. conducted experiments; D.X. and H.Z. analyzed data and wrote the manuscript. Z.L. and Y.Z. revised and perfected the manuscript. All authors have proofread the manuscript and approved the final version of the paper.

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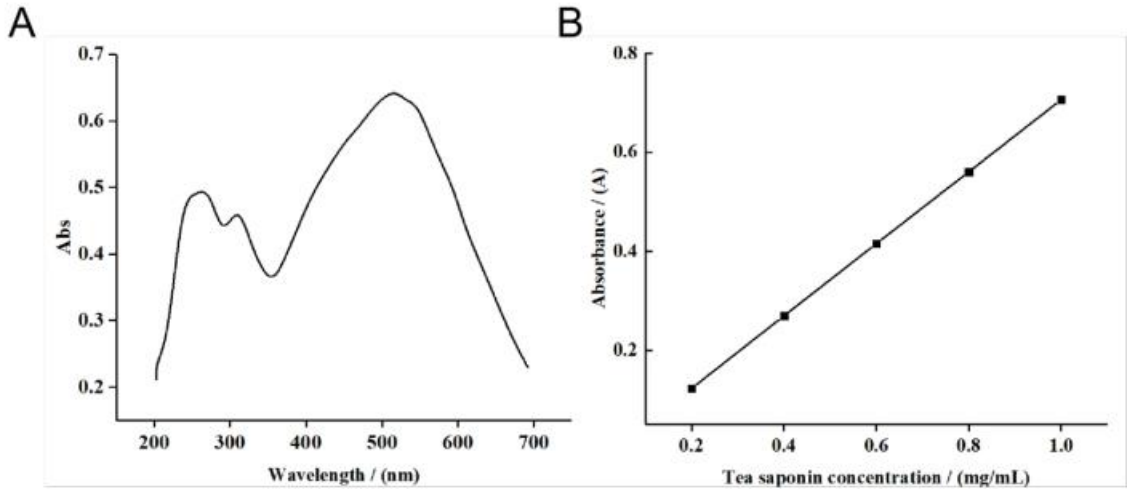
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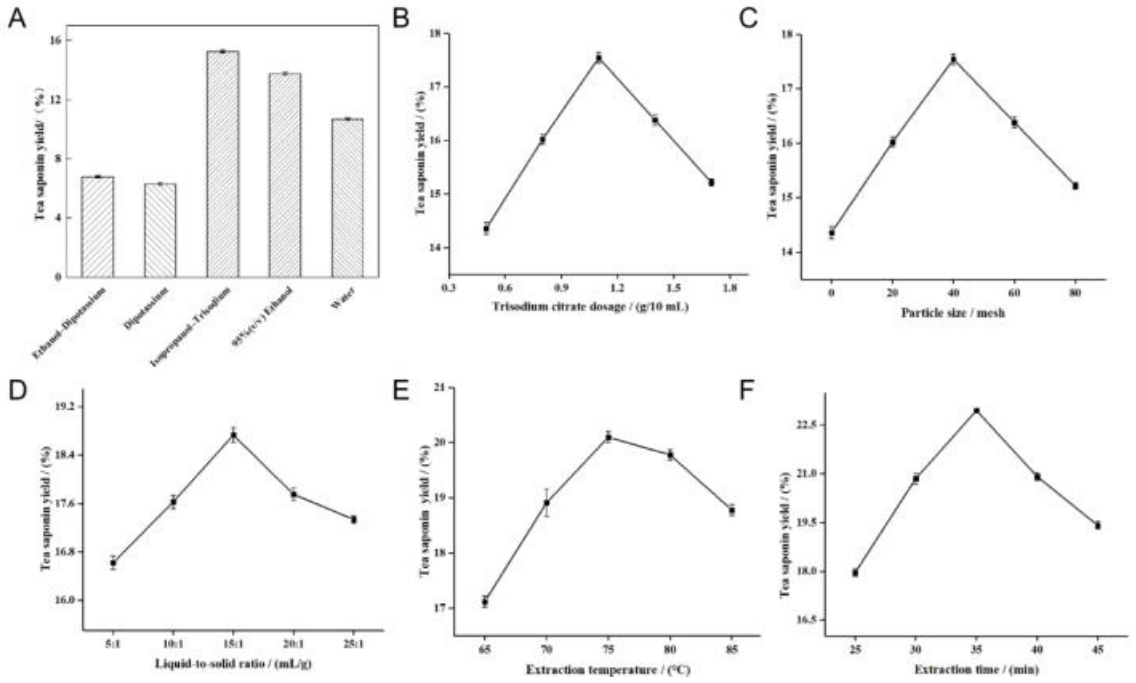
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599 **Fig. 1.** Scanning wavelength and standard curve for tea saponin determination.

600 A, Full-wavelength scan; B, Standard curve.

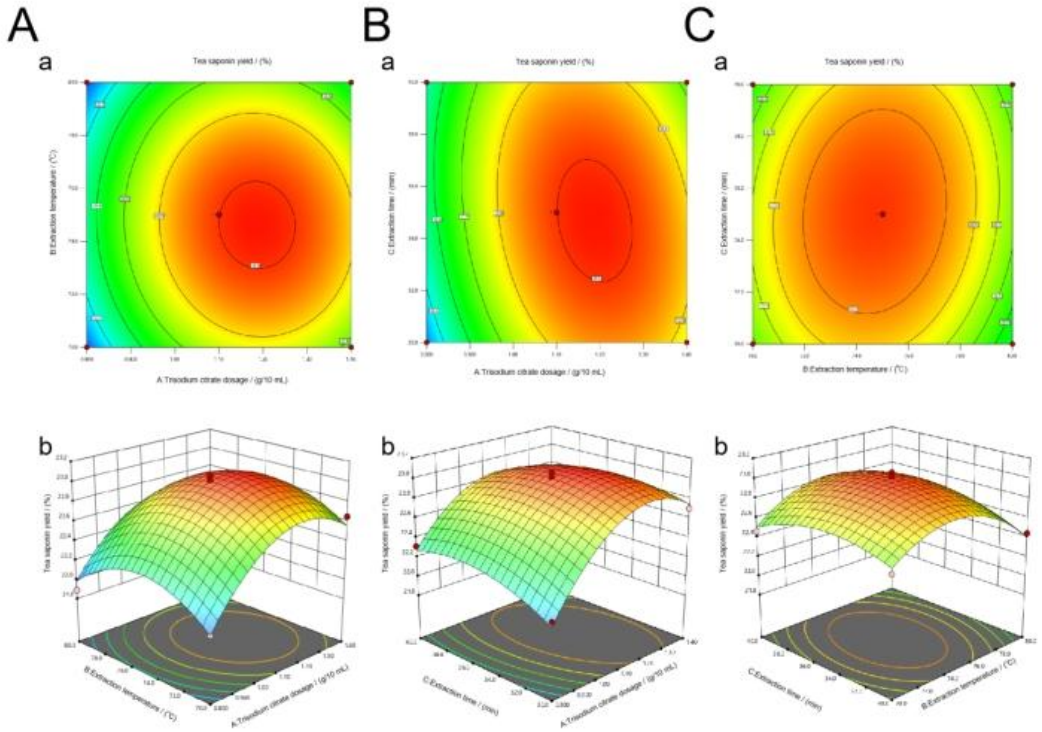


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602 **Fig. 2** Effects of single factors on tea saponin yield.

603 A, extraction solvent system; B, trisodium citrate dosage; C, particle size; D, liquid-to-solid ratio; E,

604 extraction temperature; F, extraction time.

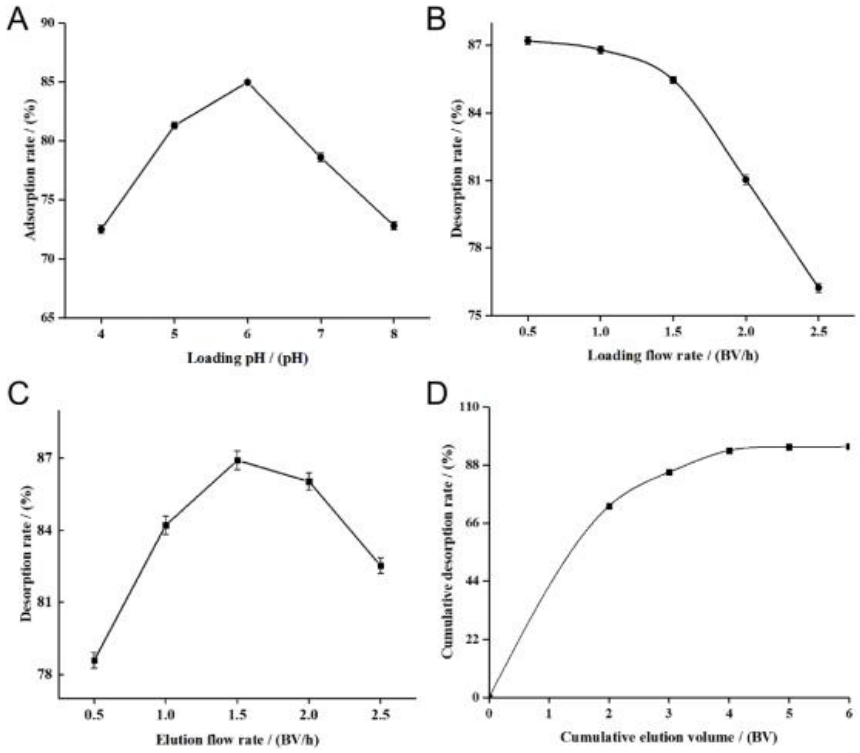


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606 **Fig. 3** Response surface plots of interactive effects on tea saponin yield.

607 A, Trisodium citrate dosage and temperature; B, Trisodium citrate dosage and time; C, Temperature and

608 time. a, Contour plots; b, 3D response surface plots.



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610 **Fig. 4** Single-factor results for dynamic purification.

A, loading pH; B, loading flow rate; C, elution flow rate; D, elution volume.

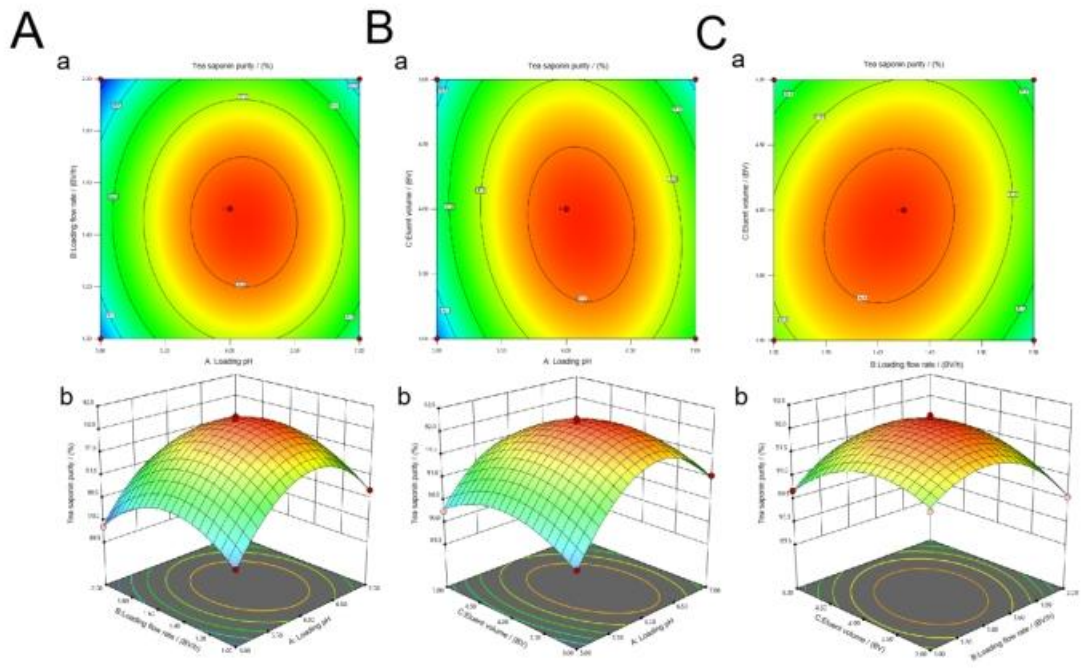


Fig.5 Response surface plots of interactive effects on tea saponin purity.

A, loading pH and loading flow rate; B, loading pH and elution volume; C, loading flow rate and elution volume. a, Contour plots; b, 3D response surface plots.

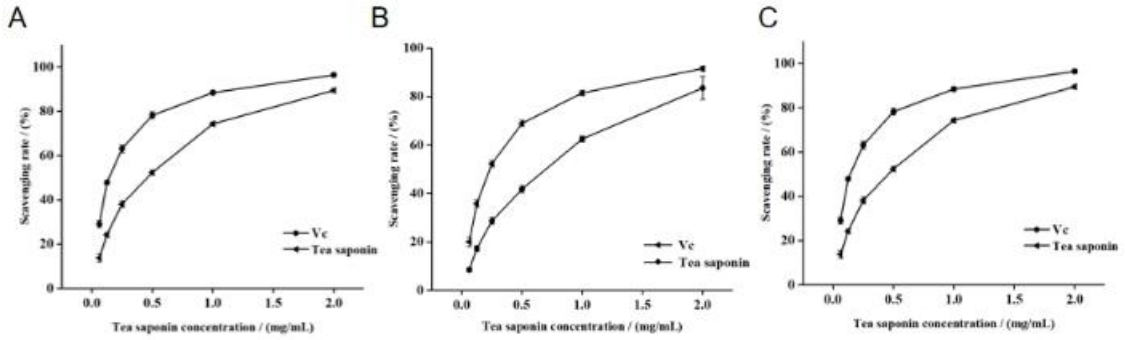


Fig. 6. Comparison of antioxidant activities between tea saponin and vitamin C.

A, DPPH radical scavenging activity; B, Hydroxyl radical scavenging activity; C, Total reducing power (A_{700}).

626 **Table 1.** Response surface results for tea saponin extraction.

Run	A Trisodium citrate dosage (g/10 mL)	B Extraction temperature (°C)	C Extraction time (min)	Tea saponin yield (%)
1	0.8	70	35	22.00
2	0.8	80	35	21.89
3	1.4	70	35	22.65
4	1.4	80	35	22.41
5	0.8	75	30	22.10
6	0.8	75	40	22.31
7	1.4	75	30	22.70
8	1.4	75	40	22.51
9	1.1	70	30	22.53
10	1.1	70	40	22.47
11	1.1	80	30	22.45
12	1.1	80	40	22.58
13	1.1	75	35	22.94
14	1.1	75	35	23.06
15	1.1	75	35	23.00
16	1.1	75	35	22.98
17	1.1	75	35	23.01

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637 **Table 2.** Response surface design and results for purification.

Run	A Loading pH	B Loading flow rate (BV/h)	C Elution volume (BV)	Tea saponin purity (%)
1	5	1.0	4	90.12
2	5	2.0	4	89.84
3	7	1.0	4	90.68
4	7	2.0	4	90.25
5	5	1.5	3	90.15
6	5	1.5	5	90.26
7	7	1.5	3	91.05
8	7	1.5	5	90.35
9	6	1.0	3	91.27
10	6	1.0	5	90.68
11	6	2.0	3	90.54
12	6	2.0	5	90.85
13	6	1.5	4	92.16
14	6	1.5	4	92.28
15	6	1.5	4	92.07
16	6	1.5	4	92.22
17	6	1.5	4	92.18

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