

Optimization of ultrasonic assisted extraction and biological activity of total polyphenols from *Toona sinensis* using response surface methodology

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Abstract

To optimize the extraction process and activity detection of total polyphenols from *Toona sinensis*. A Box–Behnken design of response surface methodology was employed to further optimize ultrasonic-assisted extraction (UAE) conditions for *T. sinensis* stems total polyphenols (*TSTP*). The results showed that optimized extraction conditions were ultrasonic power of 200 W, liquid-solid ratio of 50 mL/g, ultrasonic temperature of 60°C and ultrasonic time of 22 min. Under these conditions, the mean experimental value of extraction yield (8.13%) was achieved, which was about 49.44% higher than that of distilled water leaching extraction. As compared to *TSTP*, *TSTP* after purification (*TSPTP*) had higher purity of 57.70%, which was nearly 45.33% higher than that of *TSTP* (12.37%). *TSPTP* had higher α -glucosidase and α -amylase inhibitory activities as well as *DPPH* $\cdot$ and *ABTS* $\cdot^+$ scavenging activities with an IC_{50} value of 0.201, 0.041, 0.27 and 0.07 mg/mL. *TSPTP* from *T. sinensis* could be considered as multifunctional bioactive ingredients to be used in anti-hyperglycemic pharmaceutical formulation and antioxidant.

1. Introduction

Diabetes mellitus (DM) is a symptom of hyperglycemia caused by insufficient insulin secretion or declining insulin sensitivity, and has become one of the most challenging health problem in the 21st century (Schmidt & Hickey, 2009). There are over 422 million people with DM all over the world by 2018, of which China has about 114 million. If comprehensive treatment is not available, DM can cause multiple complications, such as chronic kidney failure, cardiovascular diseases, nonketotic hyperosmolar coma and diabetic ketoacidosis (Campbell, 2011). As we all know, controlling blood glucose level is a key factor in treating the DM. Moreover, α -amylase and α -glucosidase are typical postprandial digestive enzymes, which play important roles in the degradation of carbohydrates then causing blood glucose level elevation (Joshi et al., 2015). Acarbose is an effective α -glucosidase inhibitor in preventing postprandial hyperglycemia. However, the use of acarbose is inevitable causing some side effects (Deng, Lin-Shiau, Shyur, & Lin, 2015). Therefore, the searching for α -glucosidase and α -amylase inhibitors, derived from natural sources without or few side effects, has become a research hotspot in treating the DM (Kwon, 2006). Accumulating studies have shown that plant polysaccharides, a key bioactive component of Chinese herbs, make significant anti-diabetic effects almost without side effects or adverse drug reaction (Ren et al., 2015).

Toona sinensis Roemer (*T. sinensis*) belongs to the Meliaceae genus of *Toona*. It has been used for the treatment of enteritis, emesis, vomiting, dysentery, pruritus, carminative and heliosis in Chinese folk medicine (Wang et al., 2020). In modern clinical applications, *T. sinensis* is mainly used for the treatment of hypoglycemic, anti-oxidation, dyslipidemia, antitumor and anti-inflammatory (Hsieh et al., 2012). *T. sinensis* has been subjected to phytochemical studies, in which polyphenols are the main active compounds in *T. sinensis* (Zhao et al., 2021). So far, most of the main reported studies are to study the biological activity of *T. sinensis* extract. However, there are no available reports about the extraction of

total polyphenols from *T. sinensis* (*TSTP*) and their anti-diabetic activity. Consequently, it is of great importance to develop a high efficiency method for extracting polyphenols constituents from *T. sinensis*.

There are various methods to extract polyphenols compounds from natural products, such as soxhlet, heating, boiling, refluxing, ultrasonic-assisted and maceration extraction (Feng, García-Martín, Broncano Lavado, López-Barrera, & Álvarez-Mateos, 2020; Nishad, Saha, Dubey, Varghese, & Kaur, 2019; Xi & Luo, 2015). Among them, the ultrasonic-assisted extraction (UAE) has gained particular attention due to its efficient and easy to use, low solvent consumption, and saving money and time (Dai et al., 2021; Li, Chen, & Yao, 2005). *UAE* utilizes the energy of ultrasonic wave to promote the collision of the extracts, leading to the effective ingredients are quickly and fully dissolved in the solvent (Adjé et al., 2010). *UAE* has been recently reported as more efficient method for extraction of polyphenols from India *Moringa oleifera* L., *Diospyros kaki* and *Lobelia nicotianifolia* than conventional solvent extraction (Duan, Zhao, Zhang, Liu, & Wang, 2013; Lin, Wu, Wang, Yao, & Wang, 2021; Zimare, Mankar, & Barmukh, 2021). To the best of our knowledge, there are no data on *UAE* for isolation of *TSTP*. Therefore, in this study, ultrasonic-assisted ethanol extraction method was used to prepare polyphenols-containing extract from *T. sinensis*.

Response surface methodology (*RSM*), as an effective statistical method, is widely used for the optimization of complex process, extraction technology, and so on. Since it can depict the complete effects of variables, evaluate the interactions between multiple parameters, reduce the number of experimental trials and shorten process time. Moreover, it is more precise and effective than many approaches (Hosseinpour, Vossoughi, & Alemzadeh, 2014; Kumar, 2013).

In the present study, a three-level, three-variable (ultrasonic temperature, ultrasonic time and liquid-solid ratio) Box–Behnken design (*BBD*) of *RSM* was employed to further optimize *UAE* conditions for *TSTP*. Then, the extracted total polyphenols were purified by AB-8 macroporous resin. Finally, the α -glucosidase and α -amylase inhibitory activities of the isolated anti-diabetic total polyphenols were determined.

2. Materials And Methods

2.1. Materials and reagents

The *T. sinensis* were collected from Henan province, China. Voucher specimens were deposited in the School of Pharmacy at Guangdong Pharmaceutical University. AB-8 macroporous resin was acquired from Zhejiang taizhou luqiao tetramethyl biochemical plastics factory (China). Gallic acid, 1,1-diphenyl-2-picrylhydrazyl (*DPPH*), 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (*ABTS*), α -glucosidase, *p*-nitrophenyl- α -D-glucopyranoside (*PNPG*), α -amylase, starch, 3,5-dinitrosalicylic acid and acarbose were purchased from Guangzhou Chemical Reagent Company, China. All other chemicals and solvents were of analytical grade.

2.2. Ultrasound-assisted extraction of TSTP

The *UAE* procedure that was used in the experiment was developed by Mai et al. with some modification (Mai et al., 2020). 2 g of *T. sinensis* powder was weighted in a conical flask, the extraction solvent according to the corresponding liquid-solid ratio was added, and then the mixtures were extracted in an ultrasonic extraction reaction workstation (KQ-400KDB, Kunshan Ultrasonic Instrument Co., Ltd., China) under the corresponding ultrasonic temperature, ultrasonic time, and ultrasonic power. Finally, the extract was centrifuge at 3000 r/min for 6 min in a low-speed centrifuge (Model TDZ5-WS, Changsha Ordinary Instrument Co., Ltd., China), and the supernatant was used for the determination of the total polyphenols content (*TSTPC*).

2.3. Determination of the content of polyphenols in samples

The total polyphenols content (*TSTPC*) was investigated according to the procedure described by Amirabbasi et al. with some modification (Amirabbasi, Elhamirad, Saeediasl, Armin, & Ziaolhagh, 2020). Briefly, 100 μ L of the supernatant and 30 μ L of Folin–Ciocalteu reagent were added and then kept for 3 min. Then 120 μ L of 7.5% Na_2CO_3 solution and 500 μ L of distilled water were added and then kept for 15 min to obtain the test solution. The absorbance of the test solution was measured at 765 nm using Gallic acid as a standard on a Microplate Reader (Spectra Max M2, Molecular Devices Co., Ltd., America). The total polyphenols content of *T. sinensis* was calculated using the following formula:

$$\text{Extraction yield of total polyphenols (\%)} = \frac{C \times V \times N}{M \times 1000} \times 100\% \quad \text{Eq. (1)}$$

where C is the concentration of the total polyphenols of *T. sinensis* according to the standard curve (mg/mL); V is the volume of the extract extraction solution (mL); N is the dilution multiple; and M is the sample weight (g).

2.4. Experimental design

2.4.1 Single-factor experiment

The single-factor test was used for obtaining the preliminary range of extraction variables. The *T. sinensis* powder (2 g) was conducted in conical flask using a ultrasonic bath (KQ-400KDB, Kunshan Ultrasonic Instrument Co., Ltd., China) soaked with ethanol solvent (varying ethanol concentration from 0 to 90%, v/v; varying liquid-solid ratio from 10 to 50 mL/g) for certain ultrasonic temperature (varying from 15 to 75°C), ultrasonic time (varying from 10 to 90 min) and ultrasonic power (varying from 160 to 360 W). The extracts were centrifuged at 3000 r/min for 6 min with a low-speed centrifuge (Model TDZ5-WS, Changsha Ordinary Instrument Co., Ltd., China), and the supernatant was used for the determination of the total polyphenols content (*TSTPC*).

2.4.2. RSM experiments

A *BBD* of *RSM* was used to determine the optimal combination of extraction variables. Based on the results of single factor experiments, three independent variables (Table 1) were liquid-solid ratio (X_1 , mL/g), ultrasonic temperature (X_2 , °C) and ultrasonic time (X_3 , min), while the response variable was the

extraction yield of *TSTP*. Each variable was designated as three levels, coded + 1, 0 and – 1 for high, intermediate and low value, respectively. The response could be related to the selected variables by the following second-order polynomial model:

Table 1
The coded values and corresponding actual values of the optimization parameters.

Solvent	Code	Liquid-solid ratio(mL/g)	Ultrasonic temperature(°C)	Ultrasonic time(min)
ethanol	-1	30	45	10
	0	40	60	30
	1	50	75	50

$$Y = \sum A_0 + \sum_{i=1}^k A_{ij}X_i + \sum_{i=1}^k A_{ii}X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^k A_{ij}X_iX_j \tag{Eq. (2)}$$

Where *Y* is the response variable; *A*₀, *A*_{*i*}, *A*_{*ii*} and *A*_{*ij*} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively; *X*_{*i*} and *X*_{*j*} are the encoded independent variables (*i* ≠ *j*) affecting the response of *Y*.

2.5. Comparison of optimized UAE with distilled water leaching extraction

A necessary amount of sample (2 g) was weighted and put into a conical flask. Next, the 60 mL of distilled water was added into the flask and then soaked for 30 min. Finally, the solution in the flask was centrifuged at 3000 r/min for 6 min, and the supernatant was used for the determination of the total polyphenols content (*TSTPC*). The extraction yield of the total polyphenols from *T. sinensis* extracted by ultrasonic extraction after the response surface optimization and distilled water leaching extraction was compared.

2.6. Purification of *T. sinensis* polyphenols

2.6.1. Pretreatment of AB-8 macroporous resin

AB-8 macroporous resin was soaked in 95% ethanol for 24 hours before packing in a glass column (20 mm×40 cm). The height of resin was measured to be 18 cm, so the bed volume (BV) of the resin was about 60 mL. The macroporous resin was washed with 95% ethanol until the effluent mixed with water

(1:5) did not become white and turbid. Finally, the column was washed with distilled water until there was no detectable odor of ethanol.

2.6.2. Adsorption and elution experiments

The purification of total polyphenols was investigated according to the procedure described by Yao et al. with some modification (Yao, Zhu, Chen, Tian, & Wang, 2013). 1 BV of the sample solution was packed into the column, and then the column was washed with 2.5 BV of distilled water at a flow rate of 1.5 BV/h. Then the column was eluted with 50% ethanol at a flow rate of 1.5 BV/h. The effluents were collected and polyphenols were monitored by the color reaction with Folin–Ciocalteu reagent. The effluents containing total polyphenols were combined, concentrated and lyophilized. The purified total polyphenols (TSPTP) were stored at 4°C for the following tests. The total polyphenols purity of *T. sinensis* was calculated using the following formula:

$$Purity(\%) = \frac{C \times V}{M} \times 100\% \quad \text{Eq. (3)}$$

where C is the concentration of the total polyphenols of *T. sinensis* (mg/mL); V is the volume of the effluents (mL); and M is the sample weight (g).

2.7 Anti-hyperglycemic activity assay

2.7.1. α -Glucosidase inhibitory activity assay

The α -glucosidase inhibitory activity was determined according to the literature with a slight modification (Striegel, Kang, Pilkenton, Rychlik, & Apostolidis, 2015). A mixture of 40 μ L of sample at various concentrations (0, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/mL, dissolved in 0.1 M phosphate buffer) and 40 μ L of α -Glucosidase solution (1 U/mL, dissolved in 0.1 M phosphate buffer) were incubated in 96-well plate for 5 min at 37°C. Then, 50 μ L of PNPG (10 mM, dissolved in 0.1 M phosphate buffer) was added and kept for 30 min at 37°C. The catalytic reaction was terminated by added 90 μ L Na_2CO_3 solution (0.1 M). Acarbose was used as the positive control. The absorbance was measured at 405 nm with a microplate reader, and the inhibition percentage was calculated as follows:

$$Inhibitionpercentage(\%) = \left[1 - \frac{A_S - A_B}{A_0}\right] \times 100 \quad \% \text{ Eq. (4)}$$

where A_S represents the absorbance of the sample reaction solution; A_B is the absorbance of the reaction system without α -glucosidase; and A_0 is the absorbance of the reaction system without sample.

2.7.2. α -Amylase inhibitory activity assay

The α -amylase inhibitory activity was determined according to a previously published method (Eleazu, Eleazu, & Iroaganachi, 2016). A mixture of 40 μ L of sample at various concentrations (0, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/mL, dissolved in 0.1 M phosphate buffer) and 40 μ L of α -amylase (0.5 mg/mL, dissolved in 0.1 M phosphate buffer) were incubated in 96-well plate at 37°C for 15 min. Then, 20 μ L of 1% starch solution (dissolved in 0.1 M phosphate buffer) was added and kept for 15 min at 37°C. Next, 100 μ L of

3,5-dinitrosalicylic acid were added and heated for 15 min. The mixture was then cooled to room temperature. Acarbose served as the positive control. The absorbance was measured at 540 nm with a microplate reader, and the inhibition percentage was calculated as follows:

$$\text{Inhibition percentage (\%)} = \left[1 - \frac{A_S - A_B}{A_0}\right] \times 100 \quad \% \text{ Eq. (5)}$$

where A_S represents the absorbance of the sample reaction solution; A_B is the absorbance of the reaction system without α -amylase; and A_0 is the absorbance of the reaction system without sample.

2.8 Antioxidant activity assay

2.8.1. Determination of DPPH $\cdot$ scavenging activity

The scavenging activity of TSTP and TSPTP on DPPH $\cdot$ was determined according to the literature method with slight modification (Chen et al., 2019). Mix all samples or vitamin C (VC, 20 μ L, 0 to 3.2 mg/mL, distilled water) with DPPH $\cdot$ solution (180 μ L, 0.2 mM, absolute ethanol). After reacting at room temperature for 20 min in the dark, the absorbance was measured at 517 nm on a microplate reader. The formula for calculating the DPPH $\cdot$ scavenging rate is as follows:

$$\text{Scavenging rate (\%)} = \left[1 - \frac{A_1 - A_2}{A_0}\right] \times 100 \quad \% \text{ Eq. (6)}$$

where A_0 is the absorbance of DPPH $\cdot$ mixed with distilled water; A_1 is the absorbance of DPPH $\cdot$ mixed with the sample; and A_2 is the absorbance of anhydrous ethanol mixed with the sample.

2.8.2. Determination of ABTS $\cdot^+$ scavenging activity

The determination method of ABTS $\cdot^+$ scavenging ability is based on the method of literature and slightly modified (Rozi et al., 2019). 7 mM ABTS and 2.45 mM potassium persulfate were mixed in equal volume, and reacted at 25°C for 12 h in the dark, that is, obtain ABTS $\cdot^+$ stock solution. The stock solution was diluted so that its absorbance at 734 nm was between 0.80 ± 0.02 . Mix all samples or VC (100 μ L, 0 ~ 3.2 mg/mL) with diluted ABTS $\cdot^+$ solution (100 μ L). After reacting at 25°C for 20 min in the dark, the absorbance was measured at 734 nm. The formula for calculating the ABTS $\cdot^+$ scavenging rate is as follows:

$$\text{Scavenging rate (\%)} = \left[1 - \frac{A_1 - A_2}{A_0}\right] \times 100 \quad \% \text{ Eq. (7)}$$

where A_0 is the absorbance of ABTS $\cdot^+$ mixed with distilled water; A_1 is the absorbance of ABTS $\cdot^+$ mixed with the sample; and A_2 is the absorbance of anhydrous ethanol mixed with the sample.

2.9. Statistical analysis

All assays were performed in triplicate, and all values are expressed as means $\pm$ SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) by using Graph Pad Prism6. $p < 0.05$ was

regarded as statistically significant differences.

3. Results And Discussion

3.1. Effect of solvent concentration on yield of TSTP

Solvent concentration played prominent roles in getting high extraction efficiency of total polyphenols (Perez Gutierrez, Martinez Jeronimo, Contreras Soto, Muniz Ramirez, & Estrella Mendoza, 2022). As shown in Fig. 1d, the effect of solvent concentration on extraction yield was investigated in this study. Distilled water and ethanol solution have a certain dissolving effect on the total polyphenols of *T. sinensis*. When the ethanol concentration increased from 0–10%, the extraction yield increased slightly, and when the ethanol concentration was greater than 10%, the extraction yield gradually decreased. However, there was no significant differences between solvent concentration 0% (distilled water), 10%, and 30% ($P > 0.05$). Considering the solvent cost problem, therefore distilled water distilled water was selected as the best solvent for extracting total polyphenols from *T. sinensis*.

3.2. Effect of liquid-solid ratio on yield of TSTP

Liquid-solid ratio played outstanding roles in getting high extraction efficiency of total polyphenols during UAE (Tang et al., 2022). To investigate the effect of liquid-solid ratio on extraction yield of *TSTP*, a liquid-solid ratio range from 10 to 50 mL/g was tested while the ultrasonic power, ultrasonic temperature and ultrasonic time were kept at 160 W, 60°C and 30 min, respectively. Increasing in the tested ratio of ethanol to raw material (from 10 to 50 mL/g) could improve *TSTP* extraction, and the increase leveled off at ratio of 40 mL/g (Fig. 1e). This is because the liquid-solid ratio is increased, and the contact area between *T. sinensis* and the extraction solvent will also increase, which makes the polyphenols exudate faster (Volpi, 2004). However, with the increase of extraction solvent, the longer it takes for the total polyphenols of *T. sinensis* to diffuse. Therefore, within a limited time, with the increase of the liquid-solid ratio, the extraction yield of the total polyphenols of *T. sinensis* grows more and more slowly. When the liquid-solid ratio was 50:1, the extraction yield of total polyphenols increased slowly. As the results of statistical analysis showed that significant differences were for the liquid-solid ratio tested ($P < 0.01$). Therefore, liquid-solid ratio 40 mL/g was chosen as the optimal one in the present experiment. Similar result was obtained in Jovanović's research (Jovanović et al., 2022).

3.3. Effect of ultrasonic temperature on yield of TSTP

Ultrasound has a cavitation effect, which can promote the collision of the extracts, so that the effective ingredients are quickly and fully dissolved in the solvent (Senrayan & Venkatachalam, 2020). In the present study, the effect of various ultrasonic temperature points within 15–75°C used on extraction yield of *TSTP* was investigated, while keeping the liquid-solid ratio, ultrasonic power and ultrasonic time at 40 mL/g, 160 W and 30 min, respectively. As shown in Fig. 1a, *TSTP* content in extract assay increased with the increase of ultrasonic temperature from 15 to 60°C, and peaked at around 60°C. Further enhancing of temperature, however, resulted in decreasing *TSTP* content in extract. The possible

reason for this phenomenon is that when the temperature rises, the thermal movement of the molecules intensifies, which is conducive to the exudation and diffusion of polyphenols, resulting in the extraction yield of the total polyphenols from *T. sinensis* gradually rising below 60 °C with the increase of temperature (Pompeu, Silva, & Rogez, 2009). However, high temperature may destroy the polyphenols in *T. sinensis*, so when the temperature exceeded 60°C, the extraction yield of total polyphenols decreased instead (Jing, Dong, & Tong, 2015). As the results of statistical analysis showed that no significant differences were for the ultrasonic temperature tested ($P > 0.05$). Thus, the power chosen for *TSTP* extraction was 60°C. This was identical with the result reported by Zheng et al. (Zheng et al., 2022).

3.4. Effect of ultrasonic time on yield of TSTP

Ultrasonic time played significant roles in getting high extraction efficiency of total polyphenols (Perez Gutierrez et al., 2022). It will have an effect on the final yield of *TSTP* in the recovery, the energy cost and the efficiency of extraction. In this study, an increase of *TSTP* extraction was observed with the elevation of ultrasonic time from 10 to 90 min, reaching the highest at 70 min, but further increase of ultrasonic time resulted in decreasing *TSTP* content (Fig. 1c). It may be because the longer the ultrasound is, the structure of some polyphenols changes (Liyanapathirana & Shahidi, 2005). However, there was no significant differences between ultrasonic time 30 min, 50 min, and 70 min ($P > 0.05$). Thus, the ultrasonic time 30 min was chosen as the optimal one based on the study. This conclusion agreed with the opinion of Yu et al. (Perez Gutierrez et al., 2022).

3.5. Effect of ultrasonic power on yield of TSTP

It is very important for polyphenols extraction to keep ultrasonic at an optimal working power (Yang, Li, Wang, Chang, & Jiang, 2021). In the present study, the effect of various ultrasonic power points within 160–360 W used on extraction yield of *TSTP* was investigated, while keeping the liquid-solid ratio, ultrasonic temperature and ultrasonic time at 40 mL/g, 60°C and 30 min, respectively. As shown in Fig. 1b, *TSTP* content in extract assay increased with the increase of ultrasonic power from 160 to 200 W, and peaked at around 200 W. The reason for the increase in the extraction yield may be that the stronger the ultrasonic power, the stronger the cavitation effect produced by the ultrasonic, and the faster the polyphenols in *T. sinensis* will exude and diffuse from the cells (Zheng et al., 2022). Further enhancing of power, however, resulted in decreasing *TSTP* content in extract. The reason for the decrease in the extraction yield may be that the power is too high, which not only destroys the cell wall of the *T. sinensis*, but even destroys the structure of some polyphenols in the cells, resulting in a decrease in the extraction yield of total polyphenols (Tian et al., 2019). As the results of statistical analysis showed that no significant differences were for the ultrasonic power tested ($P > 0.05$). Thus, the power chosen for *TSTP* extraction was 200 W. This was identical with the result reported by Yang et al. (Yang et al., 2021).

3.6. Optimization of extraction conditions of TSTP

3.6.1. Statistical analysis and the model fitting

Response surface optimization method can evaluate the interaction between multiple parameters. Compared with orthogonal experiments, response surface optimization can continuously analyze all levels of the experiment, which can not only reduce the number of experiments and shorten the time, but also ensure the accuracy and effectiveness of the results. The *BBD* of *RSM* in the experimental design involves three independent variables, three levels and five replicates at the center point (Table 2), which was carried out to measure the inherent variability and process stability. The experimental conditions and the fit statistics of extraction yield of 17 runs with *BBD* design were shown in Table 2, and all tests were performed in triplicate. As shown in Table 2, the extraction yield of *TSTP* values (mg/g) varied from 4.49 to 8.10%.

Table 2
The coded experimental and predicted for RSM design using distilled water as solvent.

Run	X1	X2	X3	Extraction yield (%)
1	-1	-1	0	5.568
2	1	-1	0	7.051
3	-1	1	0	4.494
4	1	1	0	7.041
5	-1	0	-1	5.532
6	1	0	-1	8.096
7	-1	0	1	5.635
8	1	0	1	7.667
9	0	-1	-1	6.758
10	0	1	-1	6.047
11	0	-1	1	6.343
12	0	1	1	6.523
13	0	0	0	7.203
14	0	0	0	8.046
15	0	0	0	7.246
16	0	0	0	7.925
17	0	0	0	7.298

The results of extraction yield affected by liquid-solid ratio, ultrasonic temperature and ultrasonic time were fitted to a second-order polynomial equation, and the values of regression coefficients were calculated.

The effects of three variables were highly significant on extraction yield of *TSTP* (Table 3). The predicted model of the extraction yield value was obtained by the following second-order polynomial equations:

Table 3
ANOVA for the effects of liquid-solid ratio (X_1), ultrasonic temperature (X_2) and ultrasonic time (X_3) on extraction yield of *TSTP* with distilled water as solvent using predicted polynomial models.

Source	Sum of squares	Df	Mean square	F-Value	P-Value	Significant ^a
Model	15.80	9	1.76	16.17	0.0007	***
X_1	9.30	1	9.30	85.66	0.0001	***
X_2	0.33	1	0.33	3.00	0.1267	
X_3	8.799×10^{-3}	1	8.799×10^{-3}	0.081	0.7842	
X_1X_2	0.28	1	0.28	2.61	0.1504	
X_1X_3	0.071	1	0.071	0.65	0.4460	
X_2X_3	0.20	1	0.20	1.83	0.2184	
X_1^2	1.49	1	1.49	13.73	0.0076	**
X_2^2	3.49	1	3.49	32.09	0.0008	***
X_3^2	0.20	1	0.20	1.80	0.2212	
Residual	0.76	7	0.11			
Lack of fit	0.097	3	0.032	0.19	0.8948	not significant
Pure Error	0.66	4	0.17			
Cor Total	16.56	16				

$$Y_1 = 7.54 + 1.08X_1 - 0.20X_2 - 0.033X_3 + 0.27X_1X_2 - 0.13X_1X_3 + 0.22X_2X_3 - 0.60X_1^2 - 0.91X_2^2 - 0.22X_3^2 \text{ Eq. (6)}$$

The predicted values of extraction yield based on the above quadratic predictive model were shown in Table 2.

The statistical significance of regression equation was evaluated by *F*-test, *T*-test and *AVNOA* for response surface quadratic polynomial model were presented in Table 4. The results of high model *F*-value (16.17) and low *P*-value (*P*= 0.0007) turned out that the models were highly significant. The determination coefficient (*R*²) for model (0.8437) was close to 1.0, which represented the satisfactory correlation between actual and predicted values.

Table 4
Result of model validation experiments.

NO.	Optimum conditions			Extraction yield (%)	
	Liquid-solid ratio (mL/g)	Ultrasonic temperature (°C)	Ultrasonic time (min)	Experimental	Predicted
1	50	60	22	8.33	8.06
2	50	60	22	7.82	8.06
3	50	60	22	7.93	8.06
4	50	60	22	8.17	8.06
5	50	60	22	8.41	8.06
Average				8.13	
Distilled water Leaching Extraction					
6	50	60	22	5.49	
7	50	60	22	5.26	
8	50	60	22	5.58	
Average				5.44	

The lack-of-fit used to measure the failure of the model to represent the data in the experimental domain at points which were not included in the regression. The *F*-value of 0.19 and *P*-value of 0.8949 for extraction yield implied that the lack of fit was not significant relative to the pure error due to noise. Adequate precision compared the range of the predicted values at the design points to the average prediction error. The ratio greater than 4 indicated adequate model discrimination. In this research, the values were well above 4.

The P -values were used as a tool to check the significance of each coefficient, which in turn may indicate the pattern of the interactions between the variables. The smaller the value of P was, the more significant the corresponding coefficient was. It can be seen from Table 3 that linear coefficients (X_1 , X_2 , X_3) and quadratic term coefficient (X_1^2) were very significant with P values ($P < 0.01$). The quadratic term coefficient (X_2^2) was significant ($P < 0.05$).

3.6.2. Analysis of response surfaces

The 3D response surface and 2D contour plots were the graphical representations of regression equation. They provided a method to visualize the relationship between responses and experimental levels of each variable and the type of interactions between two test variables. The shapes of the contour plots, circular or elliptical, indicate whether the mutual interactions between the variables are significant or not. Circular contour plot indicates that the interactions between the corresponding variables are negligible, while elliptical contour plot indicates that the interactions between the corresponding variables are significant. In this study, the results of extraction yield of *TSTP* affected by solvent concentration, liquid-solid ratio and ultrasonic time is presented in Figs. 2, 3.

As shown in Fig. 3a, the contour plot was similar to an ellipse, indicating that the liquid-solid ratio and ultrasonic temperature have a certain interactive effect on the extraction yield of the total polyphenols of *T. sinensis*. As shown in Fig. 2a, the 3D response surface plot of liquid-solid ratio was steeper than that of ultrasonic temperature, indicating that the liquid-solid ratio has a greater influence on the extraction yield of the total polyphenols from *T. sinensis* than that of ultrasonic temperature, which is consistent with the conclusion drawn by statistical analysis and the model fitting analysis. Figures 2a and 3a, which give the extraction yield of *TSTP* as a function of liquid-solid ratio and ultrasonic temperature at fixed ultrasonic time (30 min), indicated that the extraction yield increased rapidly with increase in liquid-solid ratio from 30 to 48 mL/g, and increased with the increase of ultrasonic temperature from 45 to 60°C. However, the extraction yield decreased slowly with the liquid-solid ratio increasing from 48 to 50 mL/g and ultrasonic temperature from 60 to 75°C. It can be seen that the maximum extraction yield of *TSTP* can be achieved when liquid-solid ratio and ultrasonic temperature are around 48 mL/g and 60°C, respectively.

As shown in Fig. 2b, the 3D response surface plot of liquid-solid ratio was steeper than that of ultrasonic time, which shows that the influence of liquid-solid ratio on the extraction yield of total polyphenols is greater than that of ultrasonic time, which is the same as the conclusion drawn by statistical analysis and the model fitting analysis. The contour plot in Fig. 3b presented an ellipse, which shows that the interaction between liquid-solid ratio and ultrasonic time has a greater impact on the extraction yield of the total polyphenols from *T. sinensis*. Figures 2b and 3b, which give the extraction yield of *TSTP* as a function of liquid-solid ratio and ultrasonic time at fixed ultrasonic temperature (60°C), indicated that the extraction yield of *TSTP* increased with the increase of liquid-solid ratio from 30 mL/g to 48 mL/g, extraction yield of *TSTP* reached the plateau region where the yield was maximized and did not further increase the yield. The extraction yield of *TSTP* increased with the increase of ultrasonic time from 10 to

25 min. It can be seen that the maximum extraction yield of *TSTP* can be achieved when liquid-solid ratio and ultrasonic time are around 48 mL/g and 25 min, respectively.

The 3D response surface and 2D contour plots based on the independent variable ultrasonic temperature and ultrasonic time were shown in Figs. 2c and 3c, while the other one independent variable, liquid-solid ratio was kept at 40 mL/g. As shown in Fig. 3c, the 3D response surface plot of ultrasonic temperature was steeper than that of ultrasonic time, indicating that the ultrasonic temperature has a greater influence on the extraction yield of the total polyphenols from *T. sinensis* than that of ultrasonic time, which is consistent with the conclusion drawn by statistical analysis and the model fitting analysis. The extraction yield increased rapidly with increase in ultrasonic temperature from 45 to 62°C and decrease with increase of ultrasonic temperature from 62 to 75°C. It was observed that the extraction yield of *TSTP* increased with the ultrasonic time from 10 to 24 min, and reached the plateau region where the yield was maximized and did not further increase the yield. It can be seen that the maximum extraction yield of *TSTP* can be achieved when ultrasonic temperature and ultrasonic time are around 62°C and 24 min, respectively.

3.7. Verification of predictive model

Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw material. Response surface analysis was conducted through Design-Expert, and the optimized extraction conditions were liquid-solid ratio 50 mL/g, ultrasonic temperature 59.81°C and ultrasonic time 22.17 min. In order to validate the adequacy of the model equations, verification experiment was carried out under the actual optimal conditions: liquid-solid ratio 50 mL/g, ultrasonic temperature 60°C and ultrasonic time 22 min considering the feasibility of actual operation. Good agreement exist between the values predicted using model equations and the experimental values at the points of interest. To ensure the predicted result was not biased toward the practical value, experimental rechecking was performed using this deduced optimal condition. This set of conditions was determined to be optimal by the *RSM* optimization approach and was also used to validate experimentally and predict the values of the response using the model equation. The mean value of extraction yield (8.13%) obtained from real experiments, demonstrated the validation of *RSM* model. The predicted extraction yield of the total polyphenols of *T. sinensis* was 8.06%. The validation result revealed that there was no significant difference between experimental and predicted values, suggesting that the response model was adequate for reflecting the expected optimization. This result of analysis indicated that the experimental values were good agreement with the predicted ones, and also suggested that the model of Eq. (6) is satisfactory and accurate. So the response surface method is reliable to optimize the extraction process of *T. sinensis*.

3.8. Comparison analysis of optimized UAE with distilled water leaching extraction

The purpose of this experiment is to provide a relatively efficient and simple extraction process for the development and utilization of the total polyphenols of *T. sinensis*. Therefore, based on the extraction

yield, the optimal process conditions obtained in this experiment was compared with the traditional water extraction method to judge the effect of the optimized extraction process. Distilled water leaching extraction were compared with the UAE method, a mean value of extraction yield (5.44%) obtained from the distilled water leaching extraction. As compared to distilled water leaching extraction, UAE after the response surface optimization had higher extraction yield of 8.13%, which was about 49.44% higher than that of distilled water leaching extraction (5.44%). Therefore, this finding corroborates previous reports that with respect to total polyphenols content, ultrasonic has its superiority in improving efficiency, shortening extraction time, reducing solvent consumption. The total polyphenols content reported herein is higher than those previously reported. This may have been partly due to increased extraction efficiency.

3.9. Purification analysis of TSPTP

The collected 50% ethanol eluate was developed by the color reaction with Folin–Ciocalteu reagent to monitor the total polyphenols content. The elution profile was obtained based on the volume of elution and the concentration of solute therein and is given in Fig. 4. It can be seen from the figure that the total polyphenols were completely eluted by approximately 115 mL eluent at a flow rate of 1.5 BV/h. As compared to the purity of the unpurified total polyphenols (TSTP), the purity of the total polyphenols of *T. sinensis* after purification (TSPTP) by the macroporous resin had higher purity of 57.70%, which was about 45.33% higher than that of TSTP (12.37%).

3.10 Anti-hyperglycemic activity of TSPTP in vitro

3.10.1. Inhibitory effect of TSPTP on α -glucosidase activity

The inhibition percentage of α -glucosidase can be used to measure the anti-diabetic effect of drugs, as α -glucosidase inhibitors can significantly reduce postprandial blood glucose levels, which is a key factor in the treatment of DM (Ademiluyi, Oboh, Boligon, & Athayde, 2014). The α -glucosidase inhibitory activities of acarbose and TSPTP were determined, and the results were shown in Fig. 5. As shown in Fig. 5, TSPTP showed dose-dependent inhibitory effect on α -glucosidase activity when the concentration of total polyphenols was in the range of 0.1–3.2 mg/mL. The IC_{50} values of TSPTP was determined to be 0.201 mg/mL. TSPTP strongly inhibited α -glucosidase, with an IC_{50} value of 0.201 mg/mL, which was close to that of acarbose (0.004 mg/mL). In the range of 0.1–3.2 mg/mL, the inhibition percentage of acarbose reached more than 90%, and the inhibition percentage of TSPTP was 84.66% at a concentration of 3.2 mg/mL, which was close to that of acarbose. After purification, the inhibition percentage of the total polyphenols from *T. sinensis* was relatively high, indicating that the total polyphenols from *T. sinensis* have development value in the direction of being an α -glucosidase inhibitor. TSPTP had outstanding α -glucosidase inhibitory activity, which was even higher than that of the polyphenol-rich extracts extracted from *Malva neglecta* Wallr. (14.19 mg/mL) (Türker & Dalar, 2013). The result suggested that TSPTP may be the active component responsible for the anti-diabetic activity of *T. sinensis*.

3.10.2. Inhibitory effect of TSPTP on α -amylase activity

The α -amylase can break down dietary starch and glycogen to produce glucose and maltose. Thus, the inhibition of α -amylase activity can delay blood glucose level elevation, which is an important aspect in the treatment of DM (Lordan, Smyth, Soler-Vila, Stanton, & Ross, 2013). The α -amylase inhibitory activities of acarbose and TSPTP were determined, and the results were shown in Fig. 6. As shown in Fig. 6, the α -amylase inhibitory activities of all samples correlated positively with increasing concentrations in the range of 0.1–3.2 mg/mL. The IC_{50} value of TSPTP was determined to be 0.041 mg/mL. TSPTP strongly inhibited α -amylase, with an IC_{50} value of 0.041 mg/mL, which was less than that of acarbose (0.103 mg/mL). In the range of 0.1–3.2 mg/mL, the inhibition percentage of acarbose reached 99.44%, and the inhibition percentage of TSPTP was 88.18% at a concentration of 3.2 mg/mL, which was close to that of acarbose. After purification, the inhibition percentage of the total polyphenols from *T. sinensis* was relatively high, indicating that the total polyphenols from *T. sinensis* have development value in the direction of being an α -amylase inhibitor. TSPTP exhibited remarkable α -amylase inhibitory activity, which was even higher than that of the polyphenol-rich extracts extracted from *Lonicera caerulea* berry (0.30 mg/mL) (Liu, Yu, Guo, Fang, & Chang, 2020).

3.11 Antioxidant activity of TSPTP in vitro

3.11.1. DPPH $\cdot$ scavenging activity of TSPTP

The DPPH $\cdot$ scavenging experiment is a classic method for measuring the antioxidant capacity of foods in the food industry and agriculture. The DPPH $\cdot$ scavenging activities of VC and TSPTP were determined, and the results were shown in Fig. 7. As shown in Fig. 7, the total polyphenols of *T. sinensis* before and after purification showed dose-dependent scavenging effects on DPPH $\cdot$ scavenging activity when the concentration of total polyphenols was in the range of 0.1–3.2 mg/mL. The IC_{50} values of VC and TSPTP were determined to be 0.03 and 0.27 mg/mL. In the range of 0.1–3.2 mg/mL, the inhibition percentage of VC reached more than 90%, and the inhibition percentage of TSPTP was 82.80% at a concentration of 3.2 mg/mL, which was close to that of VC. After purification, the scavenging rate of the total polyphenols from *T. sinensis* has been improved, and the scavenging rate was relatively high, indicating that the total polyphenols from *T. sinensis* have development value in the direction of being an antioxidant. TSPTP had outstanding DPPH $\cdot$ scavenging activity, which was even higher than that of the polyphenol-rich extracts extracted from *Hypericum perforatum* (0.43 mg/mL) and *Achillea millefolium* (0.51 mg/mL) (Becker et al., 2016). The result suggested that TSPTP may be the active component responsible for the antioxidant activity of *T. sinensis*.

3.11.2. ABTS $\cdot^+$ scavenging activity of TSPTP

ABTS $\cdot^+$ is a type of free radical that can be scavenged by polyphenols. The ABTS $\cdot^+$ scavenging activities of VC and TSPTP were determined, and the results were shown in Fig. 8. As shown in Fig. 8, the ABTS $\cdot^+$ scavenging activities of all samples correlated positively with increasing concentrations in the range of

0.1–3.2 mg/mL. The IC_{50} values of VC and TSPTP were determined to be 0.05 and 0.07 mg/mL. TSPTP strongly scavenged $ABTS^{\cdot+}$, with an IC_{50} value of 0.07 mg/mL, which was close to that of VC (0.05 mg/mL). In the range of 0.1–3.2 mg/mL, the scavenging rate of VC reached 100.00%, and the scavenging rate of TSPTP was 96.50% at a concentration of 3.2 mg/mL, which was close to that of VC. After purification, the scavenging rate of the total polyphenols from *T. sinensis* was relatively high, indicating that the total polyphenols from *T. sinensis* have development value in the direction of being an antioxidant. TSPTP exhibited remarkable $ABTS^{\cdot+}$ scavenging activity, which was even better than that of the polyphenol-rich extracts extracted from *Chromolaena odorata* (1.32 mg/mL) (Srinivasa Rao, Chaudhury, & Pradhan, 2010).

4. Conclusions

In the present study, ultrasonic assisted extraction method and distilled water leaching extraction method were screened for the extraction treatment of *T. sinensis*, and the extracts exhibited different yields. Ultrasonic assisted extraction method was found to be the most effective one for improving yield. In the case of ethanol as solvent, optimal extraction conditions for ultrasonic assisted extract of *TSPTP* are obtained as follows conditions: ultrasonic power 200 W, liquid-solid ratio 50 mL/g, ultrasonic temperature 60°C and ultrasonic time 22 min. Under this condition, the mean experimental value of extraction yield (8.13%) was achieved, which corresponds well with the predicted value and was about 49.44% higher than that of distilled water leaching extraction. As compared to *TSTP*, *TSPTP* had higher purity of 57.70%, which was nearly 45.33% higher than that of *TSTP* (12.37%). *TSPTP* had higher α -glucosidase and α -amylase inhibitory activities as well as *DPPH* $^{\cdot}$ and $ABTS^{\cdot+}$ scavenging activities with an IC_{50} value of 0.201, 0.041, 0.27 and 0.07 mg/mL. The above results suggest that *TSPTP* has improved in vitro hypoglycemic and antioxidant activities, which provides a scientific basis for the further development and utilization of the total polyphenols of *T. sinensis* in the hypoglycemic and antioxidant activities and provides a new development direction for the treatment of DM. Further studies are worthy to investigate the relative structure and pharmacological mechanism. The above results suggest that *TSPTP* are the active components responsible for the anti-diabetic effects through α -glucosidase and α -amylase inhibitory activities. Therefore, *TSPTP* could be used as anti-diabetic agent for preventing and curing DM in the future, and further studies are worthy to investigate the relative pharmacological mechanism.

Declarations

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Author Contributions

Methodology, Y.W.; investigation, Y.W. and X.M.; formal analysis, Y.W. and X.M.; resources, Y.W.; writing—original draft preparation, Y.W.; writing—review and editing, Y. W.; supervision, Y.W.; project administration, Y.W.; funding acquisition, Y.W..

Ethics declarations

Ethics Approval

This article contains no studies with human participants or animals performed by any of the authors.

Consent to Participate

Not applicable. This study did not use any human or animal subjects.

Conflict of Interest

The authors declared that they have no conflicts of interest to this work.

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Figures

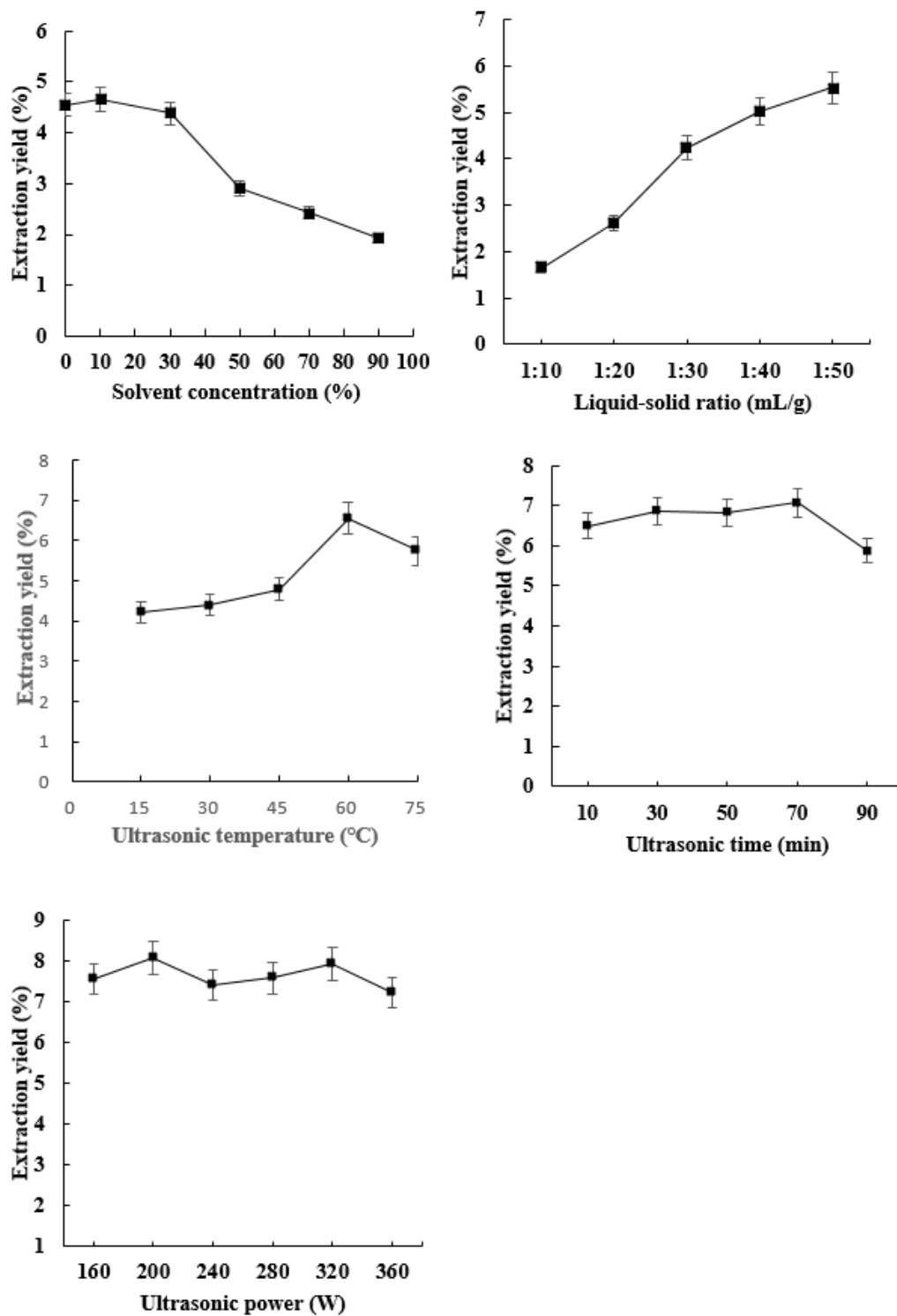


Figure 1

The effect of different solvent concentration (a), liquid-solid ratio (b), ultrasonic temperature (c), ultrasonic time (d), and ultrasonic power (e) on the extraction yield of TSTP.

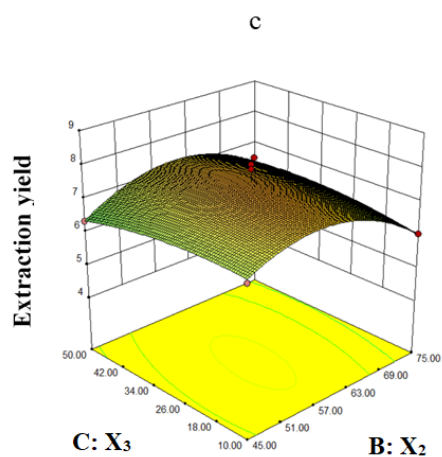
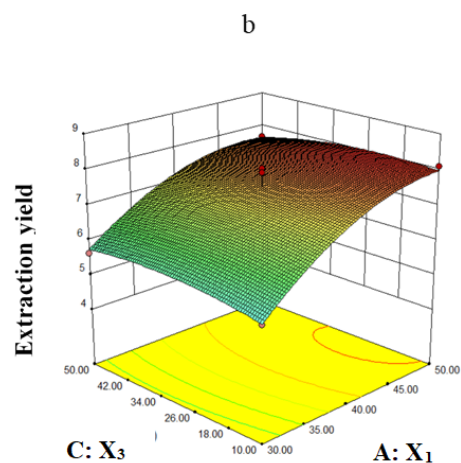
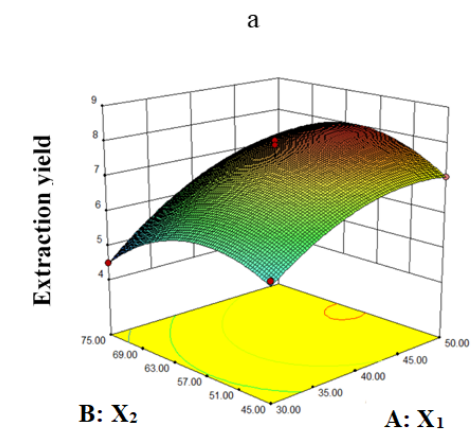


Figure 2

Response surface (3D) plots showing the effect of liquid-solid ratio and ultrasonic temperature (a), liquid-solid ratio and ultrasonic time (b), ultrasonic temperature and ultrasonic time (c) on extraction yield of *TSTP*.

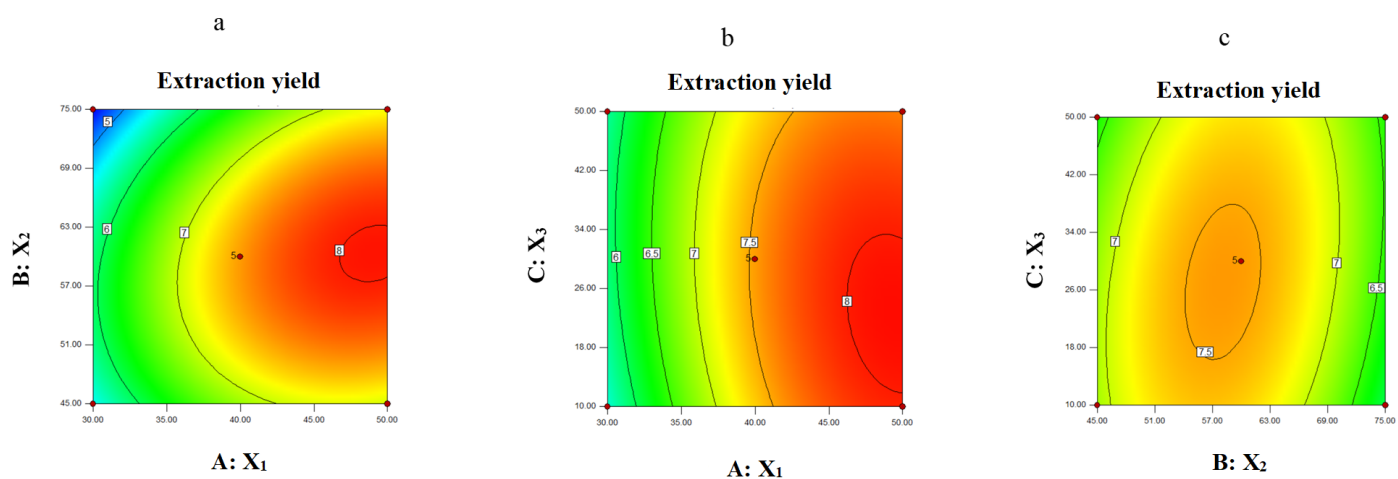


Figure 3

Contour plot showing the effect of liquid-solid ratio and ultrasonic temperature (a), liquid-solid ratio and ultrasonic time (b), ultrasonic temperature and ultrasonic time (c) on extraction yield of *TSTP*.

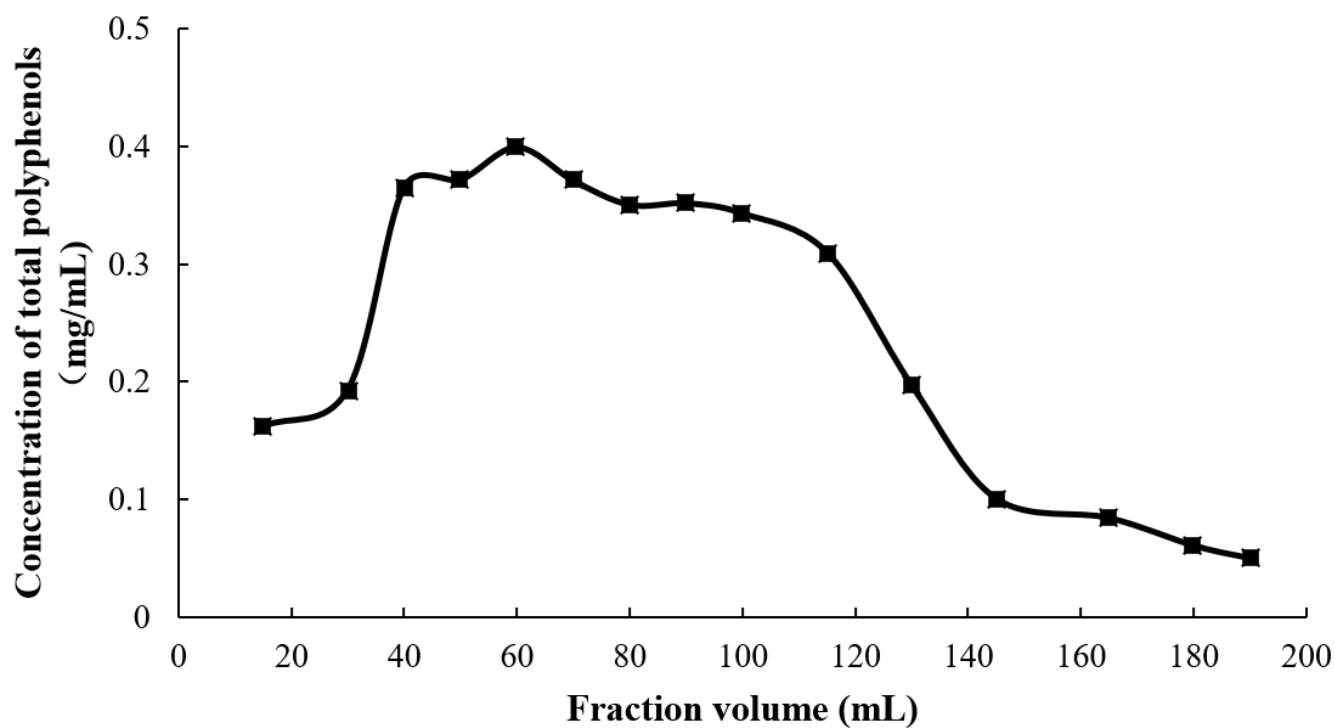


Figure 4

Elution profile of TSPTP on AB-8 macroporous resin column.

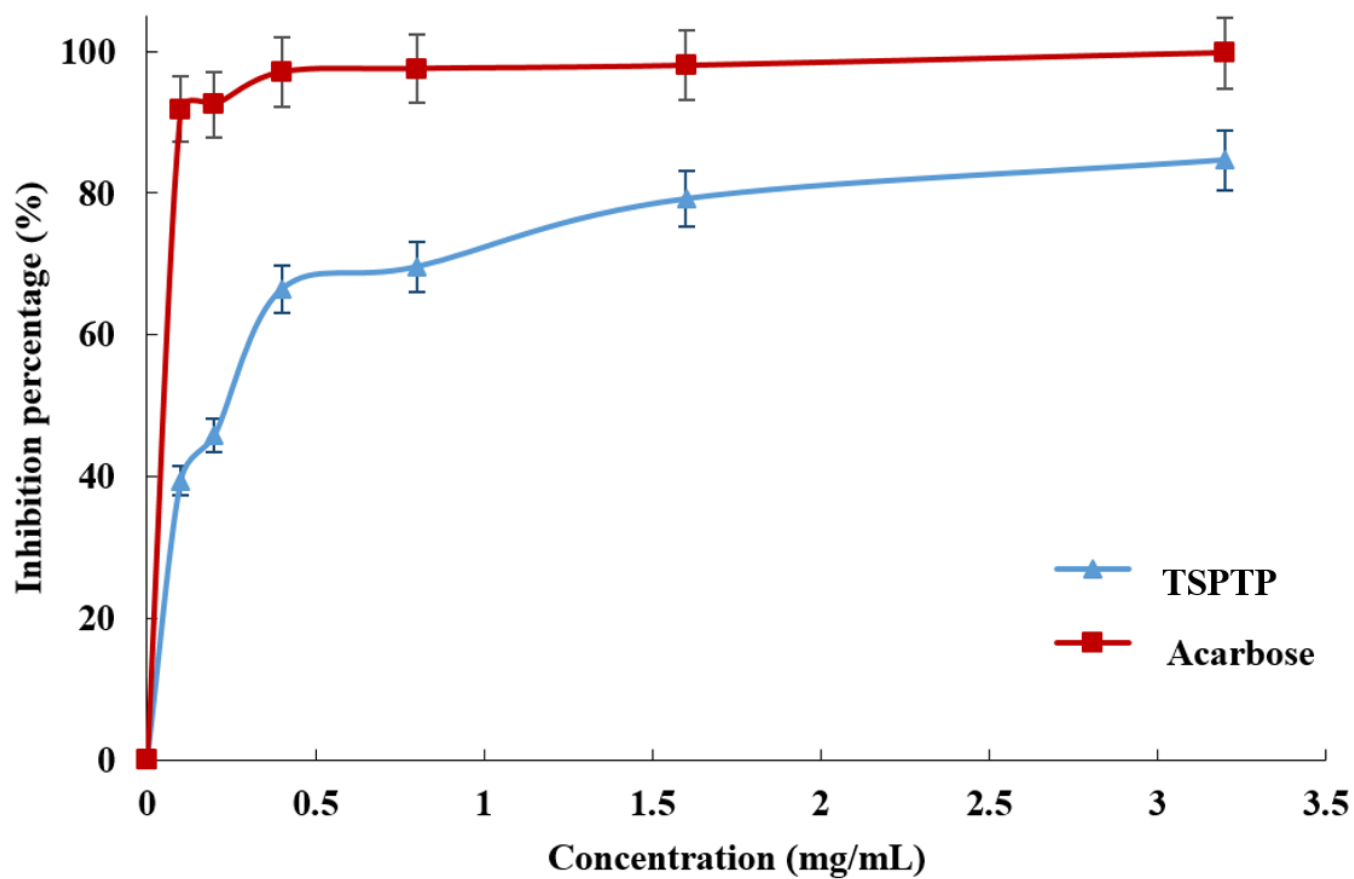


Figure 5

α -Glucosidase inhibitory activities of TSTP and TSPTP.

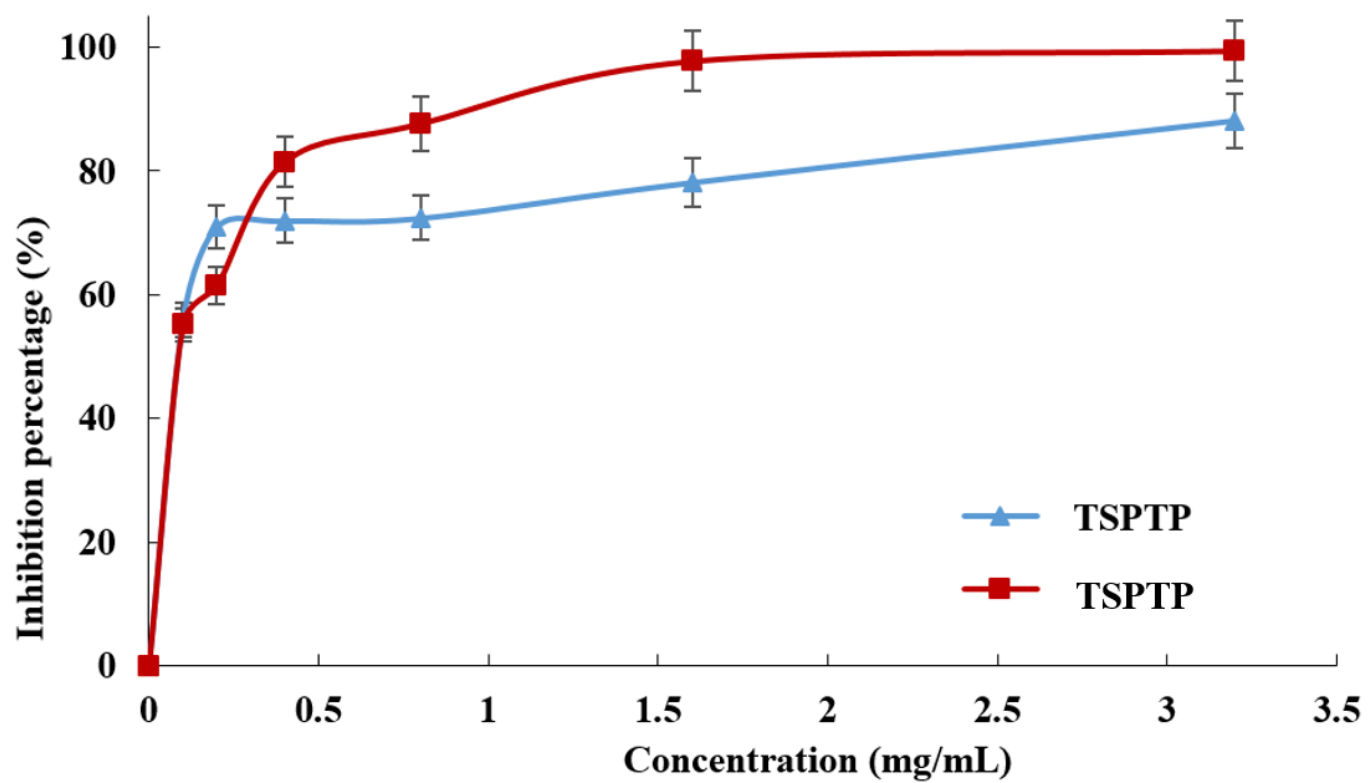


Figure 6

α -Analyse inhibitory activities of TSTP and TSPTP.

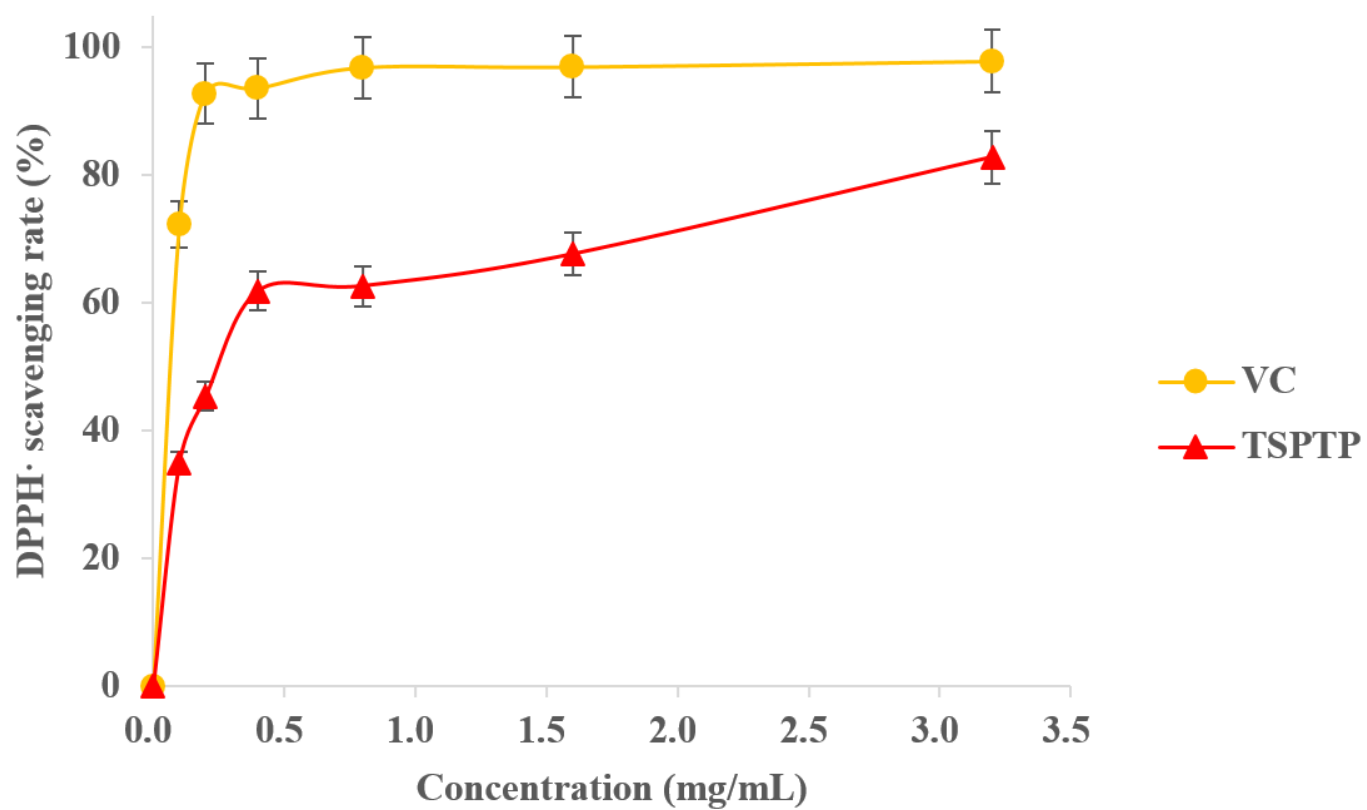


Figure 7

DPPH· scavenging activities of TSTP and TSPTP.

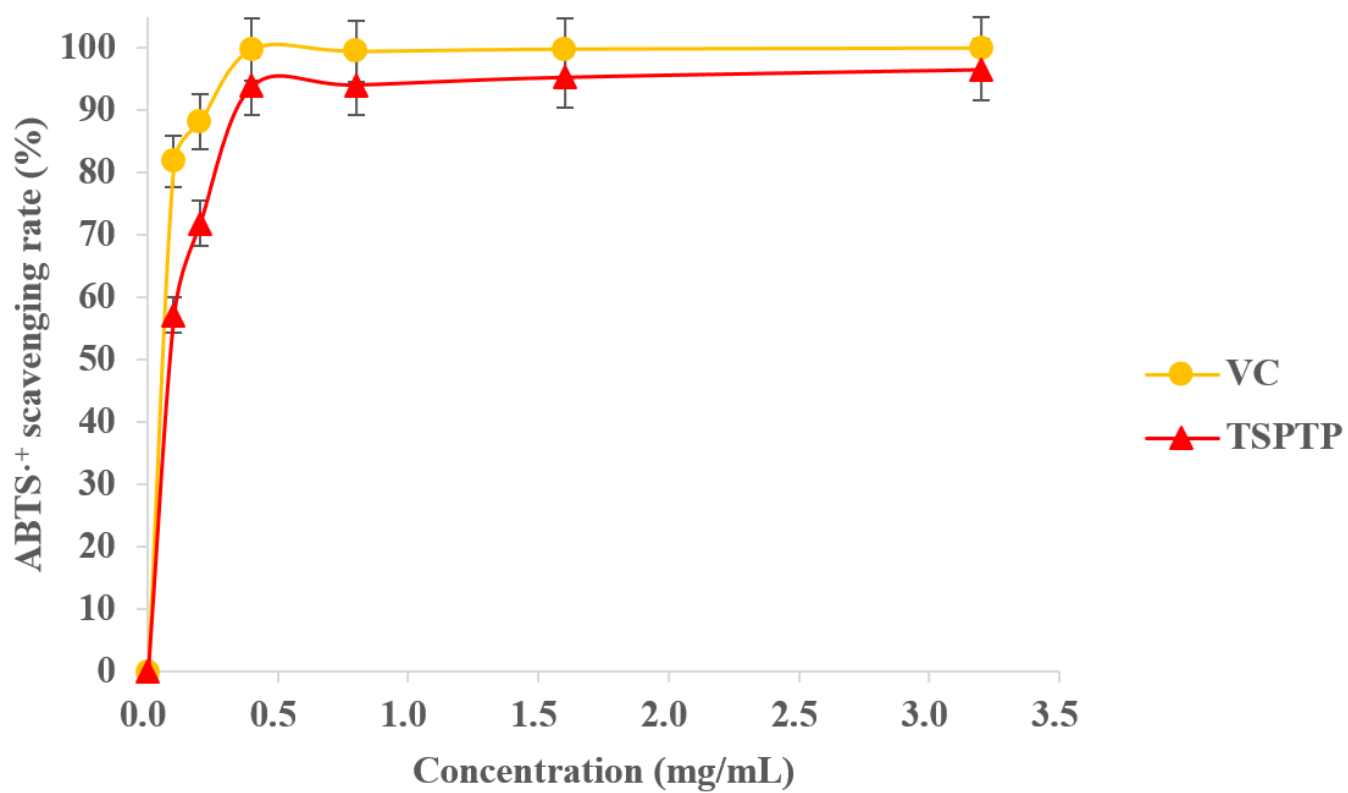


Figure 8

ABTS·⁺ scavenging activities of TSTP and TSPTP.