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ORIGINAL ARTICLE

The application of deep eutectic solvent on the extraction and in vitro antioxidant activity of rutin from *Sophora japonica* bud

Fei Peng[1,2] · Pei Xu[1,2] · Bing-Yi Zhao[1,2] · Min-Hua Zong[2] · Wen-Yong Lou[1,2]

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Abstract The extraction conditions and antioxidant activities of rutin from *Sophora japonica* bud by deep eutectic solvents were investigated. Box–Behnken design was used to optimize the extraction conditions and the scavenging activities of DPPH, O^{2-} and ·OH of purified rutin were evaluated. The highest yield of 279.8 mg/g was achieved in the extraction medium of choline chloride/triethlene glycol (1/4) under the optimum conditions: water content of the DES 18.1%, extraction time 28.3 min, extraction temperature 70 °C and liquid–solid ratio 10 mg/1 g. The highest extraction amount was slightly different from the predicted value of the established second-order polynomial equation. In addition, The EC_{50} of DPPH scavenging, O^{2-} scavenging and ·OH scavenging of rutin were 5.68 μg/mL, 0.19 and 0.28 mg/mL, respectively. The above results indicate rutin extracted by the choline chloride/triethylene glycol has excellent antioxidant activity and was an admirable free radical scavenger.

Keywords Rutin · Choline chloride/triethylene glycol · *Sophora japonica* bud · Antioxidant activity

Introduction

The external stimuli can give rise to an improvement of free radicals in the human body (Osińska-Jaroszuk et al. 2015; Ye et al. 2016), resulting in the oxidative stress increase (Adibhatla and Hatcher 2010) and high risks of many diseases, such as dementia, diabetes mellitus, dyslipidemia, hypertension (Xu et al. 2014; Wu et al. 2015). The uptake of antioxidant is in certain degree helpful to get rid of the oxidant in body and keep healthy.

Rutin is a crucial bioactive substance (Li et al. 2012; Nam et al. 2015). Numbers of excellent pharmacological properties are set in the molecular, such as anti-inflammatory, antithrombotic, anti-tumor and antidiabetic (Xu et al. 2014; Chua 2013; Niture et al. 2014; Ren et al. 2003). According to Dietary Supplement Label Database, over 860 products containing rutin are selling in America (Gullón et al. 2017). In the recent, Chinese herbal medicine has attracted considerable interest (Chua 2013) because of the existence of high bioactive compounds and low by-effect for health (Huang et al. 2016; Niture et al. 2014). *Sophora japonica* (*S. japonica*) as a conventional herb is officially listed in the Chinese Pharmacopoeia and widely planted in China, Japan, and Korea (Qi et al. 2007; Wang et al. 2003; Kim and Yun-Choi 2008). Its buds and fruits possess the stypticity (Qi et al. 2007). Also, its buds are rich in rutin. The intake amount over 50 and 500 mg of rutin dose per day would be a benefit for preventive and curative effects on the human body, respectively (Brunori et al. 2009). The disadvantages of low concentration in the raw and processing instability limit the effective recovery of rutin in the final products (Brunori et al. 2009; Cho and Lee 2015).

Among the conventional methods on the rutin extraction, abundant organic solvents, such as methanol and

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✉ Wen-Yong Lou
wylou@scut.edu.cn

[1] Laboratory of Applied Biocatalysis, School of Food Science and Engineering, South China University of Technology, Guangzhou 510640, China

[2] Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, South China University of Technology, Guangzhou 510640, China

ethanol, are often used because of poor solubility in aqueous phase (Xi and Luo 2015). Organic solvents are generally toxic, volatile and environmentally unfriendly. To overcome the shortcomings, deep eutectic solvents (DESs) are developing through hydrogen bond interaction among two or three cheap and safe components (Flores-Ferrándiz and Chinchilla 2017; Lu et al. 2016; Zhang et al. 2012). Many delightful properties are set in the solvents, for instance, low melting point, non-toxic, negligible volatility and alternatively designed. Certain publications described that rutin can be effectively extracted by DESs (Cho and Lee 2015; Faggian et al. 2016). However, the information on rutin extracted from *S. japonica* bud by DESs is still scarce. In the present work, we optimized the extraction condition of rutin by choline chloride/triethylene glycol (ChCl/TEG) and further focused on the evaluation of antioxidant activities of the purified rutin.

Materials and method

Materials

Sophora japonica bud was purchased from Zhanjiang Yizhou Medicines Co., Ltd. (Guangdong, China). The standard rutin was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). All other chemicals were from commercial sources and of analytical grade.

Rutin extraction

In the typical single factor experiment, powdered *S. japonica* bud (1.00g, mesh less than 60) was mixed with certain volume of DESs-containing appropriate water in a flask. Then, the flask was thermostatically heated in a water bath with continuous stirring at 240 rpm for relevant time. Next, the mixture was centrifuged at 12,000g for 10 min to achieve the supernatant. The supernatant was further used for determining the concentration of rutin by high-performance liquid chromatography (HPLC).

According to the single-factor experiments, three factors (water concentration of DES, extraction time, extraction temperature) were considered as the main factors on the extraction efficiency. The levels of two other factors were decided by the single-factor experiments. The optimization of the three main factors was further conducted in the Box–Behnken design (BBD). A 17-run, 3-factor, 3-level BBD was applied to establish polynomial models for the optimization process in Design-Expert soft.

Purification and structure analysis of rutin

The rutin extracts were further purified with AB-8 macroporous resin eluted with adequate distilled water, then 70% ethanol. The eluted fractions of ethanol were collected and concentrated by a rotary evaporator. The concentrated supernatant was acidized to pH 3.0 by the diluted hydrochloric acid, and the mixture was maintained at 4 °C for 18 h. The precipitate from the last step was collected by filtration, washed with distilled water to receive crude rutin. The rough rutin was heatedly dissolved in calcium hydroxide solution and filtrated. Acid solution was added into the filtrate, and the mixture was maintained at a low temperature, crystalized again, filtrated, washed with distill water and finally dried to achieve the purified rutin.

After drying under vacuum at 40 °C for 48 h, the structure determination of rutin was conducted with an Avance digital 400 MHz Bruker Spectrometer made in Germany. In addition, the purity of rutin was determined by HPLC.

Antioxidant activity of rutin

DPPH radical scavenging assay

The method determining the DPPH radical scavenging activity of rutin was referenced with previous publication (Mandade et al. 2011) with a few modifications. The rutin dissolved in methanol at different concentrations was injected into 100 mM DPPH solution (dissolved in ethanol) at equal volume. The mixture was darkly maintained at room temperature for 30 min and was measured to the absorbance at 517 nm. Vitamin C was used as the positive control. The DPPH radical scavenging activity (RSA) was calculated using the following formula:

$$\text{RSA}\ (\%) = \left(1 - \frac{A1 - A2}{A3}\right) \times 100\%$$

where A1 was the absorbance of DPPH in the mixture consisted of DPPH solution and various concentrations of rutin extracts at equal volume, A2 was the absorbance of DPPH in the mixture consisted of various concentrations of rutin extracts and distilled water at equal volume, and A3 was the absorbance of the mixture of DPPH solution and distilled water at equal volume. The effective concentration of rutin and Vc getting to RSA 50% means EC_{50}.

Superoxide radical scavenging assay

Series concentrations of rutin-methanol solutions, Tris–HCl buffer solution (pH 8.2), nitroblue tetrazolium (NBT) solution (0.98 mM), pyrogallol solution (10 mM) and HCl

solution (8 mol/L) were prepared previously. A 2.5 mL of Tris–HCl buffer was formerly hearted at a water-shaker (25 °C) for 20 min. The rutin-methanol solution (0.2 mL), NBT solution (0.6 mL) and pyrogallol solution (0.3 mL) were added into the previous heated Tris–HCl buffer solution. The above reagent was mixed and maintained at 25 °C for 4 min, then adding HCl solution (0.1 ml) to stop the reaction and measuring the absorbance at 530 nm. Moreover, the control group was conducted by the substitution of distilled water for rutin-methanol solution. Similarly, ascorbic acid was used as the positive control. The calculated formula was as following:

$$\text{RSA}\ (\%\ ) = \left(1 - \frac{A1}{A0}\right) \times 100\%.$$

where A1 was the absorbance value of positive group, A0 was the absorbance value of control group. The effective concentration of rutin and Vc getting to RSA 50% means EC_{50}.

Hydroxyl radical scavenging assay

The reaction was conducted in the system by the addition of safranine T solution (520 ug/mL, 0.2 mL), EDTA–Na–Fe(II) solution (2 mM, 0.7 mL), different concentrations of rutin solution (1.0 mL) and H_2O_2 (6%, 0.4 mL) to the phosphate buffer (pH 7.4, 2 mL), respectively. After mixing at 37 °C for 30 min, the absorbance was determined at 520 mm. Moreover, the control group was conducted by the substitution of distilled water for rutin solution. Similarly, ascorbic acid was used as the positive control. The calculated formula was as following:

$$\text{RSA}\ (\%\ ) = \left(1 - \frac{A0}{A1}\right) \times 100\%.$$

where A1 was the absorbance value of positive group, A0 was the absorbance value of control group. The effective concentration of rutin and Vc getting to RSA 50% means EC_{50}.

Results and discussion

Single-factor experiment

At first, the impact of the molar ratio of ChCl–TEG on the rutin extraction was investigated (Fig. 1a). A slight difference in the yield was observed at the different molar ratio tested. The optimum molar ratio of ChCl to TEG is 1/4, likely due to the appropriate hydrogen bond, which is the interaction forces between DESs and compounds (Dai et al. 2013a). As depicted in Fig. 1b, an increase in the extraction yield was shown at the water concentration from 0 to 20% because of the decline of viscosity and the change in the polarity of extraction solvent. For instance, Huang et al. (2017) reported that the adding water to DESs contributed to the increase of polarity of extraction system so as to influence the solubilizing ability. Further increasing water content leaded to enormous decline in extraction yield, likely due to the progressive rupture of hydrogen bond in DES (Gutiérrez et al. 2010). The results by Dai et al. (2013b) also suggest extraction capacity of DESs was obviously decided by water content. The effect of extraction time on the yield is shown in Fig. 1c. The extraction amount significantly improves with the increase of time from 5 to 10 min. The prolongation of extraction time leads to slight impact in the yield, even achieving slight low yield. The result suggests that overmuch heating leads to mild loss of rutin. Increasing temperature results in a decrease of viscosity of DESs to improve the mass transfer. As shown in Fig. 1d, the extraction amount of rutin increased with the improvement of temperature, up to the maximum at 65 °C, and further increase leaded to slight decrease of yield. Figure 1e shows 10 mL/1 g of ratio of liquid to rutin can achieve the ideal yield in rutin extraction among various ratios examined.

After the single factor experiment, the favorable extraction conditions were achieved as follows: molar ratio of ChCl/TEG 1/4, water content of ChCl/TEG 20% (v/v), extraction time 20 min, extraction temperature 65 °C, and ratio of liquid to solid 10 mL/1 g. Under the optimum extraction condition, a markedly improvement in the extraction yield (270.3 mg/g) was obtained compared with the methanol–water solution or ethanol–water solution (< 150 mg/g) (Zhao et al. 2015). This adequately proves that the superior advantages using ChCl/TEG as extraction solvent for the extraction of rutin from *S. japonica* bud compared to traditional organic solvent.

Box–Behnken design

Three main factors (water content of DES, extraction time and extraction temperature) based on the single-factor experiments, were selected as the independent variables to establish the model of rutin extraction. The other factors were at the best value in the single factor experiments.

17 runs BBD experiment was conducted to further optimize the extraction conditions (shown in Table 1). By using Design-Expert soft on the simulation of experiment data, a second-order polynomial equation was obtained as follows.

$$\begin{aligned} Y = {} & 268.40 + 5.17 \times A + 6.47 \times B + 7.64 \times C - 5.22 \\ & \times A \times B - 14.90 \times A \times C + 2.94 \times B \times C - 17.64 \times A^2 \\ & - 5.36 \times B^2 - 12.88 \times C^2 \end{aligned}$$

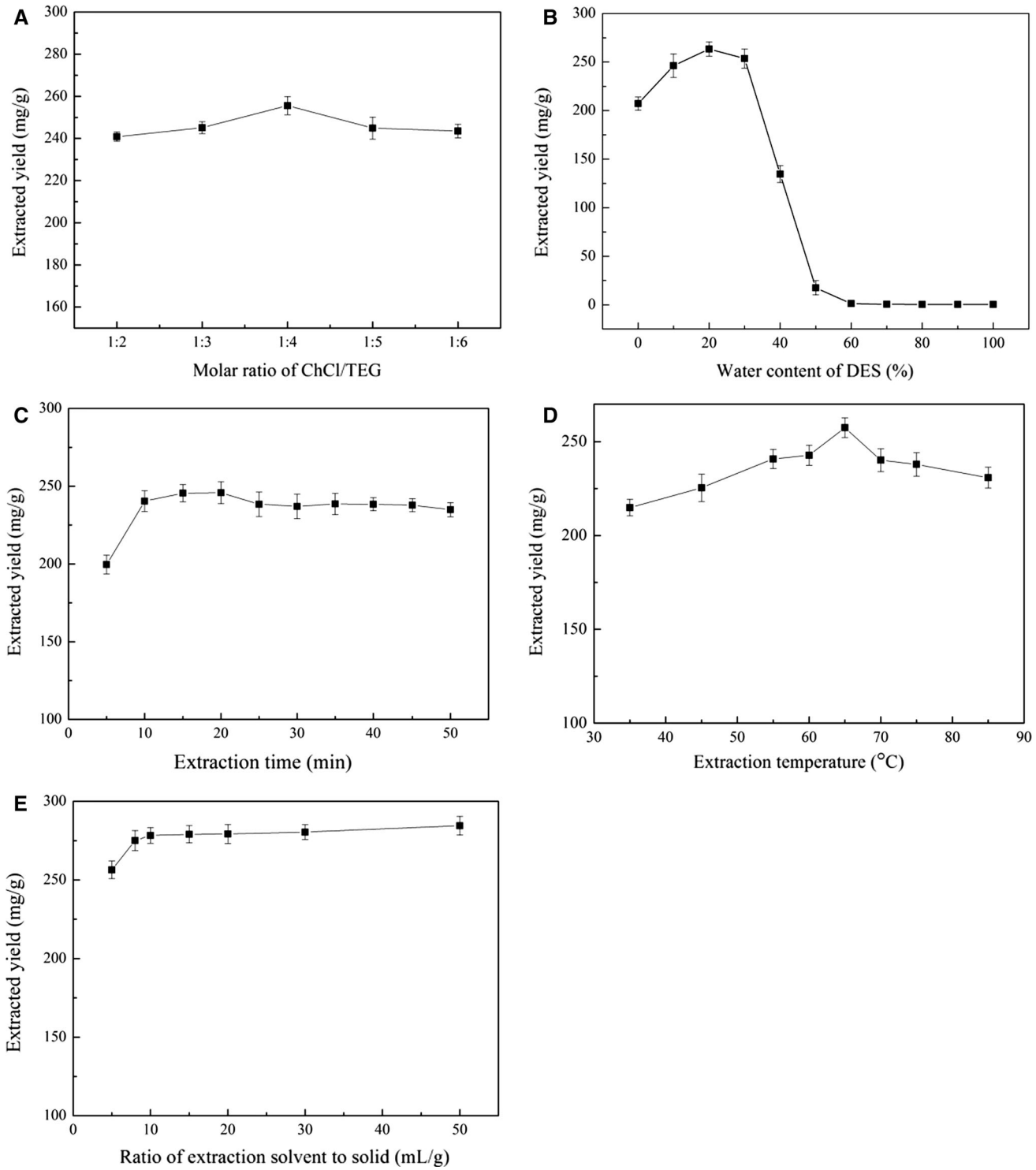

Fig. 1 Effects of various factors on the extraction yield of rutin. **a** molar ratio of ChCl/TEG; **b** water content of DES (v/v); **c** extraction time; **d** extraction temperature; **e** ratio of extraction to solid. The values were expressed as the means ± standard deviations (n = 3)

Y: predicted extraction yield of rutin (mg/g), A: code of water concentration in DES (− 1, 0, 1), B: code of extraction time (− 1, 0, 1), C: code of extraction temperature (− 1, 0, 1).

Next, one-way analysis of variance by Design-Expert soft was used for significance analysis of the model above. Model F-value of 43.89 and the *P* value less 0.001 shown in Table 2 indicate the model achieved is noteworthy. Moreover, all items examined on linear coefficients,

Table 1 The design and results of the BBD experiments

Entry	Factors			Extracted yield (mg/g)
	A	B	C	
1	1 (30)	0 (20)	− 1 (55)	249.66
2	0 (20)	− 1 (10)	1 (75)	246.92
3	0	1 (30)	1	263.88
4	1	1	0 (65)	251.01
5	0	− 1	− 1	242.30
6	− 1 (10)	0	− 1	206.04
7	1	0	1	239.93
8	− 1	1	0	254.58
9	− 1	0	1	255.89
10	1	− 1	0	246.65
11	0	1	− 1	247.52
12	1	− 1	0	229.35
13[a]	0	0	0	268.89
14[a]	0	0	0	268.23
15[a]	0	0	0	267.88
16[a]	0	0	0	268.43
17[a]	0	0	0	263.56

A Water content (v/v, %), *B* time (min), *C* temperature (°C)

[a]Central point

interaction term coefficients and quadratic term coefficients, have significant effects on the extraction yield of rutin (all P-values less 0.05). The high determination coefficient (R^2 = 0.9634) implies most of the total variations can be interpreted by the achieved model. As depicted in Fig. 2, the change in the extraction amount of rutin was investigated as two varieties was in experimental range and other variety was at zero. All contour plots showing ellipticity suggest the mutual interactions between the varieties are significant. Furthermore, the contour line in the Fig. 2d was denser than those in Fig. 2b, f, indicating that the mutual interaction between water content and extraction temperature was more important. The optimal extraction conditions were given by the resolution of regression equation as follows: water content of ChCl/TEG (1:4, molar ratio) 18.1% (v/v), extraction time 28.3 min, extraction temperature 70 °C, ratio of liquid to solid 10 ml/1 g. There is slight difference between the predicted extraction yield (272.5 mg/g) and the actual extraction yield (279.8 mg/g) under the above conditions, suggesting the model is perfect for the predication of rutin extraction from *S. japonica* bud.

Purification and structure determination

The yellow rutin extracted powder (Fig. S1A) was obtained at the yield of 62.7% after the purification process. The purity of purified rutin determined by HPLC was more than 95% (Fig. S1B). Moreover, the purified compound structure was characterized by nuclear magnetic resonance (NMR) spectroscopy (Fig. S1C). The master data given in Hz was shown as follows, and the data suggests the extract is rutin.

Rutin: δ1.00 (3H, d, CH3), 3.04-3.31 (9H, m, H2″-H6″, OH2‴-H5‴), 4.42 (1H, s, H1‴), 5.34 (1H, d, H1″), 6.20 (1H, s, H6), 6.39 (1H, s, H8), 6.85 (1H, d, H5′), 7.53-7.55 (2H, m, H2′ and H6′), 12.60 (1H, s, 5-OH).

Table 2 Analysis of variance (ANOVA) for the experimental result of BBD

Source	Sum of squares	*df*	Mean square	F value	*P* value*
Model	4372.52	9	485.84	43.89	< 0.0001
A	214.14	1	214.14	19.35	0.0032
B	335.02	1	335.02	30.27	0.0009
C	466.65	1	466.65	42.16	0.0003
AB	108.89	1	108.89	9.84	0.0165
AC	887.44	1	887.44	80.18	< 0.0001
BC	34.46	1	34.46	3.11	0.0210
A^2	1309.85	1	1309.85	118.34	< 0.0001
B^2	121.09	1	121.09	10.94	0.0130
C^2	698.53	1	698.53	63.11	< 0.0001
Residual	77.48	7	11.07		
Lack of fit	76.91	3	25.64	181.22	0.1139
Pure error	0.57	4	0.14		
Cor total	4450.00	16			
	R^2 = 0.9634		R^2adj = 0.9602		

A Water content (v/v, %), *B* time (min), *C* temperature (°C)

*Significant ($P < 0.05$)

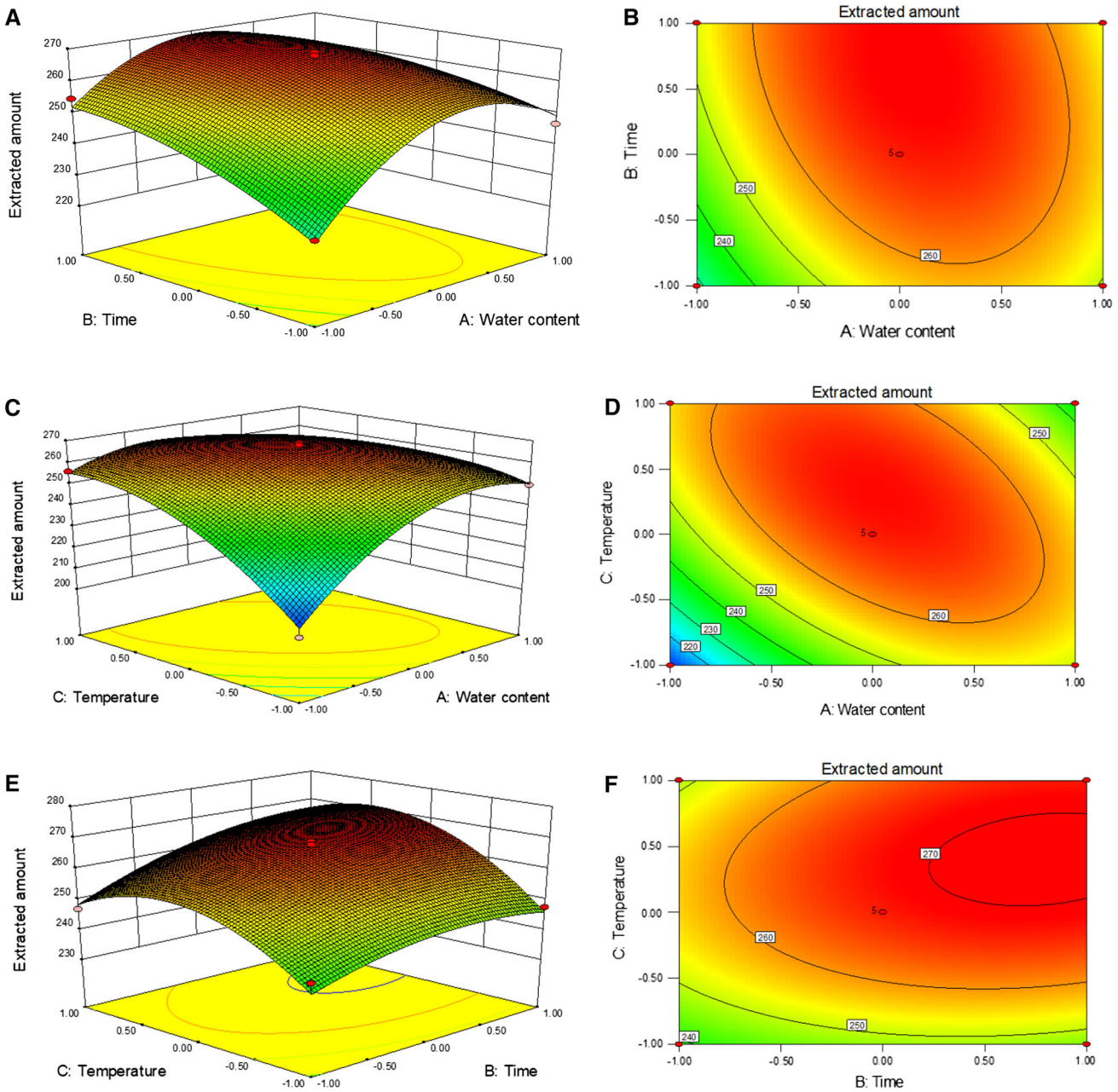

Fig. 2 Response surface plots (**a**, **c**, **e**) and Contour plots (**b**, **d**, **f**) showing the effects of variables and their mutual effects on the extraction yield of rutin. Code number: − 1, 0, 1

Free radical scavenging capacities

The excellent antioxidant capacities of rutin from *S. japonica* bud was shown in Fig. 3. DPPH-RSA of rutin and ascorbic acid at different concentrations was shown in Fig. 3a. At the tested concentrations of antioxidants, the highest DPPH-RSA was achieved at 10 μg/mL for rutin and ascorbic acid, reached up to 86.1 and 93.5%, respectively. This suggests that rutin has excellent DPPH radical scavenging ability, slightly below than that of ascorbic acid. Furthermore, DPPH radical EC_{50} values of rutin and ascorbic acid were 5.68 and 2.76 μg/mL, respectively. Compared to the conventional solvent, such as ethanol (Wu et al. 2015), the method used by us has noteworthy better effect on the DPPH radical scavenging activity (EC_{50}, 5.68 vs. 10.97 μg/mL).

As shown in Fig. 3b, the scavenging ability of rutin in the superoxide anion is obviously lower than vitamin C. The highest scavenging ability for superoxide anion is 72% at 0.39 mg/mL. In addition, a comparative EC_{50} value (0.19 mg/mL vs. 0.16–1.05 mg/mL) in the method was achieved compared with previous publications (Yang et al.

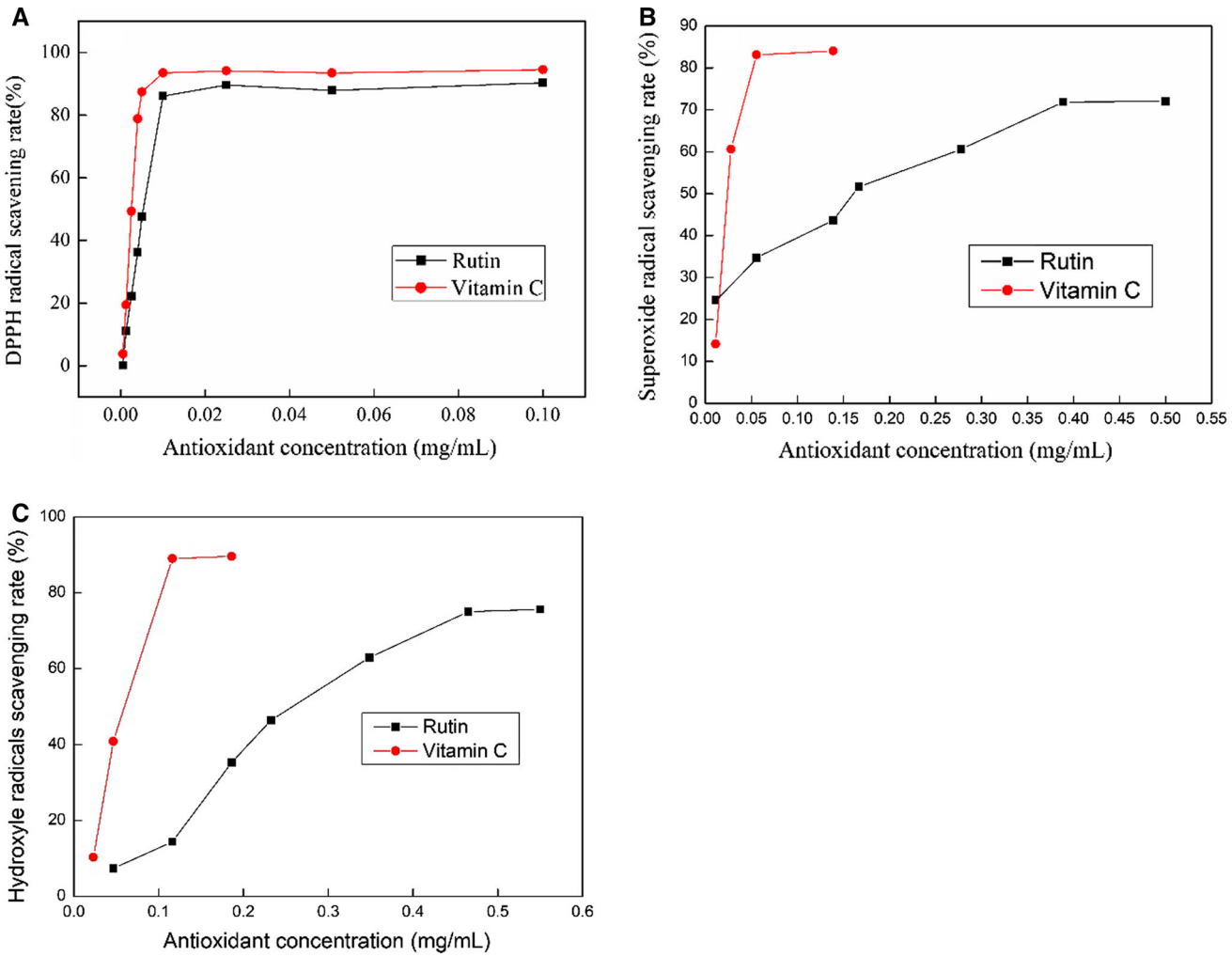

Fig. 3 Free radical scavenging rate of rutin and vitamin C. **a** DPPH; **b** O^{2-}; **c** ·OH. The values were expressed as means with less than 1% SD (n = 2)

2008; Zhang et al. 2016). The results indicate that applying the above method to extract rutin can achieve excellent superoxide anion scavenging activity.

The highest hydroxyl radical scavenging activities of rutin and ascorbic acid were 75.0 and 89.0%, respectively (Fig. 3c). Furthermore, ascorbic acid displayed a prominent scavenging activity with EC_{50} value of 0.06 mg/mL; nevertheless, the purified rutin has a relative lower scavenging activity with an EC_{50} value of 0.28 mg/mL. It was also observed in the other study that ascorbic acid had a better hydroxyl radical scavenging activity than rutin (Wu et al. 2015).

Conclusion

We have established a method for the efficient extraction of rutin by ChCl/TEG (1/4) and the antioxidant activities were also evaluated. A highest yield of 279.8 mg/g was achieved for the rutin extraction under the optimum conditions. The anti-oxidative activity of rutin proved that rutin has excellent oxidation resistance and the extraction method established has a little effect on the antioxidant activity of rutin.

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