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Application of response surface methodology to optimise the extraction of tea saponin from *Camellia oleifera*, and their verification by HPLC

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Abstract

Single factor combined with response surface methodology was used to optimise the process parameters of tea saponin extraction from *Camellia oleifera*. Four factors including material-liquid ratio, extraction temperature, extraction time, and ethanol concentration were selected as the influencing factors on the basis of single factor. The extraction rate of tea saponin was used as the response factor to analyse the response of these four factors and three levels. Results showed that extraction temperature of 81.69°C, material-liquid ratio of 1:11.85 g/mL, time of 6.17 h, and ethanol concentration of 56.69% were the best extraction conditions. The estimated yield of extraction was 7.46%. Analysis of the tea saponin samples by using high performance liquid chromatography showed that the main peak time was 6.668 min, and the absorption peaks and peaks were symmetric.

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Introduction

Camellia oleifera is rich in tea saponin (TS), but its utilisation is extremely low. The present work intended to improve the utilisation of oil tea husks, thus turning waste into wealth, and improve regional economy. TS is a class of pentacyclic triterpene compounds widely found in various tea plants (Shuo *et al.*, 2018), has general saponin-like properties, and appears mostly as white amorphous powder, but some as fine columnar crystals (Jun *et al.*, 2012). TS is a natural non-ionic surfactant with excellent performance (Yang and Huang, 2001), and widely used in industries, medical treatment, and agriculture (Yamauchi *et al.*, 2015). TS also has functional activities such as antioxidation (Hao *et al.*, 2009; 2010a; 2010b), insecticidal, antiviral (Ueda, 2001), sobering (Ye *et al.*, 2015; Liu *et al.*, 2017), detoxifying, antibacterial (Ren *et al.*, 2015), and anti-inflammatory (Hu *et al.*, 2012) activities.

China has the largest output of *Camellia*, and the annual output of *C. oleracea* is approximately 549,800 tons, whereas its by-product is approximately 183,000 tons (Ying *et al.*, 2013). However, the utilisation of TS is extremely low (Gong *et al.*, 2018). At present, the extraction of TS is mostly based on water or ethanol extraction, and foam separation method (He *et al.*, 2013; Xiong *et al.*, 2016; Wu *et al.*, 2018). However, these methods have certain drawbacks such as low TS content, deep colour, and poor quality (Lu *et al.*, 2000). Therefore, an in-depth study on the extraction of TS from the tea oil shell must be conducted.

Materials and methods

Materials

Camellia oleifera samples were obtained from Hainan Province, and then pulverised with a 40-mesh grinder. Other chemicals included standard TS

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(Solarbio, MV ≥ 98%), vanillin (Tianjin Yongda Chemical Reagent Co., Ltd.), anhydrous ethanol (Xixi Scientific Co., Ltd.), hydrochloric acid, concentrated sulphuric acid, sodium hydroxide, potassium bromide (obtained from Guangzhou), and acetonitrile (chromatographic purity; Hubei Four Seasons Spring Co., Ltd.).

Test procedure

Tea husk powder were accurately weighed and transferred into an Erlenmeyer flask with ethanol solution (55, 65, 75, 85, 95%) at a suitable pH (7, 8, 9, 10, 11), and later placed in a water bath (50, 60, 70, 80, 90°C) at a certain ratio (1:2, 1:4, 1:6, 1:8, 1:10, 1:12, 1:14 g/mL). After being submerged for several hours (1, 2, 3, 4, 5, 6, 7 h) in a water bath and filtered water, these steps were repeated three times. The filtrate was rotary-steamed to 20 mL, and dried into powder (Yi *et al.*, 2016).

Response surface methodology (RSM)

Based on the single-factor results, factors with significant influence were selected to proceed with the RSM.

Quantitative analysis methodology

Sulphuric acid-vanillin method was used to determine the concentration of TS in the flask (Hou, 2005). The TS standard was prepared in 1, 0.8, 0.6, 0.4, and 0.2 mg/mL dilutions. Distilled water was used as a blank reference solution, and absorbance measured at 550 nm (Cheok *et al.*, 2014). The fitted regression equation was $y = 0.57x + 0.053$, $R^2 = 0.99779$.

The sample solution was diluted ten times, and followed by measuring the absorbance. Shell TS extraction parameters were determined using Eqs. 1-3:

$$\text{TS quality} = [(A - 0.053) / 0.57] \times 10 \times V \times 10^{-3} \quad \text{(Eq. 1)}$$

$$\text{TS extraction yield} = (M / M_0) \times 100\% \quad \text{(Eq. 2)}$$

$$\text{TS purity} = (M / M_1) \times 100\% \quad \text{(Eq. 3)}$$

where, M: TS quality (g); M_0: *Camellia* shell quality (g); M_1: *Camellia* shell quality (g); A: Sample absorbance; and V: 500 mL constant volume.

Purification of extracts

Decolourisation and purification of TS extract was performed using hydrogen peroxide and AB-8 macroporous adsorption resin (Yuan *et al.*, 2018).

High performance liquid chromatography

The TS was chromatographed in acetonitrile to a concentration of 1 mg/mL for HPLC detection (C_{18} reverse column: 5 μm) (Yin *et al.*, 2013). The detection conditions were velocity: 1 mL/min; sample volume: 5 μL; detection wavelength: 215 nm, and column temperature: 25°C. In the first 5 min, the mobile phase was 20% acetonitrile. After 5 to 10 min, the column was flushed with 100% acetonitrile. Finally, the column was rinsed with 80% ultra-pure water for 5 min.

Fourier infrared spectroscopy

The purified sample was dissolved in anhydrous ethanol, filtered, and subjected to infrared spectroscopy.

Results and discussion

Effect of material-liquid ratio on the yield of TS

As shown in Figure 1A, as the amount of solvent increased, the lower the solute concentration, the higher the mass transfer driving force, the faster the extraction speed, the higher the TS extraction rate, and the easier the extraction; when the ratio of material to liquid was greater than or equal to 1:10 g/mL, the yield of the liquid-to-liquid ratio was negligible. Not only the cost of extraction and solvent recovery will increase greatly, but the purity of the extract will decrease. Therefore, the best condition extraction liquid ratio was found to be 1:10 g/mL.

Effect of extraction time on yield of TS

As shown in Figure 1B, when the extraction time increased, the yield of TS also increased. After the extraction time exceeded 6 h, the TS yield increased slowly, and the sample purity decreased significantly. This was because the dissolution and diffusion of TS inside the oil tea shell took a certain time, so prolonging the extraction time is beneficial to the extraction; with the increase in time, the concentration of the solute in the liquid phase increased, the mass transfer power decreased, the leaching speed decreased, and eventually reached equilibrium; the leaching time was too long, and the protein, tea polyphenols, and the likes were dissolved,

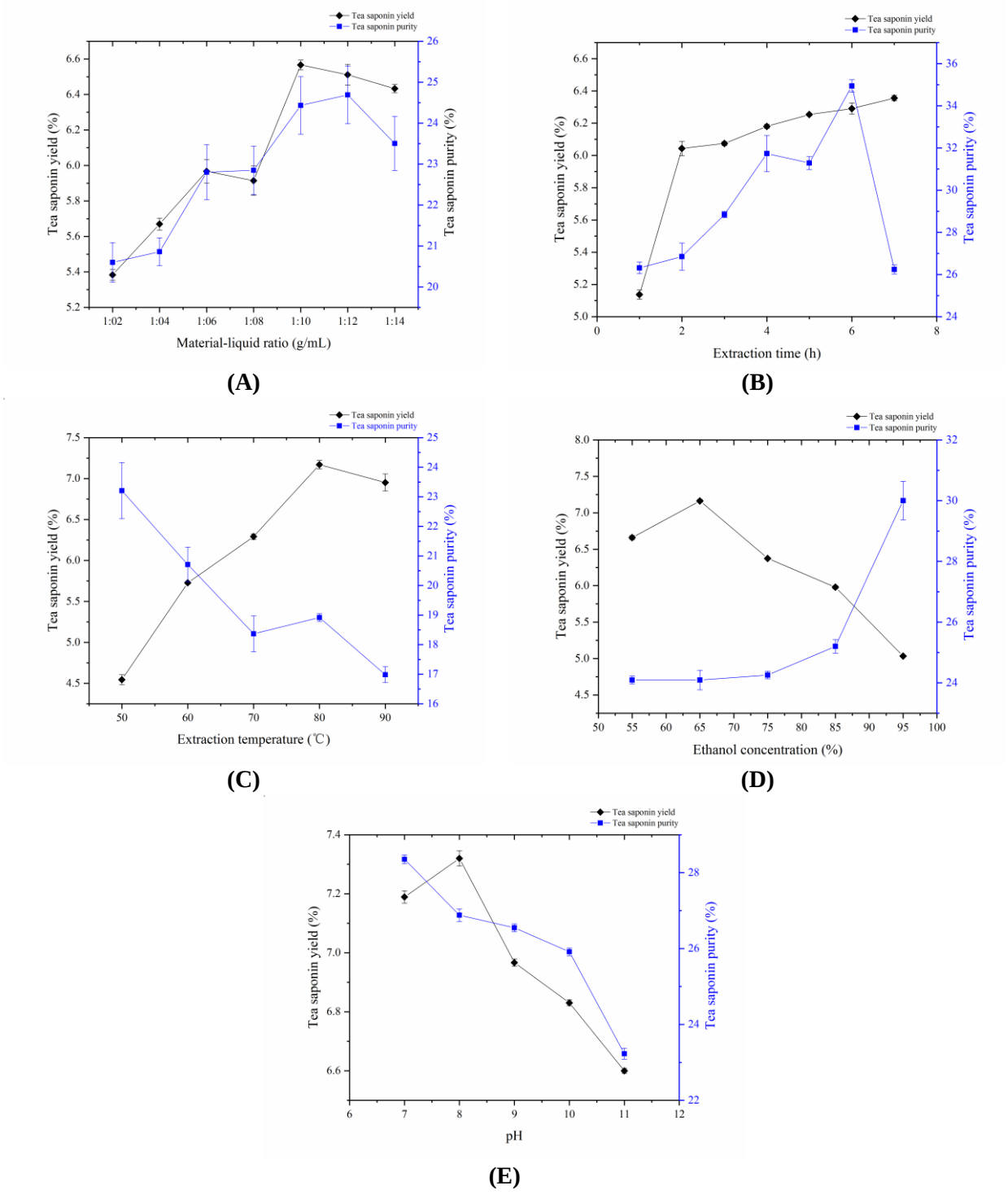

Figure 1. Effect of different factors on tea saponin yield and purity of crude TS extract. **(A)** material-liquid ratio; **(B)** extraction time; **(C)** extraction temperature; **(D)** ethanol concentration; and **(E)** pH.

thus resulting in a decrease in the purity of the extract (Lu *et al*., 2000). Therefore, the best extraction time was found to be 6 h.

Effect of extraction temperature on yield of TS

As shown in Figure 1C, when the extraction temperature was lower than 80°C, the yield of TS increased as the extraction temperature increased. When the extraction temperature exceeded 80°C, the yield of TS decreased. This was because, as the temperature rises, the solubility increases, and the rate of release is close to the maximum. At the same time, the irreversible solidification of protein in the tea seed hull is intensified, and the combination of TS and precipitation counteracts the effect of increased solubility caused by temperature increase (Gong *et al*., 2018). The best extraction temperature was therefore found to be 80°C.

Effect of ethanol concentration on the yield of TS

As shown in Figure 1D, when the ethanol concentration was lower than 65%, the yield of crude TS increased with the increase in ethanol concentration. When the ethanol concentration was higher than 65%, the yield of TS decreased linearly. Reduced polarity of extract resulted in a decrease in the dissolution rate of TS, and a decrease in the extraction rate. Moreover, when the ethanol concentration increased, the solidification rate of proteins, pectins, and the likes increased, and the extraction rate decreased. Taking into account the efficiency issues, the ethanol concentration of 65% was found to be the best extraction condition.

Effect of solution pH on the yield of TS

As shown in Figure 1E, with the increase in the pH value, the yield of crude TS showed a tendency to decrease rapidly after the first rise. The purity curve showed a gradient decrease in the value range. TS was acidic, so the solubility of TS increased in an alkaline solution. However, some alkali-soluble impurities were also excessively dissolved, and part of the saponin was decomposed under strong alkaline conditions. Therefore, the pH value of 8 was found more suitable for extracting TS.

Response surface method to optimise the extraction of TS

RSM used in the present work was an efficient statistical technique for modelling and optimisation (Liu *et al.*, 2016). Based on the results, the effects of liquid ratio (A), extraction time (B), extraction temperature (C), and ethanol concentration (D) on the extraction rate of TS were selected (Hu *et al.*, 2009). The TS yield regression model obtained using Design-Expert software is as shown in Eq. 4:

$$TS\ yield = 7.20+0.34*A+0.38*B+0.28*C-0.13*D-0.094*A*B+0.015*A*C-0.098*A*D+0.092*B*C-0.14*B*D+0.073*C*D-0.11*A^2-0.50*B^2-0.13*C^2-0.34*D^2 \quad \text{(Eq. 4)}$$

Variance analysis

Using the Design-Expert software to analyse the variance of 29 TS yield results, the model's F was 38.57, thus indicating that the model was significant. And the signal-to-noise ratio of the model was 23.113 (> 4), which further showed that the regression model had a good fit (Table 1). Based on the *p*-value, the influence degree of each factor from the largest to the smallest was the ratio of material to liquid = extraction time = extraction temperature > ethanol concentration (Figure 2).

Based on the Design-Expert software (Wang *et al.*, 2018), the optimal extraction process conditions that was used was: the material-liquid ratio (1:11.85 g/mL), the extraction time (6.17 h), the extraction temperature (81.69 °C), and the ethanol concentration (56.69%). The theoretical yield of TS under this condition was 7.46%. Five replicate experiments were performed to test the reliability of the results. The average yield of TS obtained was 7.38%, and the difference between the theoretical value and the experimental value was only 0.08%. This proved that the application of response surface method to optimise the extraction of TS from oil tea husk by aqueous ethanol could be feasible.

HPLC analysis results

As shown in Figure 3, the peak time interval between the TS standard and the sample was only 0.002 min. The main substance in the extract proved to be TS.

Fourier infrared spectroscopy results

Figure 4 shows that the strong peak at the wavenumber 3,388.84 cm^{-1} was the hydroxyl stretching vibration absorption peak, wavenumber 2,927.23 cm^{-1} was saturated C-H stretching vibration

Table 1. ANOVA for regression equation model.

Source of variance	Sum of square	Degree of freedom	Mean square	*F*-value	*p*-value	Significant
Model	6.46	14	0.46	38.57	< 0.0001	***
A	1.4	1	1.4	116.64	< 0.0001	***
B	1.7	1	1.7	141.64	< 0.0001	***
C	0.92	1	0.92	77.15	< 0.0001	***
D	0.2	1	0.2	17.04	0.001	**
AB	0.035	1	0.035	2.93	0.1088	
AC	9.44E-04	1	9.44E-04	0.079	0.7829	
AD	0.038	1	0.038	3.21	0.0947	
BC	0.034	1	0.034	2.81	0.1161	
BD	0.075	1	0.075	6.27	0.0253	*
CD	0.021	1	0.021	1.79	0.2018	
A^2	0.085	1	0.085	7.07	0.0187	*
B^2	1.65	1	1.65	137.95	< 0.0001	***
C^2	0.11	1	0.11	9.22	0.0089	**
D^2	0.73	1	0.73	60.87	< 0.0001	***
Residual	0.17	14	0.012			
Missing items	0.091	10	9.15E-03	0.48	0.8415	
Errors	0.076	4	0.019			
Total deviation	6.63	28				

*Significant at $p < 0.05$; **Highly significant at $p < 0.01$; and ***Extremely significant at $p < 0.0001$.

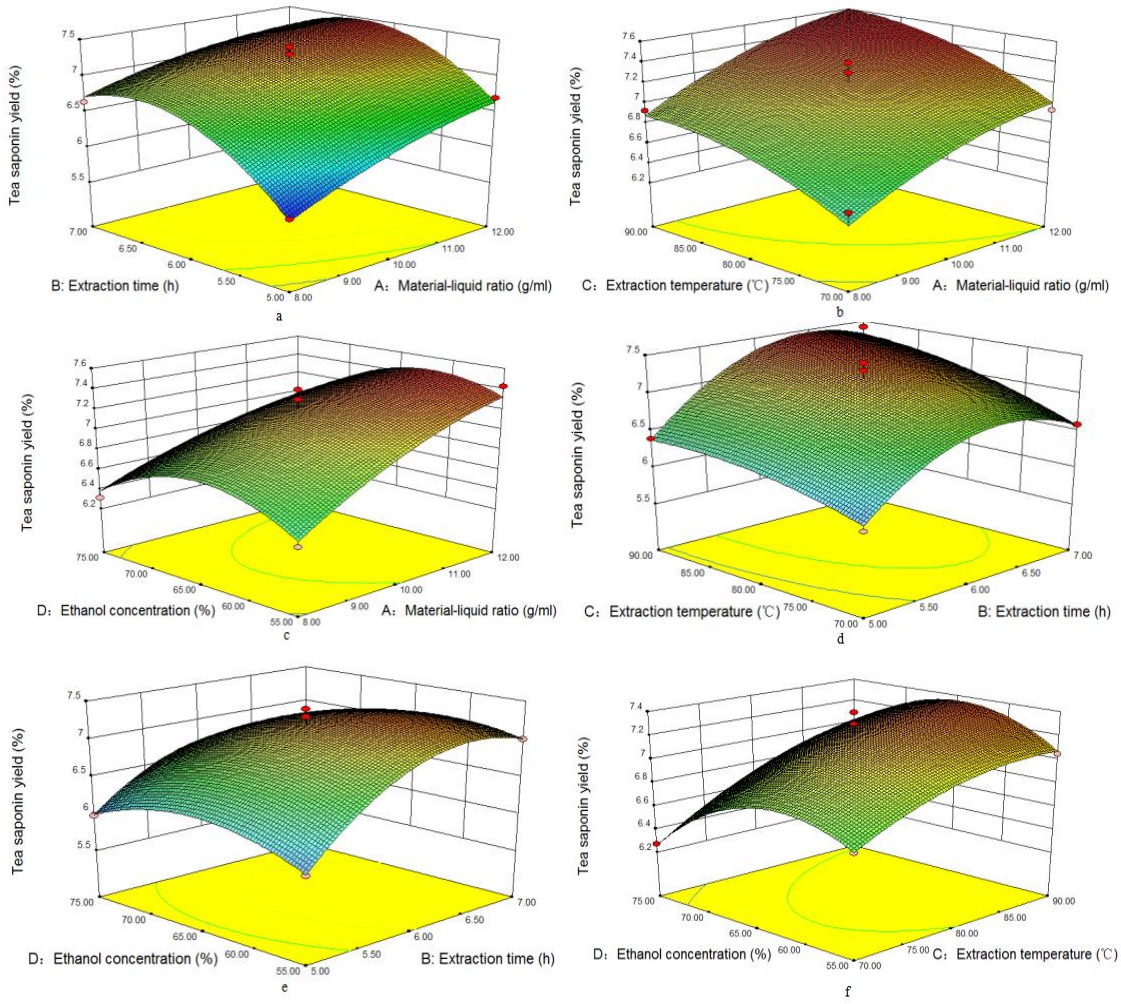

Figure 2. The contour map and response surface graph between the various factors. **(a)** Response surface diagram of the effect of material-liquid ratio and extraction time on extraction yield of TS; **(b)** response surface diagram of the effect of material-liquid ratio and extraction temperature on extraction rate of TS; **(c)** response surface diagram of the effect of material-liquid ratio and ethanol concentration on extraction rate of TS; **(d)** response surface diagram of the effect of extraction time and extraction temperature on extraction yield of TS; **(e)** response surface diagram of the effect of extraction time and ethanol concentration on extraction yield of TS; and **(f)** response surface diagram of the effect of extraction temperature and ethanol concentration on extraction yield of TS.

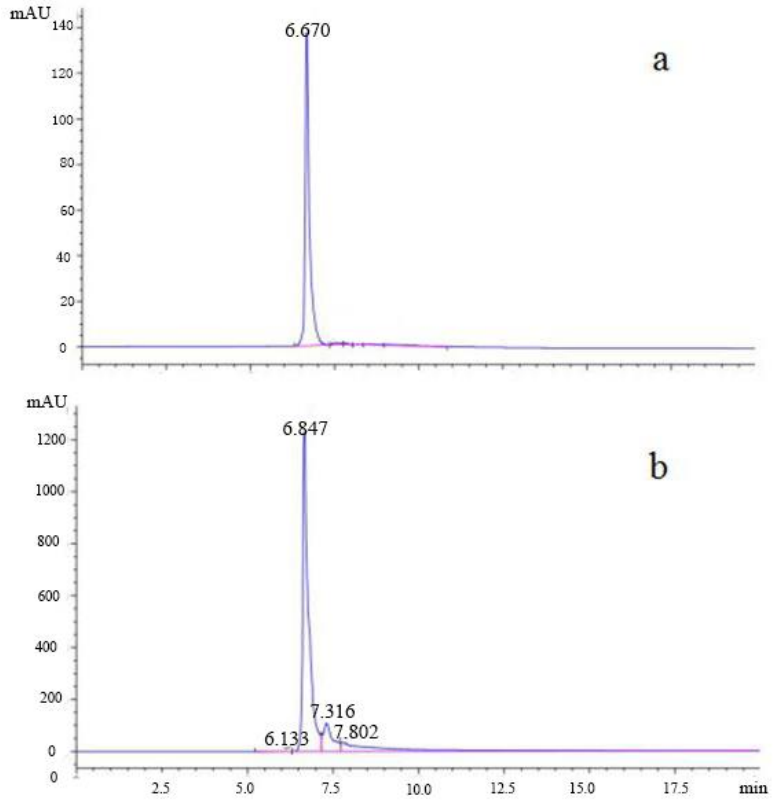

Figure 3. Chromatograms of **(a)** TS standard, and (b) TS sample by high performance liquid chromatography (HPLC).

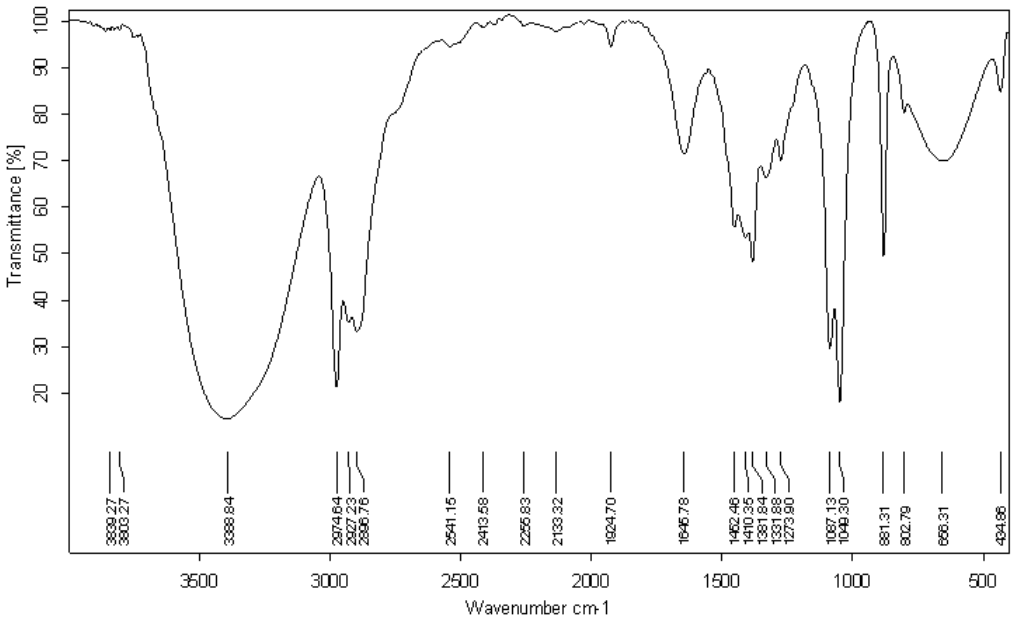

Figure 4. Infrared spectrum of TS extract.

absorption, and wavenumber 1,381.84 cm^{-1} was the stretching vibration absorption peak of the carbon-carbon double bond. These characteristic absorptions were consistent with the results reported in the literature, and with the TS structure.

Conclusion

Results of RSM showed that the extraction temperature was 81.69°C, the ratio of material to liquid was 1:11.85 g/mL, the time was 6.17 h, and the ethanol concentration was 56.69%. The verified measurement value was 7.38% (n = 5). Results of HPLC and infrared spectroscopy confirmed that the main substance contained in the extracted sample was TS.

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