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Study on Ultrasonic Extraction of Gastrodin from Gastrodia elata Bl.

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Gastrodin, a pharmacologically active constituent, was ultrasonically extracted from gastrodia elata Bl. in the aqueous solution. The effects of six parameters including ethanol–water compositions, extraction time, extraction temperature, particle size, solvent volume, and ultrasonic power on the extraction yield of gastrodin were investigated. According to the orthogonal design, the optimal extraction conditions was explored as extraction temperature 60°C, extraction time 50 minutes, ultrasonic power 126 W, solvent volume 8 mL · g^{-1}, ethanol–water compositions 70%, and particle size 10–20 mesh. Though the yield of gastrodin via ultrasonic extraction was about 0.01% lower than that from the reflux extraction, the extraction time of the ultrasonic extraction was greatly shortened. Therefore, ultrasonic extraction has high efficiency and is proved to be very valuable in the extraction of gastrodin from gastrodia elata Bl.

Keywords Gastrodia elata Bl.; Gastrodin; orthogonal design; ultrasonic extraction

INTRODUCTION

Traditional Chinese medicines (TCMs) have been used for about 20 centuries to treat different types of diseases such as pulmonary, cardiovascular, pediatric, mental illness, and so on (1). According to the Chinese Pharmacopoeia, there are about 400 TCMs widely used. Due to an increasing interest of people all over the world, theoretical and experimental research on these traditional drugs have been approached by scientists from many countries (2,3). Since the constituents of TCMs are usually complicated, accurate extraction and quality control of active components are quite necessary in the usage of TCMs.

Gastrodia elata Bl. (GE), belonging to the family of orchidaceae (4,5), is a valuable traditional Chinese drug. The investigations on the chemical composition of GE have been performed for more than 20 years, indicating that there are numerous Phenols, organic acids, and phytosterols in GE, such as gastrodin (GA), gastrodioside, parishin, vanillyl alcohol, P-hydroxy benzaldehyde, and so on (6–9). The root of GE, named "Tianma" in Chinese, is considered to be very helpful for human health. It has been demonstrated that GA (p-Hdroxyethylphenyl-β-D-glucopyranoside) is a major bioactive component in GE, and is generally used to treat rheumatism, paralysis, hemiplegia, lumbago, headaches, and other neuralgic disease (10,11). Molecular structure of the GA is shown in Fig. 1.

Extraction is a key step in the production processes of TCMs. The conventional techniques for extracting pharmacologically active compounds from TCMs include solvent extraction, steam distillation method, percolation method, and so on. However, many shortcomings have been found in the applications of these methods, such as long extraction time, great consumption of solvents, and low extraction efficiency (12). Therefore, developing an accurate and efficient extraction method of active components in TCMs is necessary.

Ultrasonic wave ranging from 20 KHz to 1 GHz is usually generated by an ultrasonic electromagnetic transducer that can converts mechanical or electrical energy into high frequency vibrations. Ultrasonic extraction (UE) is a very efficient extraction procedure (13–15). It can be used for both liquid and solid samples to extract either inorganic or organic compounds (16). In ultrasonic extraction, sonication induces cavitation and bubbles with a negative pressure. The implosion of the cavities creates shock waves near or at the surface of the sample. The shock waves are of high temperature and pressure, and can accelerate the extraction when different chemical compounds and particles escape from the matrix surface.

It has been proved that UE is an efficient method for extraction of TCMs. In our study, the UE of GA from GE was carried out and the optimum conditions of extraction were obtained.

EXPERIMENTAL

Materials and Chemicals

The UE apparatus (Fig. 2) was manufactured by Jining Jinbaite Electron Co., Ltd. and used in our study. The operation parameters are shown in Table 1. A standard substance of GA was obtained from the National Institute

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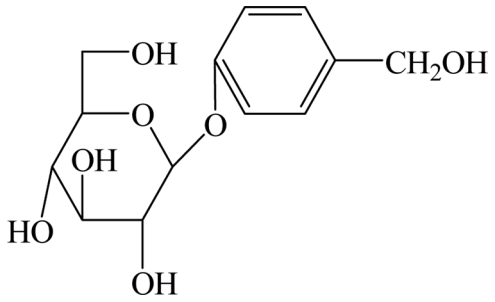

FIG. 1. Structure of the Gastrodin.

for the Control of Pharmaceutical and Biological Products. Methanol (chromatographic pure) used for HPLC analysis was obtained from Jiangsu Hanbon Science & Technology Co., Ltd. Milli-Q water was obtained from Robust (Group) Co., Ltd. GE was purchased from Nanjing Kangyi decoction pieces Co., Ltd. Before experiments, GE was treated as fine particles with different sizes, 10–20 mesh, 20–40 mesh, 40–60 mesh, 60–80 mesh, 80–100 mesh, and 100–120 mesh.

Ultrasonic Extraction

Ultrasonic Extraction

First, a certain quality of extraction solvent was added into an Erlenmayer flask. When the solvent was heated to a preset temperature, powders of GE (2.00 g) were added into the Erlenmayer flask. The Erlenmayer flask was then placed into the ultrasonic bath. As the frequency and power were adjusted at certain values respectively, GA would be extracted and the extract would be filtrated. The concentration of GA in the filtrate was measured by HPLC. The influences of the extraction temperature, extraction time, the ethanol–water compositions, the volume of the solvent, ultrasonic power and the frequency, and the particle size on the extracting yield of GA were investigated.

TABLE 1
Technical parameter and specification of UE apparatus

Type	DCTZ-2000
The most effective volume (mL)	2000
The available volume (mL)	$800 \sim 1800$
The most ultrasonic power (W)	1200
The heating power (W)	150
The controlled temperature (°C)	$10 \sim 70$
The material ultrasonic bath	SUS304
The applicable medium	water, alcohol, ether, organic solvent
The application	Preparing under laboratorial study
The overall dimension (mm)	$660 \times 600 \times 650$

HPLC Analysis for GA

GA was analyzed by HPLC-10ATvp (Shimadzu) with a C_{18} column (5 μm, 150 mm × 4.6 mm). The mobile phase consisted of 5% methanol aqueous solution for isocratic elution with a flow rate 0.8 mL · min^{-1}. The column temperature was maintained at 25°C. The injection volume was 20 μL. The detection wavelength was set at 221 nm. The chromatograms of standard GA and extract of GE were illustrated in Figs. 3 and 4, respectively, showing that the chromatographic peak of GA in the sample was primely separated from other coexistent elements under the above chromatographic condition in 40 min.

Statistical Analysis

Calibration Curve

By using the chromatographic peak area as abscissa (X) and injection amount (μg) as ordinate (Y), the equation of linear regression of the calibration curve is given as:

$$Y = 3.6348 \times 10^{-7} X - 0.0066 \quad (1)$$

Where, $R^2 = 0.9998$. As shown in Fig. 5, the linear correlation of standard GA between 0.0984–0.5904 μg was good.

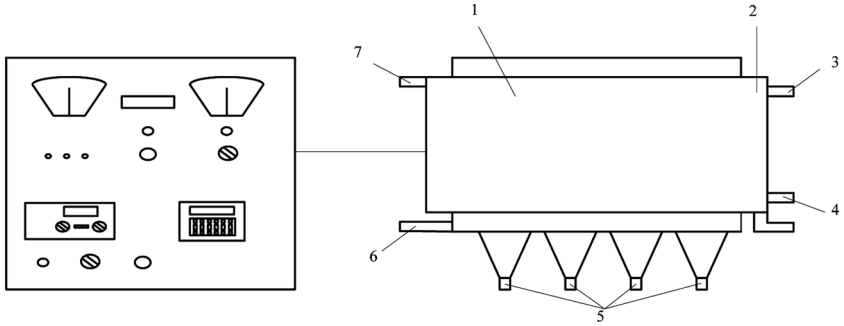

FIG. 2. Ultrasonic extraction apparatus; 1. Treatment trough; 2. Sandwich; 3. Cooling water import; 4. Cooling water outlet; 5. Ultrasonic vibrator; 6. Bleeder tube; 7. Dissection spillwater tube.

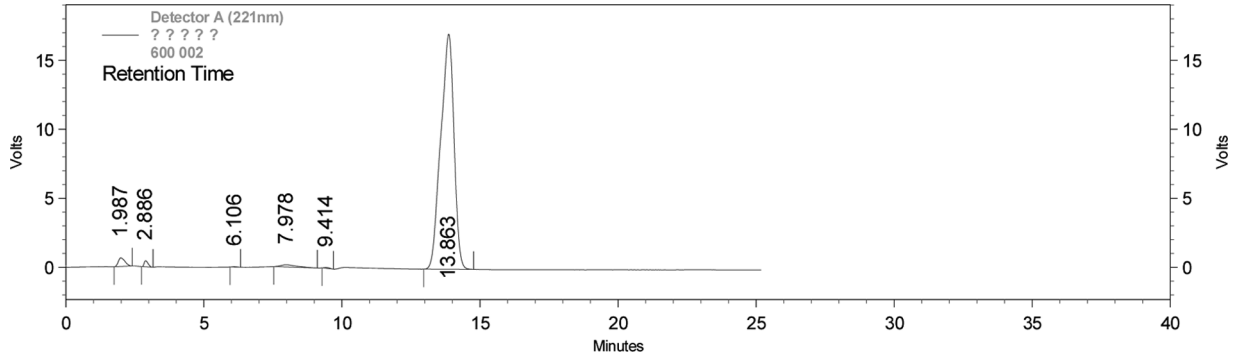

FIG. 3. Chromatogram of standard GA.

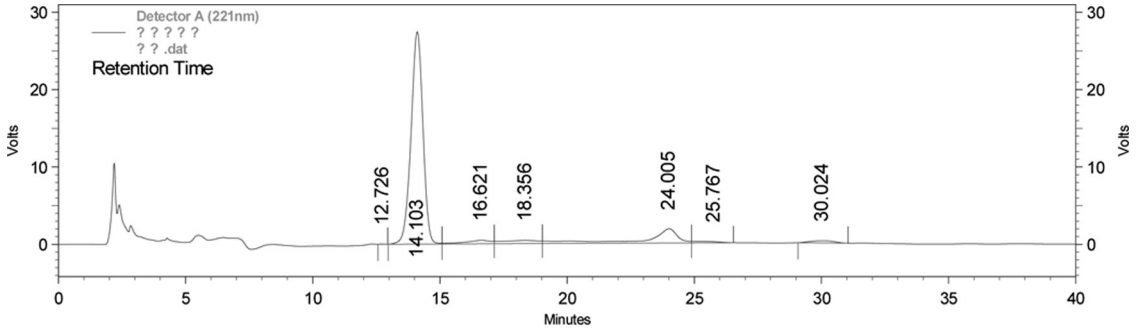

FIG. 4. Chromatogram of extract of GE.

Determination of GA in Extract

The concentration and yield of GA in extract is calculated by Equations (2) and (3) respectively.

$$\eta = \frac{Y \times \frac{5000}{20} \times \frac{V_{lo} - V'}{V_i}}{m} \times 100\% \quad (2)$$

$$C = \frac{Y \times \frac{5000}{20}}{V_i \times 1000} \quad (3)$$

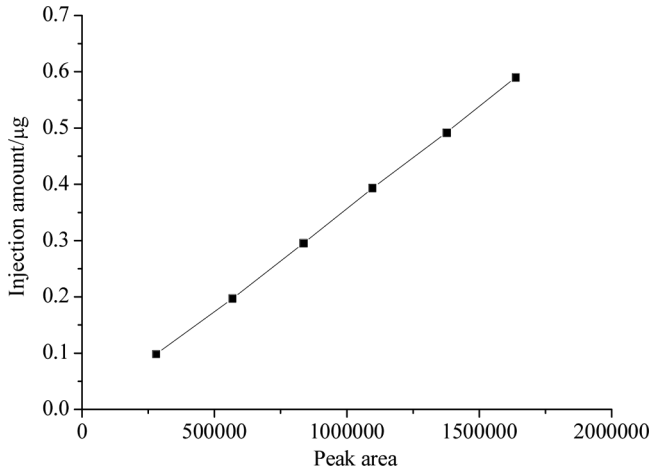

FIG. 5. Calibration curve for the correlation between HPLC peak area and injection amount.

Where, η is yield of GA extracted from GE (%) and C is concentration of GA in extract ($g \cdot L^{-1}$), Y is injection amount (μg), V' is the amount of volume permeating into interior of medicinal substances (mL). V_i is the amount volume removing extract (mL) and m is the amount of GE (g).

RESULTS AND DISCUSSION

The Effects of Operating Parameters on GA Yield

Effect of Frequency on Yield

The effect of the frequency on the GA yield was displayed in Fig. 6, showing that the yield of GA slightly increased with an increase of frequency. Obviously, the frequency barely effects on the yield of GA, due to the structure of GA and the cell of GE. It can be concluded that frequency is not a major factor that effects on the GA yield. Moreover, since the noise was notable in the operation under low-frequency, the proper ultrasonic wave was set as 70 KHz in the further experiments.

Effect of the Extraction Temperature on Yield

The influence of the extraction temperature on GA was shown in Fig. 7. It was indicated that the yield increased rapidly when the temperature rose from 30°C to 60°C, then with a further increase of temperature the yield decreased. This could be explained that when the temperature was lower than 60°C, the cavitation was enhanced by a higher

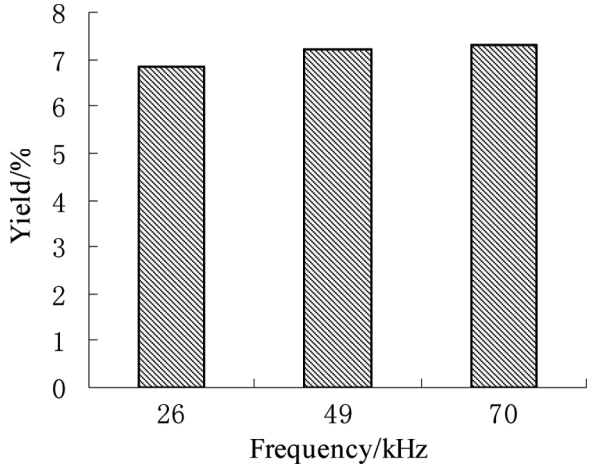

FIG. 6. The effect of frequency on GA yield (C = 50%, r = 1296 μm, M = 12 mL · g^{-1}, T = 60°C, t = 30 min, p = 258 W).

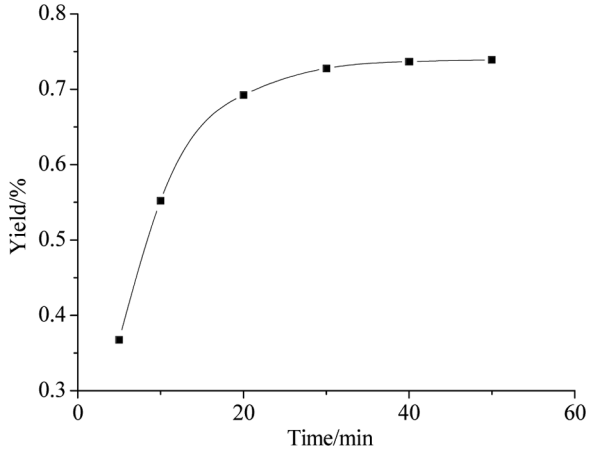

FIG. 8. The effect of extraction time on GA yield (C = 50%, r = 1296 μm, M = 12 mL · g^{-1}, T = 60°C, p = 258 W, F = 70 KHz).

temperature and the yield of GA increased accordingly. When the temperature was over 60°C, the decrease of gas solubility weakened the cavitation and then caused the decrease in the GA yield.

Effect of Extraction Time

The effect of extraction time on the yield was shown in Fig. 8, indicating that the yield increased with time from 5 increasing to 30 minutes, and kept constant when time further increased. This might be explained that most of the GA had already been extracted during the first 30 min.

Effect of Ultrasonic Power

With the rises of ultrasonic power, the cavitation was enhanced, accelerating the dissolution rate of the active substance in the cell. Figure 9 showed that with the rise

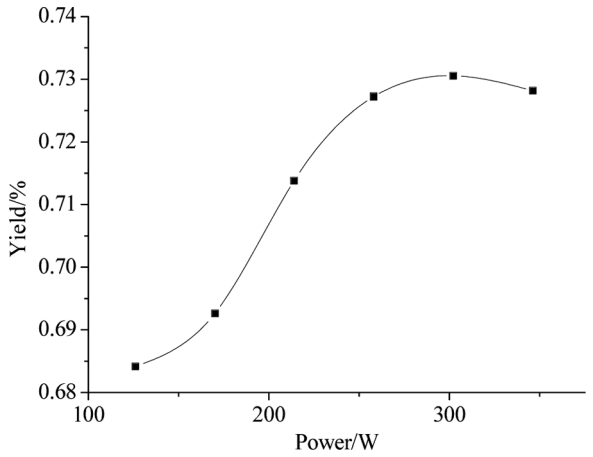

FIG. 9. The effect of ultrasonic power on GA yield (C = 50%, r = 1296 μm, M = 12 mL · g^{-1}, T = 60°C, t = 30 min, F = 70 KHz).

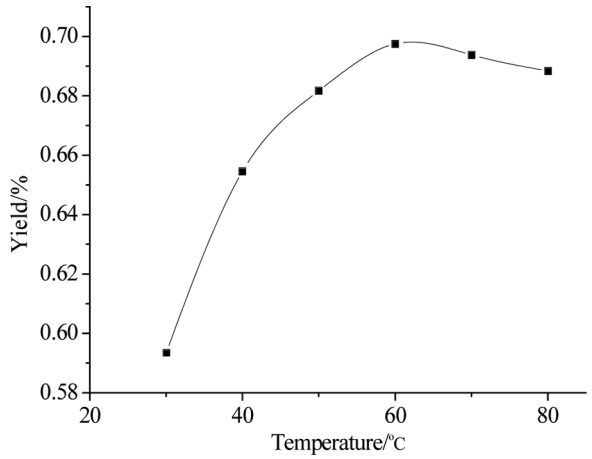

FIG. 7. The effect of temperature on GA yield (C = 50%, r = 1296 μm, M = 12 mL · g^{-1}, t = 60 min, p = 258 W, F = 70 KHz).

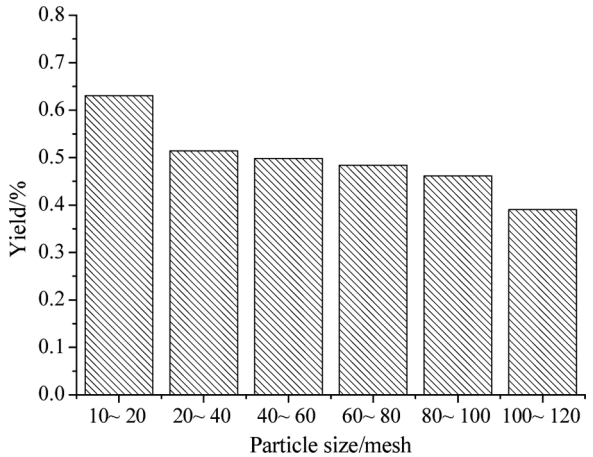

FIG. 10. The effect of particle size on GA yield (C = 50%, M = 12 mL · g^{-1}, T = 60°C, t = 30 min, p = 258 W, F = 70 KHz).

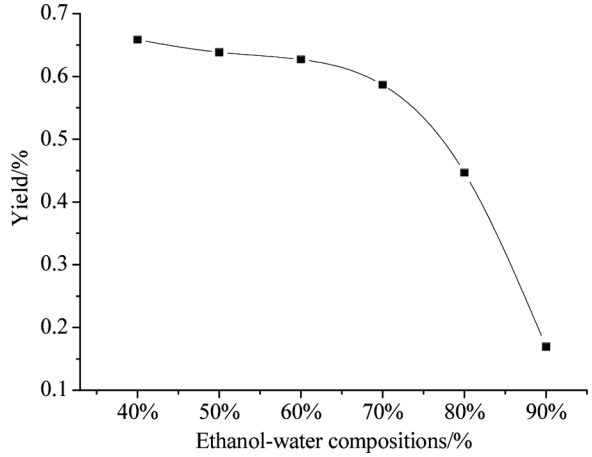

FIG. 11. The effect of ethanol–water compositions on GA yield (r = 1296 μm, M = 12 mL · g^{-1}, T = 60°C, t = 30 min, p = 258 W, F = 70 KHz).

of ultrasonic power, the extraction yield of GA increased and kept constant when the power was over 300 W. This could be explained that the ultrasonic cavitation was saturated and reached an equilibrium finally when the power was high enough.

Effect of Particle Size on Yield

The particle size generally influences on the mass transfer rate and yield of active substance. As shown in Fig. 10, the yield of GA decreased with the increase of particle size, thus the proper particle size was 10–20 mesh.

Effect of Ethanol–Water Compositions

According to the theory of "similarity and intermiscibility", the relative polarity will impact the yield of the active constituent. Moreover, ethanol aqueous solution with various concentration are of different viscosities and have different influence on the intensities of cavitation. As shown in Fig. 11, the extracting yield of GA dropped with the increase of concentration of ethanol. Since the solvent is difficult to be filtered when the ethanol concentration is lower than 50%.

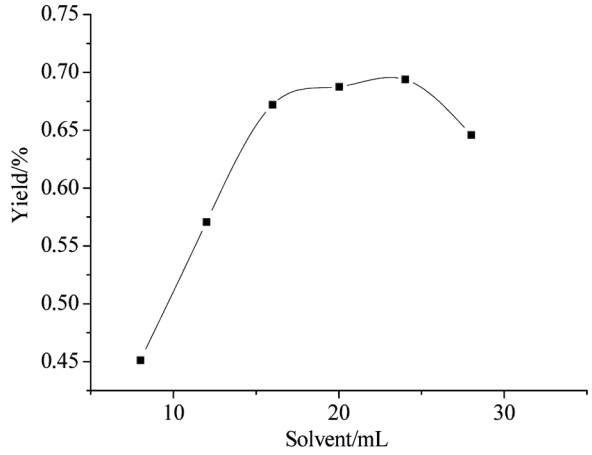

FIG. 12. The effect of ratio of solvent volume/sample on GA yield (r = 1296 μm, C = 50%, T = 60°C, t = 30 min, p = 258 W, F = 70 KHz).

Effect of the Ratio of Solvent Volume/Sample

The effect of the solvent volume on the yield of GA was shown in Fig. 12. As can be seen, the GA yield rose obviously when the volume increased from 8 mL to 24 mL. When the volume increased further, the GA yield dropped accordingly.

OPTIMIZATION OF UE BY ORTHOGONAL DESIGN

Based on the above results, the following experiments will be carried out to obtain the optimum factors by varying operating parameters according to orthogonal design. Six factors—extraction time, extraction temperature, ultrasonic power, solvent volume, ethanol–water compositions and particle size would be selected, except for frequency that had little influence on the GA yield.

An $L_{18}(3^7)$ matrix orthogonal array design was utilized to optimize the UE of GA from GE (Table 2). According to the Visual Analysis Method in Table 2, the influence order of each factor to the GA yield was ethanol–water compositions > extraction time > extraction temperature > particle size > solvent volume > ultrasonic power. The table of ANOVA was summarized in Table 3 which showed that the value p of temperature and time were both less than 0.1, the value p of ethanol–water compositions was less than 0.05, and the value p of the particle size was more than 0.1. The results imply that ethanol–water compositions were the major contributing factor to GA yield, followed by extraction temperature and time. The optimal extraction conditions were as follows: extraction temperature 60°C, extraction time 50 minutes, ultrasonic power 126 W, solvent volume 8 mL · g^{-1}, ethanol–water compositions 70%, and particle size 10–20 mesh.

COMPARISONS OF UE WITH REFLUX EXTRACTION

The comparison of UE with reflux extraction was summarized in Table 4. By using the same plant material, UE and reflux extraction (17) were both performed under the optimum conditions, respectively. It could be seen from Table 3, that the yield by UE was 0.01% lower than that by the Reflux method, since particles of GE were shattered by ultrasonic wave, absorbing more GA than was noticeable. The extraction time with UE was significantly less than the reflux extraction, indicating that the UE was still a good method for extracting GA from GE.

TABLE 2
The orthogonal array design and experimental results

No.	Temperature (°C)	Time (min)	Power (w)	Solvent volume (mL/g^{-1})	Ethanol–water compositions (%)	Particle size (mesh)	Yield (%)
1	40	10	126	4	50	10 ~ 20	0.302985
2	40	30	214	8	70	40 ~ 60	0.348849
3	40	50	302	12	90	80 ~ 100	0.136471
4	50	10	126	8	70	80 ~ 100	0.22791
5	50	30	214	12	90	10 ~ 20	0.160299
6	50	50	302	4	50	40 ~ 60	0.300032
7	60	10	214	4	90	40 ~ 60	0.094885
8	60	30	302	8	50	80 ~ 100	0.29607
9	60	50	126	12	70	10 ~ 20	0.633007
10	40	10	302	12	70	40 ~ 60	0.282785
11	40	30	126	4	90	80 ~ 100	0.06433
12	40	50	214	8	50	10 ~ 20	0.569944
13	50	10	214	12	50	80 ~ 100	0.216366
14	50	30	302	4	70	10 ~ 20	0.390963
15	50	50	126	8	90	40 ~ 60	0.152125
16	60	10	302	8	90	10 ~ 20	0.170249
17	60	30	126	12	50	40 ~ 60	0.330208
18	60	50	214	4	70	80 ~ 100	0.281164
Mean 1	0.284	0.216	0.285	0.239	0.336	0.371	
Mean 2	0.241	0.265	0.279	0.294	0.361	0.251	
Mean 3	0.301	0.345	0.263	0.293	0.130	0.204	
Range	0.060	0.129	0.022	0.055	0.231	0.167	

TABLE 3
Table of ANOVA

	Sum of squares	DF	Mean square	F-value	P-value
Temperature	0.171	2	0.086	3.37	<0.1
Time	0.204	2	0.102	4.43	<0.1
Ethanol–water compositions	0.266	2	0.133	5.78	<0.05
Particle size	0.115	2	0.058	2.52	>0.1
Error	0.137	6	0.023		
Total error	0.893	14		$F_{2,6(1-0.05)} = 5.14$, $F_{2,6(1-0.1)} = 3.46$	

CONCLUSION

UE proved to be a high efficient method in the extraction of GA from GE by experiments. The optimal conditions for extracting GA via UE were using an extraction temperature of 60°C, an extraction time of 50 min, and a frequency of 126 W, an solvent volume of $8\,mL \cdot g^{-1}$, an ethanol–water compositions of 70%, and a particle size of 10–20 mesh. Under the optimum extraction conditions, the yield of GA could reach up to 0.7228%, slightly lower than that of the reflux extraction of 0.7307%. It should be noted that the extraction time with UE was significantly less than the reflux extraction. Based on the above analysis results, UE was found to be a promising technology for the extracting of GA from GE because of its efficiency, its low cost, and its environmental acceptability.

TABLE 4
Comparisons of ultrasonic extraction and reflux extraction

Extraction method	Temperature (°C)	Time (min)	Power (w)	Solvent volume ($mL \cdot g^{-1}$)	Ethanol–water compositions (%)	Particle size (mesh)	Yield (%)
Ultrasonic extraction	60	50	214	12	50	10 ~ 20	0.7228
Reflux extraction	Room temperature	120	/	10	70	10 ~ 20	0.7307

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