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Ultrasound-assisted extraction of five isoflavones from *Iris tectorum* Maxim

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ABSTRACT

This study investigated the use of ultrasound-assisted extraction (UAE) to improve the extraction efficiency of the classical solvent extraction techniques such as maceration extraction (ME) and soxhlet extraction (SE) to extract five isoflavones (tectoridin, iristectorin B, iristectorin A, tectorigenin and iristectorigenin A) from *Iris tectorum*. The effects of various factors such as extraction solvent, solvent concentration, temperature, solvent to solid ratio, ultrasound power, extraction time and particle size on the yield of target components were investigated. The optimal UAE conditions found were: 70% (v/v) methanol solution, temperature 45 °C, solvent to solid ratio 15 ml/g, ultrasound power 150 W, extraction time 45 min and particle size 60–80 mesh. The results indicated that compared with ME at 18 h and SE at 6 h, UAE gave the highest extraction yields of tectoridin, iristectorin B, iristectorin A, tectorigenin, iristectorigenin A and total isoflavones at 45 min. The results indicated that UAE was an alternative method for extracting isoflavones from *I. tectorum*.

1. Introduction

Iris tectorum Maxim, a famous traditional Chinese medicine, is widely distributed in China [1]. Traditionally, its root and rhizome have been used for the treatment of inflammation, cough, tonsillitis and pharyngitis [2]. Recently, it has been used to fight against severe acute respiratory syndrome (SARS). Many studies found that a variety of compounds such as isoflavonoids [3–5], quinones [6] and triterpenoids [7–9] existing in the *I. tectorum*. Tectoridin, iristectorin B, iristectorin A, tectorigenin and iristectorigenin A (shown in Fig. 1), the major isoflavonoid components in *I. tectorum*, are believed to be responsible for the biological activities of anti-atherosclerosis [10], anti-oxidant [11] and anti-tumor [12].

Extraction of bioactive compounds from natural products with a solvent is a classical operation applied in many industrial processes. It is obvious that medical interest in plants derived drugs has led to an increased need for ideal extraction methods, which could obtain the maximum of the bioactive constituents in a shortest processing time with a low cost. The conventional extraction methods, such as maceration extraction (ME) and soxhlet extraction (SE), which have been employed for decades, need long extraction times and require relatively large quantities of solvent. Ultrasound-assisted extraction (UAE) has been proved to possess abilities of significantly decreasing extraction time and increasing extraction yields in many natural products [13–15].

So far, UAE has been widely applied to extract active compounds such as flavonoids [16,17], alkaloids [18], steroids [19] and anthraquinones [20,21] from plant materials. The aim of the present paper reported is to evaluate the influence of main extraction conditions including extraction solvent, solvent concentration, temperature, solvent to solid ratio, ultrasound power, extraction time and particle size on the yield of five isoflavones from *I. tectorum*.

2. Experimental

2.1. Reagents and materials

All organic solvents used for UAE extraction were of analytical grade and purchased from Tianjin Chemical Factory, Tianjin, China. Acetonitrile used for HPLC was of chromatographic grade (Fisher Scientific, USA), and water used was redistilled water. Tectoridin, iristectorin B, iristectorin A, tectorigenin and iristectorigenin A were obtained from the authors' laboratory. Their structures were identified by Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR), each at over 98% purity as determined by HPLC.

The roots of *I. tectorum* were collected from the Medicinal Plant Farm of Shandong Agricultural University in Aug 2009, Taian, China, and were identified by Doctor Jianhua Wang (College of Agronomy, Shandong Agricultural University). The herb was pulverized into powder form by a disintegrator (Taisite Instrument

Abbreviations: UAE, ultrasound-assisted extraction; ME, maceration extraction; SE, soxhlet extraction; T, tectoridin; ITB, iristectorin B; ITA, iristectorin A; TN, tectorigenin; INA, iristectorigenin A; TIF, total isoflavones.

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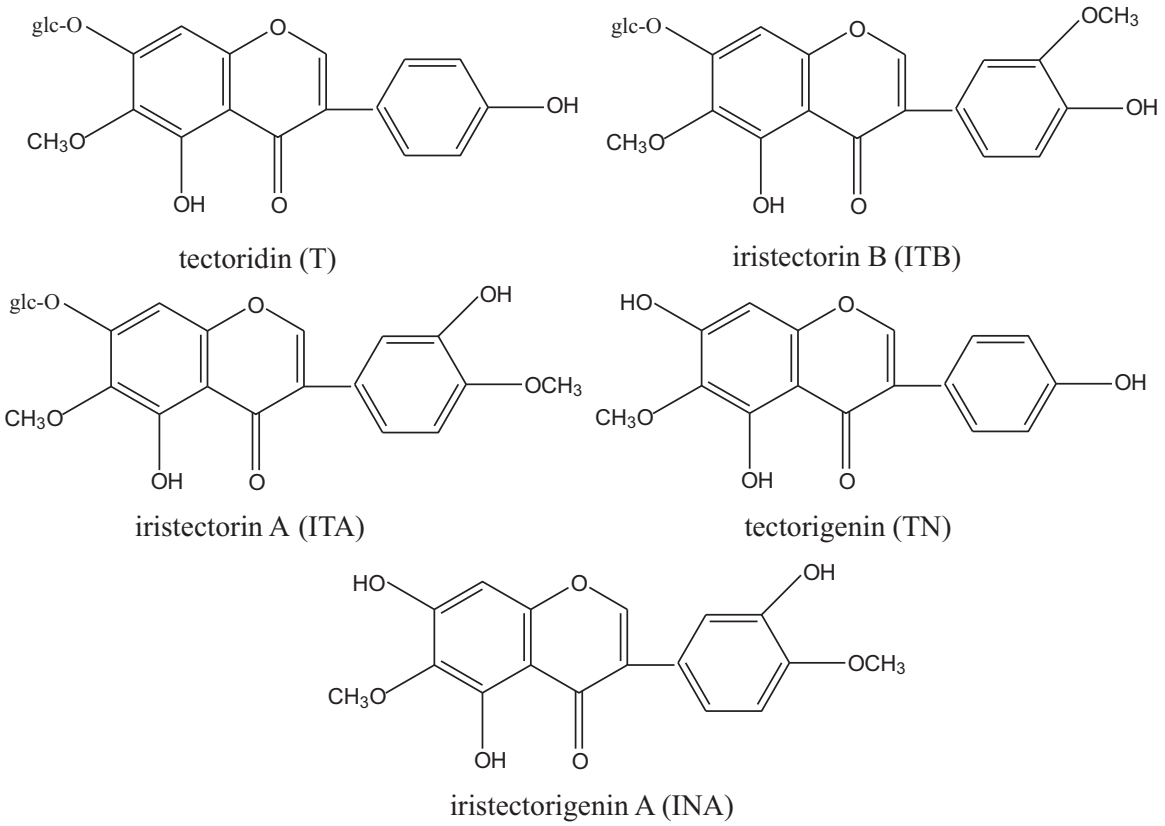

Fig. 1. Chemical structures of tectoridin, iristectorin B, iristectorin A, tectorigenin and iristectorigenin A.

Company, Tianjin, China) then sieved with stainless steel sieves to classify the particle size. The powdered samples were oven-dried at 65 °C for 12 h, and then kept in a dry and dark place until use. All UAE, ME, and SE experiments were prepared and analyzed in triplicate.

2.2. Apparatus

2.2.1. Ultrasonic instrument

For the ultrasound-assisted extraction experiments, an ultrasonic bath was used as an ultrasound source. The bath (KQ-250DE, Kunshan Ultrasound Co. Ltd., China) was a rectangular container (300 × 240 × 150 mm), to which 40 kHz transducers were annealed at the bottom. The bath power rating was 250 W on the scale of 4–10.

2.2.2. High-performance liquid chromatography (HPLC) system

The HPLC equipment used is Waters 600E (USA) HPLC system including a 4-Solvent delivery system 600E start-up kit, a 600 pump, 0–20 mL/min, a 2996 photodiode array detector, an Empower Add-on Single System, China, a Degasser in-line 4-chamber, and a 600E controller.

2.3. Extraction methods

2.3.1. Ultrasound-assisted extraction (UAE)

The extraction of five isoflavones from *I. tectorum* by ultrasound was performed by employing various different extraction conditions including solvents: chloroform, ethyl acetate, ethanol, methanol and water; percentage of methanol in water: 50–90%; temperature: 15–65 °C; solvent to solid ratio: 5–50 ml/g; ultrasound power: 100–250 W; extraction time: 5–60 min; particle size: 10–100 mesh.

2.3.2. Maceration extraction (ME)

ME was performed with 1.0 g (60–80 mesh) of dried samples and 100 ml of 70% methanol. Then mixed them at room temperature three times, each for 6 h. The extracts were combined and concentrated by a rotary vacuum evaporator. All solutions were filtered through 0.22 μm membrane filter before direct injection into the HPLC system.

2.3.3. Soxhlet extraction (SE)

60–80 mesh powder of 1.0 g was extracted with 100 ml methanol using Soxhlet apparatus for 6 h. The extract was then concentrated using rotary vacuum evaporator. SE was performed as a control for comparison with UAE.

2.4. HPLC analysis

HPLC analysis of the crude extract was performed with Hypersil C_{18} column (250 mm × 4.6 mm, i.d., 5 μm) at room temperature. The gradient elution system consisted of A (acetonitrile) and B (water), 17% A at 0–10 min, 17–46% A at 10–25 min, 46% A at 25–33 min. All solvents were filtered through a 0.22 μm filter prior to use. The sample injection volume was 20 μl. The flow rate was kept at 1.0 ml/min, and the effluents were monitored at 265 nm by a photodiode array detector. Under the above conditions, the chromatograms of standard and ultrasonically extracted *I. tectorum* are shown in Fig. 2. The chromatographic peaks of T, ITB, ITA, TN and INA were confirmed by comparing their retention time and UV spectra with those of the reference standards. Quantification was carried out by the integration of the peak using external standard method.

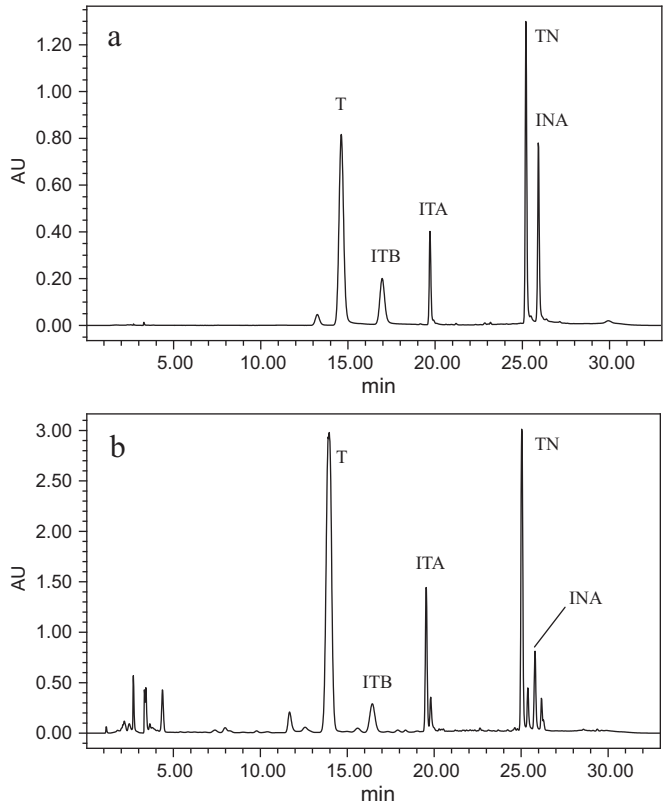

Fig. 2. The HPLC chromatograms of the standard mixture solutions and samples. (a) Standard substances; (b) crude extract by UAE from *I. tectorum*. Peaks T, ITB, ITA, TN and INA correspond to tectoridin, iristectorin B, iristectorin A, tectorigenin and iristectorigenin A, respectively.

2.5. Extraction yield determination

The extraction efficiency of UAE method and conventional extraction methods were evaluated using the extraction yield as index, which were calculated according to the following equation:

$$\text{Yield (mg/g)} = \frac{\text{weight of isoflavones extracted (mg)}}{\text{weight of dried sample (g)}}$$

3. Results and discussion

3.1. Selection of solvents in UAE

The selection of the most appropriate solvent for extracting the compounds of interest from the sample matrix is an essential step for developing any extraction method. The five different solvents (chloroform, ethyl acetate, ethanol, methanol and water) were tested under the same conditions: sample of 0.5 g, temperature of 25 °C, solvent to solid ratio of 50 ml/g, ultrasound power of 175 W, extraction time of 30 min and particle size of 40–60 mesh. The results were summarized in Table 1. The results showed that methanol gives the highest extraction yields, followed by water and ethanol. The different extraction efficiencies of these solvents could be attributed to their differing polarities and viscosities.

3.2. Effect of solvent concentration

In general, water is not a good solvent for extracting isoflavones, but it has been observed that sometimes the addition of small percentages of water to the extraction solvent helps to increase the extraction yield of the target compounds from the sample [22,23]. The five different solvents concentration have been studied for extracting isoflavones from the matrix of the sample. The results illustrated in Fig. 3 showed that the highest extraction yields of T, ITB, ITA, TN, INA and TIF were obtained at 70% methanol. When the solvent concentration of methanol was above 70%, the extraction yields for five isoflavones decreased. Thus, 70% methanol was chosen as best solvent in the following extraction experiments.

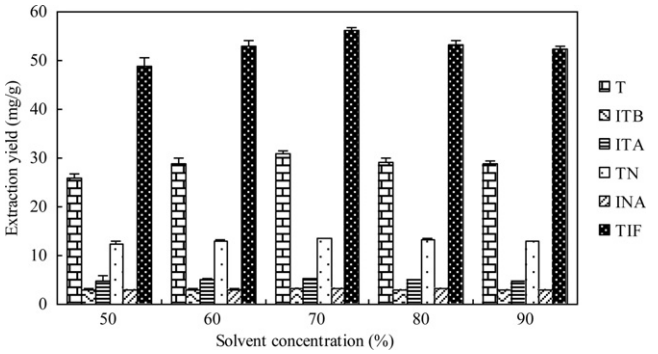

Fig. 3. Effect of the solvent concentration on the extraction yield of isoflavones from *I. tectorum*.

3.3. Effect of temperature

The effect of temperature on extraction efficiency was investigated, since that it impacts the solubility and mass transfer rate of target compounds in solvent. In this study, six different temperatures (15, 25, 35, 45, 55 and 65 °C) with 70% methanol, were selected to evaluate the influence of temperature on the extraction efficiency and quality of isoflavones from *I. tectorum*. Other conditions were of 0.5 g sample, solvent to solid ratio: 50 ml/g, ultrasound power: 175 W, extraction time: 30 min, particle size: 40–60 mesh. Fig. 4 showed the extraction yields of the five isoflavonoids increased with the increase of extraction temperature. The extraction yields of T, ITB, ITA, TN and INA at 45 °C were 33.12, 3.34, 5.44, 13.59 and 3.31 mg/g, respectively. But the increasing extraction temperature from 45 to 65 °C, the extraction yields of the dominant components T and TN, TIF in the herb, were not significant ($p > 0.05$). The results are probably because that high temperature can increase the solubility and mass transfer rate of target compounds, which was favorable to extraction. However, some degradation processes may occur at high temperature, and then lower recoveries can be obtained. Finally, 45 °C was used as the extraction temperature.

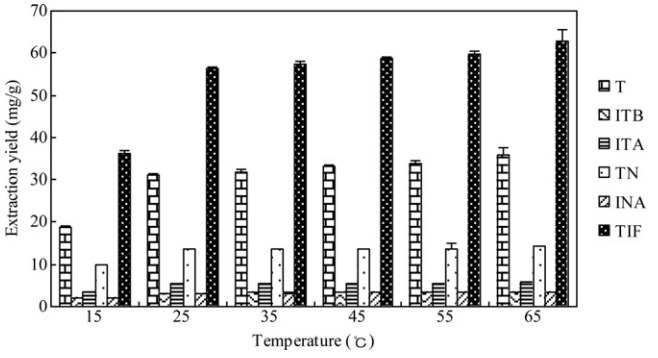

Fig. 4. Effect of the temperature on extraction yield of isoflavones from *I. tectorum*.

Table 1
Selection of solvents.

Solvent	Extraction yield (mg/g)					
	T	ITB	ITA	TN	INA	TIF
Chloroform	0.10 ± 0.01	0.04 ± 0.00	0.01 ± 0.00	1.47 ± 0.06	0.41 ± 0.53	2.04 ± 0.59
Ethyl acetate	1.06 ± 0.07	0.09 ± 0.01	0.07 ± 0.01	9.26 ± 0.34	1.63 ± 0.08	12.11 ± 0.35
Ethanol	4.33 ± 0.13	0.37 ± 0.02	2.31 ± 0.12	11.43 ± 0.07	2.38 ± 0.07	20.81 ± 0.38
Methanol	28.18 ± 0.48	2.16 ± 0.11	4.31 ± 0.06	12.85 ± 0.19	2.96 ± 0.16	50.45 ± 0.67
Water	26.07 ± 0.75	0.27 ± 0.02	1.65 ± 0.12	0.02 ± 0.00	0.12 ± 0.01	28.13 ± 0.63

3.4. Effect of solvent to solid ratio

In general, a higher solvent volume can dissolve target compounds more effectively and result in a better extraction yield. The extraction was performed with 70% methanol and 45°C at seven different solvent to solid ratio of 5, 10, 15, 20, 30, 40 and 50 ml/g, respectively. Other conditions were: 0.5 g sample, temperature: 45 °C, ultrasound power: 175 W, extraction time: 30 min, particle size: 40–60 mesh. Data shown in Fig. 5 indicated an obvious increase of extraction yield of the five major isoflavones when the solvent to solid ratio was increased from 5 to 15 ml/g. When the solvent to solid ratio was increased from 15 to 50 ml/g, however, no significant differences between the extraction yields of T, ITB, ITA, INA, and TIF ($p > 0.05$) was detected. For commercial application, a solvent to solid ratio of 15 ml/g should be optimum to avoid waste of solvent and bulky handling in the subsequent processes.

3.5. Effect of ultrasound power

The effect of ultrasound power on UAE was explored with solvent to solid ratio at 15 ml/g and other conditions fixed as mentioned previously (sample: 0.5 g, solvent: 70% methanol, temperature: 45 °C, extraction time: 30 min and particle size: 40–60 mesh). As shown in Fig. 6, the highest extraction yields of T, ITB, ITA, TN, INA and TIF were obtained at ultrasound power of 150 W, although the differences of extraction yields of T, ITB, ITA, TN and TIF were not significant ($p > 0.05$) at ultrasound power of 125 and 150 W. When the ultrasound power was above 150 W, the extraction yields for five isoflavones decreased. Therefore, ultrasound power of 150 W was suitable for the extraction.

3.6. Effect of extraction time

Traditionally, higher extraction yield requires a longer extraction period. To investigate the influence of extraction time on yield of isoflavones, 0.5 g sample was extracted at the conditions of 45 °C, 150 W and 7.5 ml of 70% methanol at different time (5, 10, 15, 30, 45 and 60 min). The results shown in Fig. 7 clearly

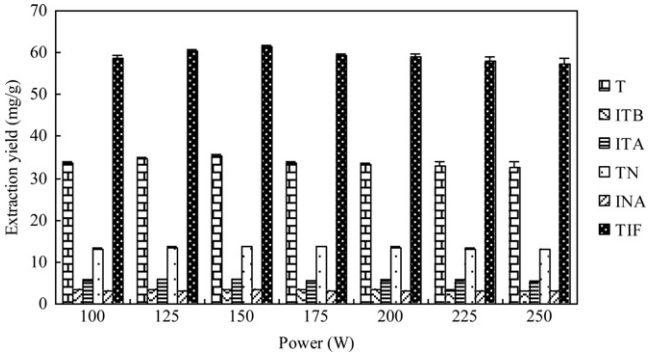

Fig. 6. Effect of the ultrasound power on extraction yield of isoflavones from *I. tectorum*.

indicated that when extraction time increased from 5 to 45 min, the extraction yields of T, ITB, ITA, TN, INA, TIF increased from 23.63 to 36.78, 2.33 to 3.56, 4.06 to 5.78, 10.45 to 14.80, 2.42 to 3.53, 42.88 to 64.46 mg/g, respectively. However the differences of the yields of five isoflavones and TIF were not significant ($p > 0.05$) when the time of UAE increased from 45 to 60 min. Because the diffusion front moved towards the interior of the tissues, the diffusion area reduced, diffusion distance increased and the diffusion rate would decrease accordingly [24]. Therefore, there was no obviously observed yields change in the prolonged time periods. Hence, 45 min was chosen as the optimum extraction time.

3.7. Effect of particle size

A particle size was the key parameter in the extraction process. An amount of 0.5 g sample was extracted at the conditions of 7.5 ml of 70% methanol, 45 °C, 150 W and 45 min at different particle size of 10–20, 20–40, 40–60, 60–80 and 80–100 mesh, respectively. As shown in Fig. 8, the yields of ITB, ITA, TN and INA increased to the maximum at 60–80 mesh, while that of T reached the peak at 80–100 mesh. But the differences of the

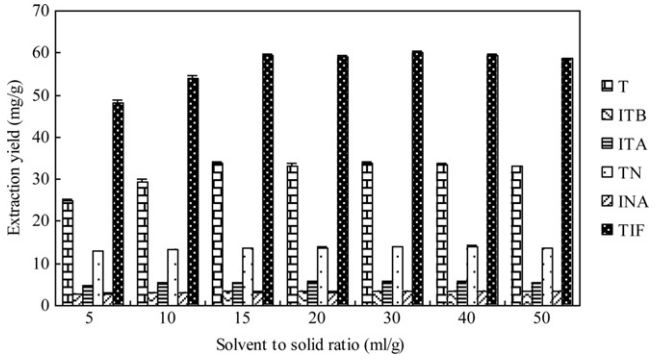

Fig. 5. Effect of the solvent to solid ratio on extraction yield of isoflavones from *I. tectorum*.

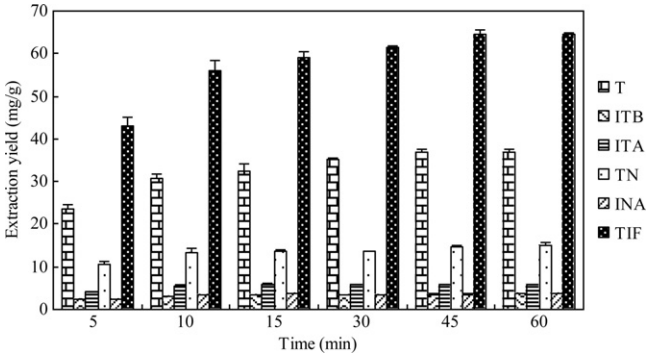

Fig. 7. Effect of the extraction time on extraction yield of isoflavones from *I. tectorum*.

Table 2
Comparison of UAE with other extraction methods.

Extraction method	Extraction yield (mg/g)					
	T	ITB	ITA	TN	INA	TIF
ME[a]	29.89 ± 0.47	2.70 ± 0.09	4.75 ± 0.08	14.60 ± 0.52	3.48 ± 0.13	55.42 ± 0.57
SE[b]	35.51 ± 1.05	3.23 ± 0.06	5.59 ± 0.06	16.32 ± 1.13	3.67 ± 0.05	64.32 ± 0.86
UAE[c]	41.36 ± 1.03	3.87 ± 0.03	6.68 ± 0.04	16.41 ± 0.98	3.87 ± 0.12	72.19 ± 2.02

[a] *Conditions*: 1.0 g sample 60–80 mesh, 100 ml of 70% methanol, at room temperature three times, each for 6 h.
[b] *Conditions*: 1.0 g sample 60–80 mesh, 100 ml methanol for 6 h.
[c] *Conditions*: 1.0 g sample 60–80 mesh, 15 ml 70% methanol, 45 °C, 150 W, 45 min.

yields of T and TIF were not significant ($p > 0.05$) with increase of particle size from 60–80 to 80–100 mesh. The results indicated that the particle size of powder has both positive and negative influence on the yield of extraction. The extraction yields of the isoflavonoids were increased with the particle size decrease. It is because that the higher amount of isoflavonoids was released as milling destroyed the plant cells, and this amount of isoflavonoids was extracted easily for direct exposure to the solvent. However, if the particle size were too small, diffusion would be a difficult step in the extraction, which was not valuable for the extraction of bioactive compounds from natural products [25,26]. Based on these results, the optimum particle size was set at 60–80 mesh.

3.8. Comparison of UAE with ME and SE

The selection of an extraction method would mainly depend on the advantages and disadvantages of the processes, such as extraction yield, complexity, production cost, environmental friendliness and safety. ME and SE are the most frequently used extraction procedures. In this study, UAE was compared with ME and SE for the extraction of five isoflavonoids from *I. tectorum* through the experiments. The conditions of different techniques and their results are summarized in Table 2. The results showed that, compared with ME at 18 h (three time) and SE at 6 h, the highest extraction yields of T, ITB, ITA, TN, INA and TIF were achieved by UAE at 45 min. UAE can save a lot of time and solvent as compared to ME and SE method and bring higher yield of isoflavonoids than ME and SE method. Therefore, UAE was proved to be suitable for the quality control of *I. tectorum* in the state pharmacopoeia [2].

3.9. Stability of UAE method

To study the stability of UAE method for T, ITB, ITA, TN and INA in *I. tectorum*, six replicates of the same ground sample (1.0 g) were processed according to the same optimal UAE protocol, i.e. 15 ml of 70% methanol, 45 °C, 150 W, 45 min, 60–80 mesh. The relative standard deviations (RSDs) for the determined extraction yields of the T, ITB, ITA, TN and INA were 1.16%, 0.82%, 0.75%, 1.04% and 1.87%, respectively. The results showed a good stability of UAE method.

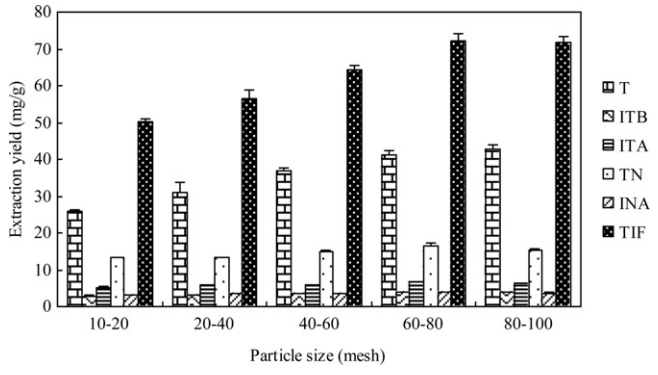

Fig. 8. Effect of the particle size on extraction yield of isoflavones from *I. tectorum*.

4. Conclusions

Ultrasound technique for the simultaneous extraction of five isoflavones from *I. tectorum* was investigated. Compared with ME and SE, UAE has been proved to be a high yield and low solvent consumption for extraction of isoflavones from *I. tectorum*. It is important to note that the extraction time of UAE is significantly shortened. In this paper, the optimal UAE conditions found were: 70% (v/v) methanol solution, temperature 45 °C, solvent to solid ratio 15 ml/g, ultrasound power 150 W, extraction time 45 min and particle size 60–80 mesh. The extraction yields of tectoridin, iristectorin B, iristectorin A, tectorigenin, iristectorigenin A and total isoflavones were 41.36, 3.87, 6.68, 16.41, 3.87 and 72.19 mg/g, respectively. The applicability of UAE to the extraction of other isoflavones from the tissue of other medicinal plants is also expected.

Acknowledgements

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