Microwave-Assisted Aqueous Two-Phase Extraction of the Total Flavonoids from *Taraxacum mongolicum*

**ABSTRACT**

A microwave-assisted aqueous two-phase extraction (MA-ATPE) technique was employed to extract total flavonoids from *Taraxacum mongolicum*. The aqueous two-phase system (ATPS) was made up with ethanol and ammonium sulfate ((NH₄)₂SO₄). The equilibrium phase diagram of the ATPS and the partition behavior of the total flavonoids in the ATPS were systematically measured. Based on the best separation effect, the ATPS with 26 and 18 wt % (NH₄)₂SO₄ was chosen for the extraction of the total flavonoids from *T. mongolicum*. Effect of irradiation power and irradiation time of microwave, as well as pH of aqueous two-phase system on the extraction yield of the total flavonoids was investigated. Response surface methodology was applied to optimize the experimental parameters of the extraction process. The optimal conditions thus obtained were as follows: ratio of ATPS liquid to dried *T. mongolicum* powder at 80 ml/g, irradiation power at 490 W, irradiation time for 45 s and ATPS pH value at 3.4. Under such conditions, the extraction yield reached 5.65%. The MA-ATPE is the most suitable technique for the extraction process studied.

**Key words:** *Taraxacum mongolicum*, total flavonoids, aqueous two-phase extraction (ATPE), microwave-assisted aqueous two-phase extraction (MA-ATPE), yield.

**INTRODUCTION**

*Taraxacum mongolicum* is a member of the family asteraceae. Its chemical compositions are very complex and varied upon its location and species. The valuable components are those bioactive compounds, including flavonoids, phenolic acids, plant sterols and triterpenoids, etc. Extensive studies and traditional medical applications revealed that the flavonoids show various bioactivities, such as anti-oxidant, anti-viral and anti-cancer (Jin and Yin, 2012; Sithisarn et al., 2013; Cao, 2013). Traditionally, *T. mongolicum* was used by Traditional Chinese Medicine (TCM) for thousand years due to their detoxification, decarburunke and sanjie activity, choleric activity and diuretic activity and it is one of the best-known natural traditional Chinese medical herbs (Schütz et al., 2006).

Various traditional extraction techniques for extracting effective medical components from natural plants have been used, such as Soxhlet extraction (SE) (Siman et al., 2002) and heat reflux extraction (HRE) (Sheng et al., 2013). These techniques, however, are of great limitations, including time-consuming, high energy consumption, low recovery rate of target components and toxic organic solvent contamination, etc. In recent decades, various novel extraction techniques have been developed, such as microwave assisted extraction (MAE), ultrasound assisted extraction (USAE), negative pressure cavitation extraction (NPCE) and pressurized solvent extraction (PSE) (Zhang et al., 2008; Brachet et al., 2002; Zhang et al., 2010).

MAE has attracted significant research attention due to its unique heating mechanism, moderate cost and good performance. Especially, microwave energy can strengthen the bioavailability of free pharmacologically active compounds by interrupting the binding of flavonoids to the plant and enhance the penetration of solvent into matrix increasing the release of bioactive compounds. The radiation of microwave has a strong destruction on fibrous tissue and cell walls of plant samples in a high-speed...
transmission process, which makes some components, dissolve into solvent easily and quickly. Moreover, instantaneous temperature increase in the plant sample will hardly change the structure and biological activities of the effective compounds extracted. In recent years, aqueous two-phase extraction (ATPE) is a widely applied separation technology. Using an aqueous polymer and one or more than one salt to form the ATPE system, targeted compounds in herbs are extracted by the designed ATPE system (Babu et al., 2008). However, the polymer in the ATPE system is difficult to recycle. Therefore, those ATPE systems with recyclable main constituents are a more favorite choice for the separation process due to environmental issues. An ATPE system consisting of short chain alcohol and salt is an obvious option with advantages over the traditional ATPE system, including cheap make-up materials and easy recycling. Such ATPE systems were successfully applied to extract and separate bioactive materials from natural plants (Liu et al., 2013, 2010).

Microwave-assisted aqueous two-phase extraction (MA-ATPE) technique takes both advantages of MAE and ATPE. This technique have been reported to effectively extract flavonoids from durian peel (Ruan et al., 2013), steroidal saponins from Dioscorea zingiberensis (Liu et al., 2009; Zeng et al., 2012) and isoflavonoids from Dalbergia odorifera T. Chen leaves (Ma et al., 2013). However, till date its application on extracting the flavonoids from T. mongolicum has not been reported.

Response Surface Methodology (RSM) was widely applied for process optimization, a popular statistical method. It can predict the results for a given set of experimental conditions and find out the maximum of the experimental process. Due to its reasonable design and superior result analysis, this technique was used in the optimization of chemical and physical processes (Draper and Smith, 1981).

In this paper, microwave-assisted aqueous two-phase extraction technology was used to extract the total flavonoids from T. mongolicum. The optimal extraction conditions were obtained by Box-Behnken design (BBD) with response surface methodology. The factors affecting the yield of total flavonoids were investigated and the extraction results compared among different techniques.

**Materials and Methods**

Plant materials and reagents

Plant samples of T. mongolicum were plucked from the University Park in Changzhou University in Jiangsu Province, China. They were washed and air-dried at 60°C until their weight remained constant. Dried samples were cut and ground with a blade-mill (FW100 medicine mill, China) to obtain relatively homogeneous plant powders and then passed through an 80 mesh stainless steel sieve for the experimental use.

Rutin (purity ≥95%) and sodium hydroxide with analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd. Ethanol, Ammonium sulfate, Aluminum nitrate and sodium nitrite were all purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. with analytical grade. Deionized water was used throughout the experiments.

**Determination of total flavonoids content of T. mongolicum**

The total flavonoids content in the extracts were measured using the method of spectrophotometry in the NaNO2-Al(NO3)3-NaOH system (Wan et al., 2014). The regression line for the flavonoids content was \( y = 11.59714x - 0.0031 \) (\( R^2 = 0.9998 \)), where \( y \) is the absorbance of the sample and \( x \) the concentration of the flavonoids.

**Aqueous two-phase system (ATPS)**

In order to prepare aqueous two-phase system with a varied concentration, a calculated mass of (NH4)2SO4 (based on its concentration) was dissolved in a coupled mass of deionized water in a glass tube with stopper. Thereafter, a designed mass of ethanol was added to the tube to form a specified ATPS. For extraction experiments, 0.5 g T. mongolicum powder samples were added to a specified ATPS in the tube. The mixture was mixed by shaking the tube and the tube was then placed into microwave extractor (Midea Group Co., Ltd, China) and irradiated under a set of conditions as designed. These conditions consist of microwave power and irradiation time. The mixture was cooled and then centrifuged at 4000 rpm for 15 min for phase separation. The top phase and bottom phase solution were collected and analyzed using spectrophotometry method.

The partitioning coefficient (\( K \)), volume ratio of the top phase to the bottom phase (\( R \)) and extraction rate (\( Y' \)) were calculated using the equation:

\[
K = \frac{C_t}{C_b} \tag{1}
\]

\[
R = \frac{V_t}{V_b} \tag{2}
\]

\[
Y' = \frac{C_tV_t}{(C_tV_t + C_bV_b)} \times 100\% \tag{3}
\]

Where \( C_t \) and \( C_b \) are the concentrations of the total flavonoids in the top phase and bottom phase, respectively. \( V_t \) and \( V_b \) are the volumes of the top and the bottom phases, respectively. The yield (\( Y' \)) was the ratio of the total
flavonoids partitioned in the top phase to the sample mass of *T. mongolicum*. It was calculated using the equation:

$$ W_{v} = \frac{C}{W} $$

(4)

Where *W* is the sample mass of *T. mongolicum*.

### Phase diagram

A phase diagram of the ATPS similar to that of Ma et al. (2013) was measured using the turbidity titration method. Ethanol was added drop by drop to (NH₄)₂SO₄ solution until the mixture became turbid; thereafter, the mass fraction of ethanol and (NH₄)₂SO₄ were calculated. A certain mass of water was added to the system until the turbidity disappeared; ethanol was added and the system became turbid once again for phase diagram calculation. The last procedures were repeated to form a serial of phase diagram points. Thus, the phase diagram of ATPS with ethanol and (NH₄)₂SO₄ were plotted. The mass fraction of ethanol (*w₁*) and (NH₄)₂SO₄ (*w₂*) were calculated as:

$$ w_{1} = \frac{m_a}{(m_a + m_b + m_c)} \times 100\% $$(5)

$$ w_{2} = \frac{m_b}{(m_a + m_b + m_c)} \times 100\% $$(6)

Where *mₐ*, *mₐ*, *mₐ* are the masses of ethanol, ammonium sulfate and water added in the system.

### Experimental design

On the basis of the single factor experiments, a Box-Behnken Design (BBD) with three independent factors (*x₁*, microwave power; *x₂*, irradiation time and *x₃*, pH) at three levels was used to optimize experiments with response surface methodology using the yield of the total flavonoids as a targeted variable. Three different levels of each independent variable were set to -1, 0, 1, respectively (Table 1).

There are 17 different runs with combinations of the three factors to form a response experiment design. Table 2 shows the experimental conditions and the yields of total flavonoids. Experimental data were fitted to the following second-order polynomial model using Design Expert, version 7.0.0.

$$ Y = B_0 + \sum_{i=1}^{3} B_i x_i + \sum_{i=1}^{3} B_i x_i^2 + \sum_{j=1}^{3} \sum_{j=1}^{3} B_{ij} x_i x_j $$

(7)
Where $Y$ is the response, that is the yield of the total flavonoids, $B_0$ is the constant term; $B_i$, $B_{ii}$, $B_{ij}$ are the linear, quadratic and interaction effects, respectively, $x_i$ and $x_j$ are the coded values of the variables, the independent variable was obtained using the equation:

$$x_i = (X_i - X_0)/\Delta X$$

(8)

Where $X_i$ is the actual value of variable; $X_0$ the actual value of $X_i$ at the centre point and $\Delta X$ the step change value.

RESULTS AND DISCUSSION

Phase diagram of ATPS

Figure 1 shows the phase diagram plotted according to the experiments. The curve in the figure divides the diagram into two regions: two-phase coexisting region above the curve and single phase region below the curve. When a composition pair for ethanol and ammonium sulfate is located in the two-phase region, an ATPS is formed. In this case, the system tends to separate into two layers of phases. The top layer is an ethanol-rich phase and the bottom layer a salt-rich phase. For an ATPE process, the ATPS has to be set in the two-phase region.

In an ATPS, it is interesting to know its phase partitioning behaviors between the top phase and the bottom phase. Figure 2 shows such behaviors when the contents of ethanol and ammonium sulfate are changed. It can be seen that when the concentration of ammonium sulfate increases, the phase volume ratio of the top phase to the bottom phase decreases. At the same time, this ratio increases with the ethanol concentration at a fixed salt concentration. The reason may be that the more the hydrophilic substance is present in one phase of the ATPS, the more the water in the system is attracted to distribute in the phase.

It was noticed that the above phase partitioning behaviors were limited by either the salt solubility capacity or the two phase coexisting requirements. For example, at the salt concentration of 20%, when the ethanol concentration was less than 22%, the ATPS would not form. Whereas, when the concentration was greater than 28%, the salt would precipitate in the vessel bottom. Therefore, all ATPS designed for the extraction process should comply with these limitations.

Partition behaviors of the total flavonoids in ATPS

Effects of the mass fractions of ethanol and ammonium sulfate in the ATPS on the partition coefficient and the total
flavonoids yield were experimentally examined (Figure 3). According to the aforementioned limitations, three different salt concentration conditions, that is, 18, 20 and 22%, respectively, were employed for the experiments. It was observed from Figure 3 that the total flavonoids go more easily into the top phase with partitioning coefficient greater than 6.5. In the ATPE extraction process, the separation of the components is according to their polarities. The chemical species with the weaker polarity go to ethanol-rich phase, whilst the chemical species with stronger polarity goes to the salt-rich phase. The polarity of flavonoids is relatively weak and therefore goes easily into...
the ethanol-rich phase. It can be seen from Figure 3a that at a fixed salt concentration the partitioning coefficient (K) firstly increases with the ethanol concentration and then slightly decreases. The reason for this phenomenon may be that at the lower end of the ethanol concentration, where there is less volume of the top phase, the total flavonoids are less extracted in the top phase. Whereas, at the higher end of the ethanol concentration, where there is more top phase, though the total flavonoids extracted in the phase may be increased, the concentration of it is decreased due to more increase in the phase volume. It also can be seen from Figure 3b that the total flavonoids yield increases overall with the ethanol concentration. It is noted that both the partitioning coefficients and the total flavonoids yields for 20% ammonium sulfate concentration are mostly much higher than these for the other two concentrations.

A set of extraction experiments was designed to examine extraction rate of total flavonoids into the ATPS (Figure 4). The extraction rate here is a ratio of total flavonoids extracted into both the top and the bottom phase to the weight of sample powders. The conditions for these experiments were similar to the procedures earlier described but without microwave processing and with the extraction time of 12 h at 25°C. It can be seen that the extraction rate of the total flavonoids in the ATPS increases overall with ethanol concentration. Also, the best extraction rate occurs at the 20% ammonium sulfate concentration. The reason is still unknown.

**Optimization of the extraction process by microwave-assisted aqueous two-phase extraction (MA-ATPE)**

**Effect of solvent to powders ratio**

Figure 5 shows the effect of the ratio of solvent to sample powders (ml/g) on the total flavonoids yield. Sample powders (0.5 g) were added in the ATPS, which consists of 26% ethanol and 20% (NH₄)₂SO₄. The microwave power was set at 350 w, the time at 45 s and the system pH at 3.5. It can be seen that when the ratio is less than 80:1, the yield rapidly increases with the ratio. Whereas, when the ratio is greater than 80:1, the yield keeps almost constant. The reason is obvious. The flavonoids fixed in the sample powders are transported to the ATPS in the MA-ATPE process. When there is less solvent, the extracted amount of flavonoids is limited due to the thermodynamic balance between the solution and the sample tissue. When the volume of solvent is increased, more flavonoids are extracted into the solution and this result in yield increase. However, if excessive solvent is present in the system, the flavonoids extracted into the solution increases very little.  

![Figure 4. Effect of mass fraction of ethanol and (NH₄)₂SO₄ on the extraction rate of total flavonoids.](image-url)
Figure 5. Effect of different ratio of solvent/solid (ml/g) on the yield (Y) of the total flavonoids.

Figure 6. Effect of different irradiation power (W) on the flavonoids yield (Y).

Effect of irradiation power

Figure 6 shows the effect of the microwave power on the flavonoids yield (Y).

with drop in their concentrations (Zhang et al., 2013). Therefore, for the following experiments, the ratio of solvent to sample powders was selected at 80:1.
total flavonoids yield. The results were obtained with 0.5 g sample powders, ATPS concentrations of 26% ethanol and 20% \( (\text{NH}_4)_2\text{SO}_4 \), the ratio of solvent to sample powders of 80:1, irradiation time of 45 s and pH of 3.5. As can be seen from Figure 6, the yield increases with the microwave power for the first stage reaches a highest value at the microwave power of 490 W and then drops sharply. This is because the microwave intensity at lower power range would help to destroy the plant tissues and release the flavonoids. However, when the microwave intensity is excessively strong, this may decompose the flavonoids structure (Gao et al., 2006). As such appropriate microwave power was chosen at 490 W.

### Effect of irradiation time

Figure 7 shows the effect of irradiation time on the yield of total flavonoids. The results were obtained with 0.5 g sample powders, the ATPS of 26% ethanol and 20% \( (\text{NH}_4)_2\text{SO}_4 \), the ratio of solvent to sample powders of 80:1, irradiation power of 490 W and system pH of 3.5. It can be seen that the yield increases with the irradiation time to a peak at 45 s and then decreases. At the beginning of the extraction process, the extract concentration difference between the system and the sample was larger and the transport of the extract from the sample to the ATPS faster. Subsequently, the yield significantly increased with time. However, too much irradiation time will make the sample interior over heated and may destruct the bioactive materials. Similar results were reported when the system was used for extraction of flavonoids from Radix Astragali (Xiao et al., 2008). Therefore, 45 s was selected as the appropriate irradiation time.

### Effect of system pH value

Obviously, system pH value will act its impact on the hydrophilic property of the three phases (two liquid phases and one solid phase) in a ATPE process and therefore affect the flavonoids distribution in the phases. Using either HCl or NaOH to adjust the system pH value, the effect of the system pH value was investigated (Figure 8). In order to keep the system stable, acidic condition should be maintained due to ammonium sulfate decomposition at alkaline conditions. The results showed that the yield increases with the pH to a peak value at pH of 3.5 and then decreases. The reason may be that at the lower end of the pH value, the flavonoids combined with H\(^+\) and was gradually released as pH value increased and thus more flavonoids entered into the top phase. However, at the higher end of the pH value, more flavonoids may be attracted into the bottom salt-rich phase. Thus, pH of 3.5 was selected as being appropriate.

### Optimization of the MA-ATPE

According to the single factor experiments, effect of
independent variables and their interaction on the total flavonoids yield was studied by Box-Behnken.

We chose the range of irradiation power (420 to 560 W), irradiation time (35 to 55 s), pH (3 to 4) for the following experiments.

**Table 3. ANOVA results of the regress model.**

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>Df</th>
<th>Mean square</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>5.52</td>
<td>9</td>
<td>0.64</td>
<td>42.95</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>0.055</td>
<td>3</td>
<td>0.018</td>
<td>5.37</td>
<td>0.0692</td>
</tr>
<tr>
<td>Pure error</td>
<td>0.014</td>
<td>4</td>
<td>0.0034</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Correlation total</td>
<td>5.59</td>
<td>16</td>
<td>R²=0.9878</td>
<td>R²_adj=0.9720</td>
<td>R²_pred=0.972</td>
</tr>
</tbody>
</table>

**Table 4. Test results of significance for regression coefficient of total flavonoids.**

<table>
<thead>
<tr>
<th>Model</th>
<th>Coefficient estimate</th>
<th>Standard error</th>
<th>95% CI low</th>
<th>95% CI high</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>5.65</td>
<td>0.044</td>
<td>5.55</td>
<td>5.75</td>
<td>-</td>
</tr>
<tr>
<td>x₁</td>
<td>0.042</td>
<td>0.035</td>
<td>-0.040</td>
<td>0.13</td>
<td>0.2631</td>
</tr>
<tr>
<td>x₂</td>
<td>0.074</td>
<td>0.035</td>
<td>-8.846E-03</td>
<td>0.16</td>
<td>0.0729</td>
</tr>
<tr>
<td>x₃</td>
<td>-0.081</td>
<td>0.035</td>
<td>-0.16</td>
<td>1.346E-03</td>
<td>0.0529</td>
</tr>
<tr>
<td>x₁x₂</td>
<td>-7.500E-03</td>
<td>0.049</td>
<td>-0.12</td>
<td>0.11</td>
<td>0.8836</td>
</tr>
<tr>
<td>x₁x₃</td>
<td>-0.028</td>
<td>0.049</td>
<td>-0.14</td>
<td>0.089</td>
<td>0.5951</td>
</tr>
<tr>
<td>x₂x₃</td>
<td>0.030</td>
<td>0.049</td>
<td>-0.087</td>
<td>0.15</td>
<td>0.5628</td>
</tr>
<tr>
<td>x₁²</td>
<td>-0.64</td>
<td>0.048</td>
<td>-0.76</td>
<td>-0.53</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>x₂²</td>
<td>-0.85</td>
<td>0.048</td>
<td>-0.97</td>
<td>-0.74</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>x₃²</td>
<td>-0.20</td>
<td>0.048</td>
<td>-0.31</td>
<td>-0.081</td>
<td>0.0049</td>
</tr>
</tbody>
</table>

**Fitting the models**

All experimental data were chosen for the program Design-Expert with 17 runs (Table 2). The experimental data were analyzed using multiple regressions, the yield and variables of predicted data were obtained from the following regression model equation:

**Figure 8.** Effect of pH on the yield(Y) of total flavonoids.
$Y = -54.40063 + 0.13234x_1 + 0.76113x_2 + 5.41250x_3 - 1.07143 \times 10^{-5} x_1 x_2 - 7.85714 \times 10^{-4} x_1 x_3 + 6.00000 \times 10^{-3} x_2 x_3 - 1.31122 \times 10^{-4} x_1^2 - 8.55000 \times 10^{-3} x_2^2 - 0.78000x_3^2$

**Analysis of variance (ANOVA) for the extraction yield of total flavonoids**

Table 3 shows the variance analysis of the yield ($Y$) model. The model F-value is 42.95 and the regression of the model is highly significant ((Prob>F) <0.0001). That the lack of fit was not significant (p=0.0692>0.05) indicates that the model chosen is adequate to explain the experiment data. The determination coefficient ($R^2=0.9878$) shows that the model can explain 98.78% of the variables and only 1.22% of the total can not be explained. The adjusted determination coefficient ($R^2_{adj}=0.9720$) is also in reasonable agreement with the predicted ($R^2_{pred}=0.9720$). Low coefficient of variation is also clearly demonstrated indicating that the experimental values have a high degree of precision and reliability. Therefore, the model obtained from the response surface method is appropriate for predicting the yield of the total flavonoids.

Table 4 shows that the test results of significance for regression coefficient of total flavonoids extraction. The linear effects of all the variables were not so significant (P>0.05). It was observed from Table 4 that pH was the most significant factor followed by irradiation time and power. The quadratic terms ($x_1^2$, $x_2^2$ and $x_3^2$) were extremely significant (P<0.01). The interaction terms were not significant (P>0.05). Standard error of regression coefficients are in the range of 0.035 to 0.049. All these indicated that the effect of these factors on the total flavonoids extraction is not a simple linear relationship. According to the regression model, the predicted maximum yield obtained is 5.66%. The optimal combination thus obtained is as follows: microwave power 492.64 W, microwave time 45.39 s and system pH 3.4.

**Effect of variables and theirs interaction on the yield of the total flavonoids**

The relationships among variables and responses were obtained from the response surface method. The strength of interaction effect is reflected by the shape of contour. Oval represents a significant interaction effect and on the contrary, circular is not significant.

Figure 9 shows the effect of irradiation power ($x_1$) and irradiation time ($x_2$). As the irradiation power increases from 420 to 490 W, the yield of flavonoids also increases with the irradiation time. However, the yield decreases with the irradiation time over the irradiation power of 490 W. Meanwhile, as the irradiation time increases from 35 to 45 s, the yield of flavonoids increases with the irradiation power. Afterwards, the yield decreases.

Figure 10 shows the influence of the interaction between irradiation power ($x_1$) and pH value ($x_3$). It is noted that pH
value had a significant effect on the flavonoids yield. The influence of the interaction between irradiation time ($x_2$) and pH value ($x_3$) was demonstrated in Figure 11. Note that similar to Figure 10, the pH value has a great impact on the flavonoids yield. Therefore, pH value set in the process is crucial to the flavonoids extraction. It also noted from Figures 9 to 11 that the interaction between $x_1$ and $x_2$ is circular whilst the interactions between $x_1$ and $x_3$, and $x_2$ and $x_3$ are oval. Therefore, the interaction effects of $x_1x_3$ and $x_2x_3$ are superior to $x_1x_2$. The $P$ values for these three interactions in Table 4 also indicate this feature.

**Optimization of extraction parameters and verification**

According to the aforementioned optimal conditions, we designed the best extraction conditions for extracting total flavonoids from *T. mongolicum*, which were irradiation power at 490 W, irradiation time for 45 s and pH at 3.4. The experimental yield $Y$ was 5.65%, which was in agreement with the predicted value (5.66%) earlier mentioned. Therefore, the regress model is suitable for explaining the extraction process.

**Comparison of MA-ATPE with heat reflux (HRE) and ultrasound-assisted extraction (UAE)**

Heat reflux extraction (HRE) is a conventional method for the extraction of bioactive components from natural material. Ultrasound-assisted extraction (UAE) was widely...
used for extraction of flavonoids recently. At the optimization conditions, Table 5 shows a comparison of MA-ATPE with HRE and UAE. Compared with HRE, the MA-ATPE has obvious advantage of less solvent usage, shorter time and higher extraction yield. In addition, as compared to UAE, the extraction time for MA-ATPS is significantly reduced and the yield is improved. By the way, ethanol and ammonium sulfate can be recycled by precipitation and distillation processes (Wu et al., 2011).

**Conclusions**

A safety green and highly efficient method was developed for extracting the total flavonoids from *T. mongolicum* using the MA-ATPE process. Response surface methodology was used to optimize the conditions of the experiment. The ATPS was made up with recyclable ethanol and ammonium sulfate. The optimized MA-ATPE conditions were in the ratio of solvent/solid 80:1, irradiation power 490 W, irradiation time 45 s and pH of system 3.4. Under these conditions, the flavonoids yield reached 5.65%. The pH value in the ATPS is crucial to the flavonoids extraction. The MA-ATPE is the best option for the extraction processes in study.

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**REFERENCES**


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Table 5. Comparison of aqueous two-phase extraction with other extraction methods.

<table>
<thead>
<tr>
<th>Extraction methods</th>
<th>Extraction time</th>
<th>Solvent</th>
<th>Ratio of ethanol to sample plant (ml/g)</th>
<th>Y (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA-ATPE</td>
<td>45 s</td>
<td>ethanol/(NH₄)₂SO</td>
<td>26.3</td>
<td>5.65</td>
</tr>
<tr>
<td>UAE</td>
<td>30 min</td>
<td>60%ethanol</td>
<td>36.0</td>
<td>5.25</td>
</tr>
<tr>
<td>HRE</td>
<td>1 h</td>
<td>60%ethanol</td>
<td>36.0</td>
<td>4.63</td>
</tr>
</tbody>
</table>

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