Optimized extraction of polysaccharides from *Taxus chinensis* var. *mairei* fruits and its antitumor activity

Chunjian Zhao\(^a,b\), Zhao Li\(^a\), Chunying Li\(^a,e\), Lei Yang\(^a\), Liping Yao\(^a\), Yujie Fu\(^a\), Xin He\(^a\), Kunming Shi\(^a\), Zhicheng Lu\(^a\)

\(^a\) Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin 150040, China
\(^b\) Collaborative Innovation Center for Development and Utilization of Forest Resources, Harbin 150040, China

**A R T I C L E   I N F O**

Article history:
Received 24 November 2014
Received in revised form 18 January 2015
Accepted 25 January 2015
Available online 31 January 2015

**Keywords:**
*Taxus chinensis* var. *mairei* polysaccharides
Optimized extraction
Antitumor activity

**A B S T R A C T**

The simultaneous ultrasonic/microwave-assisted extraction (UMAE) method is potentially useful for the extraction of polysaccharides from *Taxus chinensis* var. *mairei* fruits (TCFPs). In this study, we used a response surface methodology to identify optimal TCFPs extraction conditions. Optimal parameters were determined as follows: a liquid to raw material ratio of 33 mL/g, an extraction time of 10 min, a microwave power level of 560 W, and a fixed ultrasonic power of 50 W. Under the optimized conditions, TCFPs yields obtained by UMAE were 4.33 ± 0.15%, a 1.79-fold increase compared with conventional heating reflux extraction (HRE). In addition, the extraction time used in UMAE was shorter than that required for HRE: 10 versus 90 min. UMAE is therefore a rapid and efficient method for the extraction of TCFPs. The inhibitory effect of TCFPs on S180 tumor growth in vivo was also studied. The tumor inhibition rate of TCFPs was 76.33%, indicating a tumor-inhibiting effect. Analysis of organ weights demonstrated that TCFPs exhibited no toxicity to liver, kidney, spleen, heart, or lung relative to a positive control group. TCFPs thus showed antitumor activity with no organ toxicity.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

*Taxus chinensis* var. *mairei*, also known as Mairei yew, is a valuable, natural anti-cancer plant species endemic to China [1]. Since the discovery of taxol with its significant anti-cancer biological activity in the bark of *T. chinensis* var. *mairei*, the chemical components of this species have been of considerable worldwide research interest [2–4]. Recent reports have suggested that polysaccharides in *T. chinensis* var. *mairei* leaves have potent antitumor, glycemia-inhibiting, and immunity-enhancing properties [5,6].

Many extraction methods have been used to extract polysaccharides, such as heating reflux [7], ultrasonic [8,9] and microwave [10,11] extraction. These methods have many disadvantages, such as long extraction time, low yields, and unsatisfactory recoveries. A novel and efficient extraction method that avoids these disadvantages is therefore needed.

A combination of microwave radiation and ultrasound can accelerate the extraction process and thereby improve the extraction efficiency of bioactive compounds [12]. The main advantage of the microwave approach is speed [13,14], but a disadvantage is inhomogeneous heating. The possible benefits of ultrasonic extraction are mass transfer intensification, cell disruption, and improved penetration effects [15]. Consequently, the combination of ultrasound and microwave radiation is a complementary technique that may exhibit the advantages of both methods. Simultaneous ultrasonic/microwave-assisted extraction (UMAE) is a recent approach that couples the advantages of microwave and ultrasonic extraction, and has been used for the extraction and separation of some natural plant compounds [16,17]. Although a few reports have appeared regarding the optimization of UMAE of polysaccharides [18,19], no such studies exist for polysaccharides from *T. chinensis* var. *mairei* fruits (TCFPs). In this study, we used response surface methodology (RSM) to optimize the UMAE of TCFPs and to initially evaluate the inhibitory effect of TCFPs on S180 tumor growth in vivo.

2. Materials and methods

2.1. Materials

*Taxus chinensis* var. *mairei* fruits were collected in the city of Nanping, Fujian Province, China in November 2013. After drying at
2.2. Equipment

To extract polysaccharides, we used a UMAE apparatus (CW-2000; Shanghai Xintuo Analytical Instrument Technology Co., Shanghai, China) with a maximum microwave power of 800 W equipped with an ultrasonic transducer with a fixed power of 50 W. An ultraviolet spectrophotometer (UV2550; Shimadzu, Japan) was used for the determination of polysaccharides. To examine morphological changes in materials, we used a scanning electron microscope (Quanta-200; FEI, USA).

2.3. Extraction procedures

2.3.1. Heating reflux extraction (HRE)

According to reference [20], powdered T. chinensis var. mairei fruit (10.0 g) was mixed with 300 mL of distilled water and boiled using a heating jacket for 30, 60, 90, or 120 min. The mixture was then centrifuged for 5 min at 4000 × g. This extraction procedure was performed three times.

2.3.2. Ultrasonic-assisted extraction (UAE)

UAE was performed as described by reference [20] with minor modification. Powdered fruit samples (10.0 g) were placed in a flask with 300 mL of distilled water. Each sample was extracted three times by ultrasonication for 10, 20, 30, or 40 min at 50 °C. The obtained extraction solutions were combined and cooled to room temperature.

2.3.3. Microwave-assisted extraction (MAE)

Powdered fruit samples (10.0 g) were mixed with 300 mL of distilled water, according to reference [20]. The suspensions were then irradiated under microwave heating. Each sample was microwave extracted three times for 10, 20, 30, or 40 min at 560 W. After each irradiation, obtained extraction solutions were combined and cooled to room temperature.

2.3.4. UMAE of polysaccharides

Polysaccharides from T. chinensis var. mairei fruits was extracted using the UMAE apparatus described as Section 2.2. With cold water running through the UMAE system’s condenser, samples were mixed with 10 mL of water. The suspensions were then irradiated under microwave heating with ultrasonication at a fixed ultrasonic power of 50 W. After each irradiation, the obtained extraction solutions were combined and cooled to room temperature.

2.4. Calculation of polysaccharides yields

After extraction, the mixtures were centrifuged at 4000 × g for 5 min. The supernatants were collected and condensed using a rotary evaporator at 60 °C under vacuum. Condensed supernatants were added to 4-fold volumes of cold absolute alcohol (4 °C) and allowed to sit overnight. The precipitated crude polysaccharides were prepared by centrifugation at 4000 × g for 5 min and freeze-drying.

Polysaccharides content was calculated using the phenol-sulfuric acid method [21]. The yield of polysaccharides was calculated using the following equation:

\[
Y = \frac{W_2 \times C}{W_1} \times 100%
\]

where \(Y\) is the TCFPs yield (%), \(W_1\) is the weight of raw material (g), \(W_2\) is the weight of crude polysaccharides (g), and \(C\) is the polysaccharides content in the crude polysaccharides extract (%).

2.5. Experimental design

2.5.1. Single-factor experimental design

The effect of liquid to raw material ratio (15, 20, 25, 30, and 35 mL/g), extraction time (5, 10, 15, 20, and 25 min), microwave power (200, 300, 400, 500, and 600 W), and pre-soaking time (0, 1, 2, 4, 6, and 8 h) were all studied using a single-factor design. In each experiment, one factor was changed while the others were kept constant.

2.5.2. Box–Behnken design

On the basis of the single-factor experimental results, we performed a Box–Behnken design (BBD) analysis with three independent variables \(X_1\), \(X_2\), and \(X_3\) (ratio of liquid to raw material; \(X_2\), extraction power; \(X_3\), extraction time) at three levels and with yield \(Y\) as the response variable. The range of independent variables, the BBD matrix, and values of the response variable used in the model construction are listed in Table 1. Based on the BBD experimental data, a regression analysis was carried out using the following quadratic equation model [22]:

\[
Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j
\]

Table 1

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Symbol</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio of liquid to raw material (mL/g)</td>
<td>(X_1)</td>
<td>−1 0 1</td>
</tr>
<tr>
<td>Microwave power (W)</td>
<td>(X_2)</td>
<td>250 500 750</td>
</tr>
<tr>
<td>Extraction time (min)</td>
<td>(X_3)</td>
<td>15 20 25</td>
</tr>
</tbody>
</table>

where \(Y\) is the response variable, \(\beta_0\), \(\beta_i\), \(\beta_{ii}\), and \(\beta_{ij}\) are the regression coefficients of the intercept, linear, quadratic, and interaction terms, respectively, and \(X_i\) and \(X_j\) are the levels of the independent variables influencing the response variable \(Y\). The coefficients of the polynomial model and the response surfaces obtained from the experimental design were subjected to multiple nonlinear regression analyses using the software package Design-Expert 8.0.5 (Stat-Ease, USA).
2.6. Scanning electron microscopy (SEM)

Morphological changes in the pulverized fruit as a result of the different extraction methods (HRE, UE, ME, and UMAE) were observed by SEM [23]. The test samples were fixed on a specimen holder with conductive tape and then sputtered with gold using an ion sputter coater. All samples were scanned under high vacuum at an accelerating voltage of 12.5 kV.

2.7. Antitumor in vivo activity analysis

2.7.1. Tumor model generation and experimental design

A solid tumor model was established by inoculating mice with 0.2 mL of a cell suspension containing \(1 \times 10^7\) S180 cells/mL via subcutaneous injection of the right axilla [24]. The mice were then randomized into four groups: a negative control (orally fed with physiological saline), a positive control (orally fed with 100 mg/kg of cyclophosphamide), and two groups administered different doses of TCFPs (orally fed with 200 or 600 mg/kg of TCFPs). Each group comprised eight mice. Mice in the non-control groups were orally fed with 0.5 mL of TCFPs once daily for 7 days.

2.7.2. Statistical data analysis

Mice were weighed daily during the experimental period. At the end of the experiment, the animals were sacrificed by cervical dislocation. The solid tumor and organs (heart, liver, spleen, lung, and kidney) were separated and weighed.

Tumor inhibition rate was calculated from the following formula [25]:

\[
\text{TIR} \text{ (%)} = \left(1 - \frac{W_1}{W_0}\right) \times 100, \tag{3}
\]

where TIR (%) is the tumor inhibition rate, \(W_1\) is the tumor weight of the treatment group, and \(W_0\) is the tumor weight of the control group.

Change in mouse body weight was calculated as follows [25]:

\[
\text{RBWC} \text{ (%)} = \frac{W_2 - W_1}{W_1} \times 100, \tag{4}
\]

where RBWC (%) is the percentage change in mouse body weight following drug treatment and \(W_1\) and \(W_2\) are mouse body weights on the first and last days of the experimental period, respectively.

Relative organ weight was calculated as follows [25]:

\[
\text{ROW} \text{ (%)} = \frac{W_0}{W_5} \times 100, \tag{5}
\]

where ROW (%) is the relative organ weight, \(W_0\) is the organ weight, and \(W_5\) is the mouse body weight.

The data of tumor inhibition rate, the percentage change in mouse body weight and relative organ weight were subjected to single-factor analysis of variance (ANOVA) using SPSS10.0 statistical software.

3. Results and discussion

3.1. Effect of different ratios of liquid to raw material on TCFPs yield

The effect of different ratios of liquid to raw material on polysaccharides yield was investigated. As shown in Fig. 1, yields increased significantly when the ratio of liquid to raw material was increased from 15:1 to 30:1. Further increases in the ratio of liquid to raw material had little additional effect. Within a certain range, raising the ratio of liquid to raw material can facilitate complete immersion of raw material into the liquid and increase mass transfer, resulting in higher yields of target compounds [26]. On the basis of these results, a 30:1 ratio of liquid to raw material was selected as the optimum ratio for subsequent experiments.

3.2. Effect of microwave power on TCFPs yield

Selecting a proper microwave power is very important for improving TCFPs yields. Fig. 2 illustrates the effect of different microwave power levels (200, 300, 400, 500, and 600 W) on TCFPs yields. As can be seen in the figure, yields of polysaccharides rapidly increased between 200 and 500 W. This increase was owing to the fact that microwave irradiation energy enhances solvent penetration into the matrix and enables its efficient delivery to target materials via molecular interaction with the electromagnetic field; this rapid transfer of energy to the solvent and matrix improves dissolution of the components to be extracted [22]. At microwave power levels above 500 W, yield increases leveled off, with no additional significant changes observed. Consequently, 500 W was chosen as the optimal microwave power level for TCFPs extraction.

3.3. Effect of different extraction time on TCFPs yield

Extraction of TCFPs was carried out for different time durations (5, 7.5, 10, 12.5, and 15 min). The effect of extraction time on TCFPs yields is shown in Fig. 3. Yield increased as the extraction time was lengthened from 5 to 10 min, and then decreased at longer UMAE treatment time. We therefore chose a 10-min extraction time as optimal for TCFPs extraction.
We first used a pure liquid to raw material ratio of 1:30 and a microwave power of 500 W, and an extraction time of 10 min were used.

Fig. 3. Effect of extraction time on the yield of polysaccharides from Taxus chinensis var. mairei fruits (TCFPs). Error bars indicate standard deviations (n = 3). A 30:1 ratio of liquid to raw material and a microwave power of 500 W were used.

Fig. 4. Effect of pre-soaking time on the yield of polysaccharides from Taxus chinensis var. mairei fruits (TCFPs). Error bars indicate standard deviations (n = 3). A 30:1 ratio of liquid to raw material, a microwave power of 500 W, and an extraction time of 10 min were used.

3.4. Effect of different pre-soaking time on TCFPs yield

Pre-soaking time is an important factor in the extraction of dry materials, as soaking can increase moisture content and enhance the ability of cells to absorb microwave irradiation [27]. The effect of different pre-soaking time on TCFPs yields is shown in Fig. 4. We observed that TCFPs yield increased as pre-soaking time was lengthened from 0 to 2 h, with negligible changes at longer time. We therefore chose 2 h as the appropriate pre-soaking time.

Table 2

<table>
<thead>
<tr>
<th>Run</th>
<th>X1 (ratio of liquid to raw material, mL/g)</th>
<th>X2 (microwave power, W)</th>
<th>X3 (extraction time, min)</th>
<th>TCFPs yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>500</td>
<td>15</td>
<td>3.92</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>500</td>
<td>20</td>
<td>4.30</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>500</td>
<td>25</td>
<td>3.92</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>600</td>
<td>20</td>
<td>4.31</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>400</td>
<td>20</td>
<td>3.82</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>400</td>
<td>15</td>
<td>3.59</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>400</td>
<td>25</td>
<td>3.67</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>500</td>
<td>20</td>
<td>4.20</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>400</td>
<td>20</td>
<td>3.50</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>500</td>
<td>20</td>
<td>4.30</td>
</tr>
<tr>
<td>11</td>
<td>35</td>
<td>500</td>
<td>15</td>
<td>4.22</td>
</tr>
<tr>
<td>12</td>
<td>30</td>
<td>500</td>
<td>20</td>
<td>4.22</td>
</tr>
<tr>
<td>13</td>
<td>30</td>
<td>500</td>
<td>20</td>
<td>4.18</td>
</tr>
<tr>
<td>14</td>
<td>30</td>
<td>600</td>
<td>25</td>
<td>4.25</td>
</tr>
<tr>
<td>15</td>
<td>35</td>
<td>500</td>
<td>25</td>
<td>4.15</td>
</tr>
<tr>
<td>16</td>
<td>25</td>
<td>600</td>
<td>20</td>
<td>4.13</td>
</tr>
<tr>
<td>17</td>
<td>30</td>
<td>600</td>
<td>15</td>
<td>4.15</td>
</tr>
</tbody>
</table>

3.5. Optimization of TCFPs extraction by RSM

3.5.1. Statistical analysis and model fitting

Methods such as factorial design, BBD provide powerful and efficient way to optimize extraction conditions using a reduced number of experiments [17,18]. In the study, according to the single-factor experimental results, three independent factors—extraction time, liquid-solid ratio, and microwave power—and a dependent variable (TCFPs yield) were investigated by RSM base on BBD.

The experimental results are shown in Table 2, which lists 17 different experimental combinations and the response values (TCFPs yields). From the five center points, a pure error sum of squares was obtained. Analysis of the experimental data with Design-Expert software demonstrated that the response variable Y (the yield of TCFPs) could be characterized by the following second-order polynomial equation:

\[
Y = 4.24 + 0.13X_1 + 0.28X_2 + 0.015X_3 - 0.036X_1X_2 - 0.018X_1X_3 + 0.0038X_2X_3 - 0.018X_{12} - 0.22X_{22} - 0.1X_{32},
\]

where Y represents TCFPs yield (%) and X₁, X₂, and X₃ represent the ratio of liquid to raw material (mL/g), ultrasonic power (W), and extraction time (min), respectively.

Table 3

Results of analysis of variance (ANOVA) for a fitted quadratic polynomial model of the yield of polysaccharides from Taxus chinensis var. mairei fruits.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F-Value</th>
<th>p-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>1.08</td>
<td>9</td>
<td>0.12</td>
<td>40.52</td>
<td>0.000</td>
<td>**</td>
</tr>
<tr>
<td>X₁</td>
<td>0.13</td>
<td>1</td>
<td>0.13</td>
<td>45.00</td>
<td>0.000</td>
<td>**</td>
</tr>
<tr>
<td>X₂</td>
<td>0.64</td>
<td>1</td>
<td>0.64</td>
<td>215.44</td>
<td>0.000</td>
<td>**</td>
</tr>
<tr>
<td>X₃</td>
<td>1.69 × 10⁻¹</td>
<td>1</td>
<td>1.69 × 10⁻¹</td>
<td>0.57</td>
<td>0.474</td>
<td>n.s.</td>
</tr>
<tr>
<td>X₁X₂</td>
<td>5.15 × 10⁻¹</td>
<td>1</td>
<td>5.15 × 10⁻¹</td>
<td>1.74</td>
<td>0.229</td>
<td>n.s.</td>
</tr>
<tr>
<td>X₁X₃</td>
<td>1.31 × 10⁻¹</td>
<td>1</td>
<td>1.31 × 10⁻¹</td>
<td>0.44</td>
<td>0.527</td>
<td>n.s.</td>
</tr>
<tr>
<td>X₂X₃</td>
<td>5.89 × 10⁻¹</td>
<td>1</td>
<td>5.89 × 10⁻¹</td>
<td>0.02</td>
<td>0.892</td>
<td>n.s.</td>
</tr>
<tr>
<td>X₁²</td>
<td>0.03</td>
<td>1</td>
<td>0.03</td>
<td>9.40</td>
<td>0.018</td>
<td>*</td>
</tr>
<tr>
<td>X₂²</td>
<td>0.20</td>
<td>1</td>
<td>0.20</td>
<td>68.40</td>
<td>0.000</td>
<td>**</td>
</tr>
<tr>
<td>X₃²</td>
<td>0.05</td>
<td>1</td>
<td>0.05</td>
<td>15.34</td>
<td>0.006</td>
<td>*</td>
</tr>
<tr>
<td>Residual</td>
<td>2.96 × 10⁻¹</td>
<td>7</td>
<td>2.96 × 10⁻¹</td>
<td>0.73</td>
<td>0.585</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>7.34 × 10⁻³</td>
<td>3</td>
<td>7.34 × 10⁻³</td>
<td>0.73</td>
<td>0.585</td>
<td>n.s.</td>
</tr>
<tr>
<td>Pure error</td>
<td>0.01</td>
<td>4</td>
<td>3.34 × 10⁻³</td>
<td>0.73</td>
<td>0.585</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total</td>
<td>1.10</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a X₁: liquid/solid ratio; X₂: microwave power; X₃: extraction time.

b *Significant, p < 0.05; **Highly significant, p < 0.001; n.s., not significant.
Results of the ANOVA for the response surface quadratic model are also shown in Table 3. As can be seen from Table 3, the low p-value (<0.0001) of the model indicates that the regression equation was ideal. At the same time, the lack of fit was not significant relative to the pure error. Consequently, the model was proved to be suitable for analysis of the experimental data and the experimental performance evaluation.

The p-value was used as a standard to check the significance of each independent variable and the degree of correlation between each independent variable in the model. As indicated in Table 3, the linear terms (X₁ and X₂) and the quadratic term (X²) were highly significant (p < 0.001). The quadratic terms X²₁ and X²₂ were found to be significant (p < 0.05). However, the interaction terms (X₁X₂, X₁X₃, and X₂X₃) were found to be non-significant (p > 0.05), indicating that the interactions of any two of three variables were not significant. Analysis of the regression equation using Design-Expert software allowed us to generate contour plots and 3D response-surface plots to visualize the interaction between variables. The relationship between TCFPs yield and any two independent variables (with the other variable set to “0” level) is accordingly shown in Fig. 5.

3.5.2. Verification of the predictive model

Optimal parameter values for maximal TCFPs yields according to regression Eq. (6) were a liquid to raw material ratio of 33.31 mL/g, ultrasonic power of 559.04 W, and an extraction time of 10.06 min. The predicted yields of TCFPs were 4.36%. Considering the precision of the extraction device, we chose a liquid to raw material ratio of 33 mL/g, ultrasonic power of 560 W, and extraction time of 10 min as the optimal conditions for the TCFPs extraction. Using these parameter conditions, the actual yield of TCFPs was 4.33 ± 0.15% (n = 3), which was very close to the predicted value. The predictive model was accordant with the actual experiment. The polysaccharides content in crude TCFPs extract obtained under the optimal conditions was determined to be 75.2%.

3.6. Comparison of different extraction methods

UMAE has been used for the extraction of polysaccharides from Schisandra chinensis Baill. fruits and results showed that the extraction yield of polysaccharides using this method was higher than other methods [17]. In this study, TCFPs extraction by UMAE was compared with that of three other methods (HRE, UAE, and MAE). As shown in Fig. 6, the yields of TCFPs obtained by UMAE were higher than those obtained using the other three extraction methods; in addition, UMAE involved the shortest extraction time. The TCFPs yield obtained by UMAE was 4.33%, a 1.79-fold increase compared with conventional HRE. The UMAE extraction time was 10 min, which was 9-fold shorter than that required for conventional HRE.

Compared with the other three extraction methods, UMAE gave the highest yields of TCFPs in the shortest period of time. The cavitation and microwave irradiation of UMAE resulted in high effective temperatures and pressures at the interphase between the solvent and solid matrix. In addition, the microwave irradiation caused a rapid temperature rise that disrupted the structure of the vegetal cell [28], leading to higher TCFPs yields.
3.7. Morphology before and after extraction

Changes in sample residue morphology as a result of the various extraction methods (HRE, UAE, MAE, and UMAE) were investigated by SEM. Parenchyma was undamaged and cells were intact in untreated samples. An increasing level of cell damage increased in the following order: HRE < UAE < MAE < UMAE. In particular, most of the sample appeared completely disrupted and collapsed following the UMAE treatment. The results are consistent with results of previous studies [29].

3.8. Antitumor activity assay

3.8.1. Tumor inhibition rates

Mice models inoculated with S180 tumor cells have been used to evaluate antitumor effects of polysaccharides from plants in some literatures [30,31]. In the study, an S180 tumor-burdened mouse model was used to investigate TCFPs antitumor activity. The tumor formation rate was 100%, and all mice were alive during the treatment period. The results of this portion of the study are shown in Fig. 7 and Table 4. Compared with the negative control, tumor sizes in the two TCFPs-treated groups and the positive control group decreased significantly (p < 0.01). At TCFPs doses of 200 and 600 mg/kg, the tumor inhibition rate was 46.04% and 76.33%, respectively, revealing obvious tumor inhibitory and concentration-dependent effects. As shown in Table 4, the percentage change in mouse body weight from highest to lowest in the four treatment groups was as follows: negative control > low TCFPs dose (200 mg/kg) > high TCFPs dose (600 mg/kg) > positive control. Mouse body weights decreased in the positive control group, possibly a result of cyclophosphamide-induced immune injury. Mouse body weights in TCFPs-treated groups increased, demonstrating that TCFPs have no evident toxicity in mice.

3.8.2. Relative organ weights

In toxicological experiments, a comparison of relative organ weights between treated and untreated groups of animals have been used to evaluate the toxic effect [32]. And, relative organ weight is an endpoint used by regulatory agencies to develop toxicity reference values for use in human health risk assessments [33].

The results of animal dissection revealed no abnormalities in organs (liver, kidney, spleen, heart, and lung) in any treatment groups, but the sizes of some organs were changed. As shown in Table 5, the relative spleen weight of the cyclophosphamide-treated group was significantly decreased (p < 0.01) compared with the negative control group, but no significant difference (p > 0.01) was observed in the relative weight of the other organs (liver, kidney, heart, and lung). No significant difference (p > 0.01) was observed in the relative weight of any organ (liver, kidney, spleen, heart, and lung) between the negative control group and the two TCFPs-treated groups (200 and 600 mg/kg). According to these results, cyclophosphamide showed significant toxicity to the spleen, but TCFPs exhibited no toxicity to any organs.

![Fig. 7. Photograph of excised tumors from mice dosed with Taxus chinensis var. mairei fruit polysaccharides (TCFPs) or cyclophosphamide (positive control) or subjected to no treatment (negative control).](image-url)
4. Conclusions

UMAE is a rapid and efficient technique for TCFPs extraction. Optimized extraction parameters based on the single-factor and BBD analyses were as follows: 33 mL/g liquid to raw material ratio, 10-min extraction time, 560 W microwave power, and 50°C ultrasonic power. TCFPs yield obtained by UMAE was 4.33 ± 0.15%, a 1.79-fold increase compared with conventional HRE. The extraction time used for UMAE (10 min) was shorter than that required for HRE (90 min). After separation, the polysaccharides content in the crude TCFPs extract obtained under the optimized conditions was 75.2%. We also studied the inhibitory effect of TCFPs on S180 tumor growth in mice and found that TCFPs showed good tumor inhibitory activity (76.33% inhibition rate) that was concentration dependent. According to an analysis of relative organ weights, TCFPs were not toxic to livers, kidneys, spleens, hearts, or lungs. TCFPs thus have great potential as a desirable antitumor agent for therapeutic and immunomodulatory applications.

Acknowledgements

This work was financially supported by the Fundamental Research Fund for Central Universities (DL13CA06), the National Natural Science Foundation (31200478), the Natural Science Foundation of Heilongjiang Province (C201412) and China Postdoctoral Science Foundation funded project (2013M530773).

References