Optimization of Ultrasonic-Assisted Total Flavonoids Extraction from Flower of *Albizia Julibrissin Durazz.* by Response Surface Methodology for Characterization of Chemical Profile and Evaluation of Antioxidant and Tyrosinase Inhibitory Activities

Yijie Cheng, Yuwen Zhang, Hanzhang Sun, Dongting Huang, Bo Jiang and Jingyuan Xu

Changshu Institute of Technology, Changshu, China
Changshu Hospital Affiliated to Soochow University, Changshu, China

**Abstract:** To investigate the flavonoids in flower of *Albizia julibrissin* (‘He Huan Hua’ in Chinese), the ultrasonic-assisted extraction of total flavonoids from ‘He Huan Hua’ was optimized by response surface methodology and the chemical profile was characterized by HPLC-QTOF-MS/MS. What’s more, the antioxidant and tyrosinase inhibitory activities of ‘He Huan Hua’ were also evaluated in this study. The extraction time, extraction temperature and ethanol concentration were optimized by single factor experiments and response surface methodology. The three factors were optimized to be 43 min, 40°C and 49% (v/v) respectively. Under the optimized extraction condition, the yield of total flavonoids from ‘He Huan Hua’ was 3.31% (W/W). There were 32 different flavonoids identified from the extracts of ‘He Huan Hua’, which mainly were flavonol glycosides and the principal component was quercitrin. The antioxidant activity of the extracts of ‘He Huan Hua’ was evaluated by Diphenyl Picryl Hydrazinyl Radical (DPPH), 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and reducing power assays. The extracts of ‘He Huan Hua’ exhibited good antioxidative activity with IC$_{50}$/EC$_{50}$ of 90.91±3.36 (DPPH assay), 15.09±0.17 (ABTS assay) and 67.69±2.97 (reducing power assay) μg/mL, respectively. Most importantly, the extracts of ‘He Huan Hua’ also showed good tyrosinase inhibitory activity (IC$_{50}$:1.32±0.01 mg/mL). The higher antioxidant and tyrosinase inhibitory activities of ‘He Huan Hua’ might be related to the presence of rich flavonol compounds. In summary, flavonoids in ‘He Huan Hua’ have great value for developing antioxidants and tyrosinase inhibitors and are deserved to be applied for prevention of premature skin aging.

**Keywords:** *Albizia Julibrissin* Durazz, Chemical Profile, Antioxidant Activity, Tyrosinase Inhibitory Activity, Response Surface Methodology

**Introduction**

Excessive production of Reactive Oxygen Species (ROS) in skin cells can induce oxidative stress and has been verified as a major cause of premature skin aging (Cruciani *et al.*, 2021; Lee *et al.*, 2021). Elimination of excessive ROS by exogenous supplement of antioxidants can prevent skin from oxidative stress-induced damage (Lavigne *et al.*, 2022). Hyperpigmentation also can result in different dermatological disorders such as premature skin aging (Haldys *et al.*, 2018). It is well known that tyrosinase is the key enzyme for melanin synthesis in skin and inhibiting tyrosinase activity can relieve...
pigmentation (Bourhim et al., 2021; Arroo et al., 2020). Thus, the development of effective antioxidants and tyrosinase inhibitors is of great significance for prevention of premature skin aging.

The flower of Albizia Julibrissin Durazz., ‘He Huan Hua’ in Chinese, is widely consumed as scented tea and Chinese medicine in China (CPC, 2020). Previous studies have shown that ‘He Huan Hua’ is rich in flavonoids (Kang et al., 2000; Yahagi et al., 2012). Substantial evidences have suggested that flavonoids in herbal medicine always exhibit good antioxidant and tyrosinase inhibitory activities (Li et al., 2021). It can be speculated that the flavonoids in ‘He Huan Hua’ may have potential value for developing antioxidants and tyrosinase inhibitors. However, the chemical profile and related bioactivities of ‘He Huan Hua’ have not been fully investigated so far. It has limited the further application of ‘He Huan Hua’ for solving skin health issues.

In order to characterize chemical profile or assess bioactivities of natural products in plants, extraction method should be optimized firstly to make sure that the target compositions are adequately extracted. Compared with other conventional extraction methods, ultrasonic-assisted extraction method has obvious advantages such as shorter extraction time, lower extraction temperature and higher yield rate (Yuan et al., 2015). Due to the above characteristics, ultrasonic-assisted extraction method has been used for flavonoids extraction from medicinal plants (Zuo et al., 2022). At present, few studies have investigated the ultrasonic-assisted total flavonoids extraction method from ‘He Huan Hua’ and the influences of extraction factors on extraction method have not been clarified. Response Surface Methodology (RSM) is a powerful statistical technique. Since it can predict the optimum experimental factors by mathematical model with few tests, RSM has been widely used to optimize extraction scheme and provide information on independent variables on one or more responses (Cui et al., 2018).

Therefore, in this research, the total flavonoids in ‘He Huan Hua’ were extracted by ultrasonic-assisted extraction method and extraction factors were optimized by RSM. The chemical profile of the extracts of flower of A. julibrissin (EFA) was characterized by HPLC-QTOF-MS/MS and its antioxidant and tyrosinase inhibitory activities were assessed in vitro. The flow chart of research methodology was showed in Fig. 1. This study will provide a basis for screening antioxidants and tyrosinase inhibitors from ‘He Huan Hua’ and discover the application value of ‘He Huan Hua’ for prevention of premature skin aging.

Fig. 1: Flow chart of research methodology in this study

Materials and Methods

Plant Materials

‘He Huan Hua’ was collected from the campus of Changshu Institute of Technology (Changshu, China) and identified by Professor Minjian Qin. The herbarium samples (Voucher No.AJD-01) were deposited at School of Biotechnology and Food Engineering, Changshu institute of Technology, Changshu, China. The samples were placed in a cool and ventilated room for natural drying. The dry samples were ground to 50 meshes and stored at room temperature before extraction.

Chemicals and Reagents

Quercitrin, quercetin and rutin purchased from Baoji Herbest Biotechnology (Baoji, China) were used as reference standards. The purities of the reference standards were over 98% determined by HPLC analysis. The methanol used for high resolution mass spectrometry was purchased from Merck (Darmstadt, Germany). Preparation of deionized water was through Merck Millipore Direct-Q3 (Marlborough, MA, U.S.A.). DPPH and ABTS were purchased from Sangon Biotech Co. Ltd. (Shanghai, China). Tyrosinase (from mushroom) was purchased from Sigma-Aldrich (St Louis, MA, U.S.A.). The other analytical grade reagents were purchased from Nanjing Chemical Regents Co. Ltd. (Nanjing, China).

Ultrasonic-Assisted Extraction Method

Firstly, the extraction conditions (extraction solvent, extraction time and extraction temperature) were optimized through single factor experiments. The accurately weighed dry sample powder (0.3 g) was ultrasonicated in ethanol aqueous (25 mL) for a period of time and cooled to room temperature. After that, the extraction solution was centrifuged at 4,000 g for 10 min and the supernatant was transferred. Repeat this step once. The supernatant was mixed and adjusted to 50 mL with ethanol solution. Then, the extraction solution of ‘He
Huan Hua’ was stored at 4°C until use.

**Determination of the Extraction Yield of Total Flavonoids**

The concentrations of total flavonoids in extraction solution of ‘He Huan Hua’ was determined according to the published literature with modification (Sarikurkcu et al., 2015). 1 mL of extraction solution and 8 mL of aluminum chloride solution (dissolved in methanol, 1%, w/v) were mixed. After that, the volume of the mixture was adjusted to 25 mL with ethanol solution (48%, V/V). After reacting for 15 min at room temperature, the mixture was measured at 272 nm by ultraviolet spectrophotometry. Different concentration solutions of quercetin were used as standard substance for calibration.

The concentration of total flavonoids in sample solution was calculated by calibration curve (Eq. 1) and the extraction yield of total flavonoids was calculated by Eq. (2).

The calibration curve for determination of total flavonoids concentration:

\[
A = 2.5429C - 0.0132, R^2 = 0.9999
\]  

(1)

where:

- \(A\) = The absorbance
- \(C\) = The concentration of total flavonoids in extraction solution (mg/mL)

The extraction yield of total flavonoids (%) was calculated as follows:

\[
R(\%W/W) = \frac{C \times 50\text{mL}}{W_s} \times 100\%
\]  

(2)

where:

- \(C\) = The concentration of total flavonoids in sample solution (mg/mL)
- \(W_s\) = The weight of sample (mg)
- \(R(\%W/W)\) = The extraction yield of total flavonoids

**RSM Experimental Design and Statistical Analysis**

The RSM was used to optimize the extraction conditions by analyzing the relationship between variables and response. Box-Behnken (BBD) is one of the most commonly used RSM experimental design that had been widely used to optimize extraction conditions (Ahmad et al., 2015). Therefore, the extraction process of total flavonoids was optimized by a three-factor three-level BBD. The regression analysis of experimental results was performed by Design Expert software (version 8.0.6, Stat-Ease Inc., Minneapolis, Minnesota). The three independent variables were extraction temperature (\(X_1\)), extraction time (\(X_2\)) and ethanol concentration (\(X_3\)). The ranges and center point values of the three variables were showed in Table 1. The model was developed by the following equation:

\[
Y = A_0 + \sum_{i=1}^3 A_i X_i + \sum_{i=1}^3 A_i^2 X_i + A_i A_j
\]  

(3)

where:

- \(Y\) = The predicted extraction rate of total flavonoids
- \(A_0\) = The constant coefficient
- \(A_i, A_j\) = The linear, quadratic and interaction coefficients, respectively
- \(X_i\) and \(X_j\) = The levels of independent variables

**Chemical Composition Qualitative Analysis by UPLC-QTOF-MSMS**

Waters ACQUITY UPLC (Waters, Massachusetts, USA) was used for chromatographic separation. An ACQUITY UPLC BEH-C18 column (3.0×50 mm, 1.7 μm, Waters, Ireland) was used for analysis at 30°C. The mixture of 0.1% formic acid-water (A) and methanol (B) was used as mobile phase for chromatographic separation. The flow rate was 0.3 mL/min and the gradient conditions were: 0 min - 5 min, 20 - 30% (B), 5 min - 15 min, 30 - 55% (B), 15 min - 20 min, 55 - 95%, 20 min - 21 min, 95% - 20%, 21 min-25 min, 20%. The injection volume was 5 μL.

The qualitative analysis of EFA was performed by AB SCIEX Triple TOF™ 5600+ system (AB SCIEX Technologies, Redwood City, CA, USA) in negative ion mode. The mass system was equipped with an electrospray ion source. The mass range was set from 100 to 2000 m/z. The electrospray ionization temperature was 50°C. The nebulizer gas pressure, ion spray voltage and collision energy were 60 psi, 4.5 KV and -50 V respectively.

**Antioxidant Activity**

**DPPH Scavenging Assay**

To assess the antioxidant activity of EFA, DPPH scavenging assay was used with slight modifications (Khochapong et al., 2021). Firstly, EFA was prepared at different concentrations. After that, 0.5 mL of EFA solution was mixed with 6 mL of 0.1 mmol/L DPPH solution. The mixed solution was left to react for 30min at room temperature, protected from light. Subsequently, the absorbance of the mixed solution was measured at 517 nm. Ascorbic acid (Vc) was used as a positive control. The DPPH radical scavenging activity was calculated as follows:

\[
\text{scavenging activity(\%) = } \frac{A_i - (A_i - A_j)}{A_i} \times 100\%
\]  

(4)
where:

\[ A_0 = \text{The absorbance of control} \]
\[ A_1 = \text{The absorbance of EFA (or } V_C) \text{ group} \]
\[ A_2 = \text{The absorbance of blank without DPPH} \]

**ABTS Scavenging Activity**

The reported ABTS scavenging assay was used to evaluate the antioxidant activity of EFA (Ha et al., 2021). The ABTS stock solution was prepared by mixing the same volume of ABTS solution and potassium persulfate solution. Before used, the ABTS stock solution should be stored at room temperature for 16 h and then diluted until its absorbance at 734 nm close to 0.7. After that, the EFA solution and the diluted ABTS solution was mixed and incubated in dark at 30°C for 30 min. The absorbance of the mixture was measured at 734 nm. The ABTS radical scavenging activity was calculated as follows:

\[
\text{scavenging activity(%) = } \frac{A_0 - (A_1 - A_2)}{A_0} \times 100\%
\]

(5)

where, \( A_0, A_1 \) and \( A_2 \) were the control, the absorbance values of the EFA (or \( V_C \)) group and the blank without ABTS, respectively.

**Reducing Power Assay**

The antioxidant activity of EFA was also evaluated by reducing power assay (Xie et al., 2021). Firstly, the EFA solution was mixed with 1.5 mL phosphate buffer (0.2 mol/L, pH 6.6) and 1.5 mL potassium ferricyanide (1%, w/v) solution. Then, the mixture solution was incubated at 50°C for 20 min. After incubation, 1.5 mL of trichloroacetic acid (10%, w/v) was added to the mixture and centrifuged. The supernatant of the mixture was recovered and mixed with 10 mL of anhydrous Iron (III) chloride (FeCl₃) (1 mg/mL) and 3 mL of distilled water. After incubated at room temperature for 30 min, the absorbance of the mixture was measured at 700 nm and its EC₅₀ value was calculated. \( V_c \) was used as a positive control.

**Tyrosinase Inhibitory Activity**

The tyrosinase inhibitory activity of EFA was evaluated by the published method with a slight modification (Yang et al., 2019). Before determination, the evaporated EFA was dissolved in phosphate buffer (0.2 mol/L, pH 6.8, PBS) and diluted to different concentration. Then, 70 μL of EFA solution, 200 μL of tyrosinase solution (dissolved in PBS, 200 U/mL) and 360 μL of L-DOPA (5 mmol/L, dissolved in PBS) was added in the mixture. After reacting for 20 min, the absorbance of the mixed solution was measured at 475 nm by using a Microplate Reader (Epoch, Biotek, USA).

### Table 1: Experimental values of response variables for BBD.

<table>
<thead>
<tr>
<th>Run</th>
<th>( X_1 )(^a)</th>
<th>( X_2 )(^b)</th>
<th>( X_3 )(^c)</th>
<th>( Y )(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>-1</td>
<td>1</td>
<td>1.51</td>
</tr>
<tr>
<td>2</td>
<td>-1</td>
<td>0</td>
<td>-1</td>
<td>2.47</td>
</tr>
<tr>
<td>3</td>
<td>-1</td>
<td>0</td>
<td>1</td>
<td>1.16</td>
</tr>
<tr>
<td>4</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>2.68</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>-1</td>
<td>0</td>
<td>2.86</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.27</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.34</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3.09</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>-1</td>
<td>-1</td>
<td>2.76</td>
</tr>
<tr>
<td>10</td>
<td>-1</td>
<td>1</td>
<td>0</td>
<td>2.96</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1.77</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>0</td>
<td>-1</td>
<td>2.56</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.23</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.19</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.33</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>1</td>
<td>-1</td>
<td>2.96</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1.96</td>
</tr>
</tbody>
</table>

\(^a\) \( X_1 \), extraction temperature (°C), three different levels (-1, 0, 1) represented 16, 37 and 58

\(^b\) \( X_2 \), extraction time (min), three different levels (-1, 0, 1) represented 10, 30 and 50

\(^c\) \( X_3 \), ethanol concentration (%), three levels (-1, 0, 1) represented 20, 60 and 100

\(^d\) \( Y \), extraction rate of total flavonoids from ‘He Huan Hua’ (%)
Vc was used as a positive control. Tyrosinase inhibition activity was calculated as follows:

\[
\text{Inhibition activity} (\%) = \left( 1 - \frac{A_e - A_c}{A_e - A_d} \right) \times 100\% \quad (6)
\]

where,

- \(A_e\) = The absorbance of the reaction system including EFA or Vc, tyrosinase and L-DOPA
- \(A_b\) = The absorbance of the reaction system without tyrosinase
- \(A_c\) = The absorbance of the reaction system without EFA (or Vc)
- \(A_d\) = The absorbance of the reaction system only containing L-DOPA

Statistical Analysis

In this study, each experiment was performed in triplicate. The data were illustrated as mean ± standard deviation. Statistical analysis was performed using SPSS (v. 14.0; SPSS Inc., Chicago, IL, USA). Values were considered significant when \(p\) value was lower than 0.05.

Results

Single-Factor Experiments for Optimizing Ultrasounds Assisted Extraction Condition

The extraction rate of flavonoids from crude plant materials was affected by many factors. It has been reported that the extraction solvent, ultrasonic time and ultrasonic temperature were key factors to improve extraction rate by ultrasound assisted extraction method (Yu et al., 2021). Therefore, in this study, the influence of these factors on yield rate of total flavonoids were firstly evaluated by single factor experiments.

When extraction solvent has similar polarity with target components, natural products will dissolve in extraction solvent more sufficiently. As the polarity of ethanol aqueous changed with the concentration of ethanol, the appropriate concentration of ethanol must be investigated. In this part, the extraction rate of the total flavonoids was compared under different concentrations of ethanol. As shown in Fig. 2A, the extraction rate of total flavonoids changed with different concentrations of ethanol. The extraction rate of total flavonoids increased significantly when the concentration of ethanol solution increased from 20% to 40%. After that, the extraction rate remained stable even the concentration of ethanol increasing continuously. When the concentration of ethanol reached to 100%, the yield rate of the total flavonoids decreased. Yuan et al. (2012) also found similar change rule of the extraction yield of total flavonoids in He Huan Hua when extracted with different concentration ethanol solutions. For environmental protection and saving solvent, the optimum concentration of ethanol was set at 40%.

The extraction time was the second factor investigated. The extraction time was set from 10 to 50 min. When extraction time changed, the concentration of ethanol was kept at 40% and the extraction temperature was kept at 25°C, consistently. The results indicated that the extraction rate of total flavonoids elevated gradually within 30 min and reached to the maximum when extracted for 30 min (Fig. 2B). After that, the extraction rate of total flavonoids decreased. Therefore, 30 min was the appropriate time for sufficient extraction of total flavonoids.

In addition to extraction solvent and extraction time, extraction temperature could also influence the yield rate of total flavonoids when using ultrasound assisted extraction method. In this research, extraction temperature was set from 15°C to 60°C with concentration of ethanol at 40% and extraction time for 30min. The extraction rate of the total flavonoids increased significantly when extraction temperature rising from 15 to 30°C and kept consistently when extraction temperature changed from 30°C to 45°C. However, with the extraction temperature rising continuously, the extraction rate of the total flavonoids decreased (Fig. 2C). The partial reason of this phenomenon might be due to that high temperature induced the decrease of dissolved gas in extraction solution, which leaded to the weakening of cavitation and the decrease of extraction rate (Song et al., 2014). Except that, more non-flavonoid compounds in ‘He Huan Hua’ would be extracted at higher extraction temperature (Yuan et al., 2013). Thus, the best extraction temperature was set at 30°C.

Optimization of Ultrasounds Assisted Extraction Method by RSM

As the optimal extraction condition was the result of the interaction of multiple factors, the three factors were optimized by RSM subsequently based on the results of single factor tests. The BBD matrix was consisted by 17 experiments and three factors (ethanol concentration, extraction time and extraction temperature). The results of all 17 experiments were presented in Table 1. Based on the experimental data,
quadratic regression analysis was performed. The predicted regression equation was as follows:

\[ Y = 3.27 + 0.15X_1 + 0.12X_2 - 0.54X_3 \\
- 0.012X_1X_2 + 0.18X_1X_3 + 0.016X_2X_3 \\
- 0.29X_1^2 - 0.083X_2^2 - 0.94X_3^2 \]  
(7)

Where:

\[ Y = \text{The extraction rate of the total flavonoids} \]
\[ X_1 = \text{The values of extraction temperature} \]
\[ X_2 = \text{The values of extraction time} \]
\[ X_3 = \text{The values of ethanol concentration} \]

The RSM model coefficients were validated by ANOVA. As shown in Table 2, the \( F \) value of the model was 59.72 and the \( P \) value of the model was less than 0.0001. It indicated that the model was statistically significant (Sunday, 2020). The lack-of-fit analysis was always applied to judge the difference between the obtained model and the actual situation. In this study, the \( P \) value of the lack-of-fit was larger than 0.05. It indicated that the extraction rate predicted by the regression equation had no significant difference from the actual experimental results. In addition, the coefficient of determination \((R^2)\) and adjusted coefficient of determination \((\text{Adj.}R^2)\) were 0.9871 and 0.9706, which showed that the difference between obtained mode and actual situation was less than 3%. All above results indicated that the regression model (Eq. 7) was appropriate to predict the optimal condition of ultrasonic-assisted extraction method.

Through the RSM, the contour plots and the three-dimensional response curves were obtained to visualize the interaction effect of two independent extraction factors on extraction rate. The shape and curvature of response surface plot can reflect the influence of the interaction between different factors on response value to some extent (Sady et al., 2019). The shape of the response surface can reflect the change pattern of response value when interaction factors changing and the curvature of the response surface could reflect the degree of influence of the interaction factor on response value. Greater curvature of response surface meant more obvious influence of the interaction of related factors on response values (Xie et al., 2017). As shown in Fig. 3, the similar shape of response surface plots meant the similar change pattern of the yield rate of total flavonoids in three different interactions. In addition, the response surface plot in Fig. 3B showed a steeper profile than the other two plots. It meant that the interaction between ethanol concentration \((X_1)\) and extraction temperature \((X_2)\) had the most significant effect on the extraction rate of total flavonoids when using ultrasound assisted extraction method. It was consistent with the results of ANOVA in Table 2.

According to the established response surface model, the optimal condition of ultrasound-assisted total flavonoids extraction was predicted as follows: Extraction temperature of 40.32\(^\circ\)C, extraction time of 43.85 min and ethanol concentration of 49.30\%. Under this condition, the predicted yield rate of the total flavonoids was 3.40\%. To evaluate the accuracy of the predicted optimal extraction condition, the total flavonoids was extracted based on the predicted extraction condition with slight modification. The extraction condition was as follows: Extraction temperature of 40\(^\circ\)C, extraction time of 43 min and ethanol concentration of 49\%. The actual yield rate of the total flavonoids was 3.31\% with a deviation from the predicted value less than 3\%. Therefore, the established response surface model could be effectively used to optimize the ultrasonic-assisted extraction condition.

The Chemical Profile of EFA

‘He Huan Hua’ was rich in flavonoids, but the chemical profile of ‘He Huan Hua’ was still unclearly. In this study, we developed a simple, rapid and sensitive UPLC-QTOF-MS/MS method to characterize the chemical profile of ‘He Huan Hua’ within 25 min (Fig. 4A). A total of 32 compounds were identified in EFA and eight of these compounds were reported in ‘He Huan Hua’ for the first time (as shown in Table 3). These identified compounds were almost belonged to flavonol glycosides.

The compounds were identified as follow process. Firstly, the detected accurate mass values of deprotonated molecular ions and the empirical molecular formula was matched with reported data. Subsequently, the confirmation was further performed by comparing fragmentation ions. Under the negative mode, the identified compound showed [M-H]- ions and flavonol glycosides were deduced by successive losses of glycosyls, such as hexose (m/z 162 or m/z 146), pentose (m/z 132), or rutinose (m/z 308), while the information of additional fragment ions was used as supplementary for further identification. The compound 16 was characterized as a typical flavonol glycoside, which showed a deprotonated molecular ion at m/z 447.0955 with the
molecular formula \( \text{C}_{21}\text{H}_{19}\text{O}_{11}^- \). The fragment ion at m/z 301.0379 showed the deprotonated molecular ion lost a rhamnose residue \([\text{M-146}^-]\) and the fragment ion at m/z 300.0301 showed the deprotonated molecular ion lost a rhamnose residue and a hydrion \([\text{M-147}^-]\). Further dissociation of the aglycone ion m/z 300.0301 \([\text{M-146}^-]\) might yield a serial of fragments. The fragment ions at m/z 271.0272 and 255.0321 might come from the aglycone ion m/z 300.0301 with losses of neutral fragments CHO and \([\text{CO}+\text{OH}]^-\) respectively. The fragment ions at m/z 271.0272 might further lost neutral fragments CO and generated the fragment ions 243.0317 \([\text{M-147}^-\text{CHO-CO}^-]\) and 227.0363 \([\text{M-147}^-\text{CHO-2CO}^-]\). The extracted ion chromatogram and the fragmentation pattern were shown in Fig. 4B and 4C. In comparison with the reference standard (Fig. 5A) and the published data (Zhang et al., 2015), this compound was deduced as quercitrin. Other compounds were identified by the similar method. The detected accurate mass values of deprotonated molecular ion and fragment ions were compared with those published data in literatures or/and the mass spectrometric data of reference standards showed in Fig. 5. In general, flavonol glycosides were the main flavonoids in EFA and the principal component was quercitrin.

**Antioxidant Activity of EFA**

As redundant ROS may damage skin cellular and accelerate skin aging, the antioxidant effects of extracts isolated from plants should be ascertained to make sure whether the natural products are suitable for developing skin-care products. For this reason, the antioxidant activity of EFA was evaluated by DPPH method, ABTS method and reducing capacity.

The DPPH method is a typical antioxidant assay for evaluating the antioxidant activity of natural products in vitro. In the reaction, DPPH is applied as a free radical and used to evaluate the ability of antioxidants to bind to free radicals. It was found that EFA reduced DPPH in a dose-dependent fashion (Fig. 6A) and the DPPH scavenging potential of EFA (IC\(_{50}\): 90.91±3.36μg/mL) was lower than that of Vc (IC\(_{50}\): 43.08±0.60μg/mL) (Table 4). In the case of ABTS\(^+\) scavenging assay, which is a combined electron transfer and hydrogen atom transfer assay, the ABTS\(^+\) scavenging capacity of EFA was also in a dose-dependent manner (Fig. 6B). However, the ABTS\(^+\) scavenging capacity of EFA (IC\(_{50}\): 15.09±0.17μg/mL) was higher than that of Vc (IC\(_{50}\): 29.67±0.05μg/mL) (Table 4). The scavenging of ABTS\(^+\) radical by EFA was relatively higher than DPPH radical scavenging activity. Similar with these results, You et al. (2011) also reported that the extracts of *Psidium guajava* exhibited potent ABTS radical scavenging activity which was higher than that of DPPH radical scavenging activity. This might be due to that the stereoselectivity of the radicals or the solubility of the extract in different testing systems affect the capacity of extracts to react and quench different radicals (Yu et al., 2002). Furthermore, the antioxidant activity of EFA was also evaluated by the reducing power assay. As shown in (Fig. 6C), the Fe\(^{3+}\) could be reduced to Fe\(^{2+}\) by EFA in a dose-dependent manner. The concentration for 50% of maximal effect (EC\(_{50}\)) of EFA was 67.69±2.97μg/mL which is slightly higher than that of Vc. All the above results indicated that EFA had good antioxidant activity.

**Fig. 2:** The results of single-factor experiments. (A) ethanol concentrations (%); (B) extraction time (min); (C) extraction temperature (°C).
Fig. 3: The three-dimension response surface graphs and contour plots showing the correlative effects of extraction temperature, extraction time and ethanol concentration on extraction rate of total flavonoids. (A) extraction temperature versus extraction time; (B) extraction temperature versus ethanol concentration; (C) extraction time versus ethanol concentration.
Fig. 4: Mass spectra of EFA and the fragmentation pattern of quercitrin. (A) Base peak chromatogram of EFA in negative mode based on UPLC-QTOF-MS/MS; (B) Extracted ion chromatogram of quercitrin in EFA; (C) MS/MS spectra and postulated fragmentation pattern of quercitrin by means of ESI-QTOF in negative mode.

Tyrosinase Inhibitory Activity of EFA

Tyrosinase is the rate-limiting enzyme for biosynthesis of melanin in skin. It can catalyze the hydroxylation of monophenols to diphenols and further oxidize diphenols to o-quinone which is synthesized to melanin ultimately (Tian et al., 2019). The mushroom tyrosinase inhibitory assay is an effective method to screen melanogenesis inhibitors. Hence, the mushroom Tyrosinase Inhibitory Activity of EFA was tested to evaluate its anti-melanogenic activity. As shown in Fig. 7, the EFA suppressed tyrosinase activity in a dose-dependent manner, with an IC$_{50}$ value of 1.32±0.01mg/mL (Table 4). Although the IC$_{50}$ value of EFA was higher than that of Vc, the EFA presented greater inhibitory activity against tyrosinase than those plant extracts previously reported owning good tyrosinase inhibitory activity (Sarikurkcu et al., 2020). From these results, it could be clearly stated that the EFA showed good tyrosinase inhibitory activity.
Fig. 5: The extracted ion chromatogram and MS/MS spectra of the reference standard by means of ESI-QTOF in negative mode. (A) quercitrin; (B) quercetin; (C) rutin.
Fig. 6: Antioxidant activities of EFA and Vc. (A) the result of DPPH scavenging assay; (B) the result of ABTS scavenging assay; (C) the result of reducing power assay.
Fig. 7: The tyrosinase inhibitory activities of EFA and Vc. (A) the tyrosinase inhibitory activity of EFA; (B) the tyrosinase inhibitory activity of Vc.

Table 2: ANOVA results of the RSM.

<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>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>7.11</td>
<td>9.00</td>
<td>0.79</td>
<td>59.72</td>
<td>&lt; 0.0001</td>
<td>Significant</td>
</tr>
<tr>
<td>X₁</td>
<td>0.18</td>
<td>1.00</td>
<td>0.18</td>
<td>13.55</td>
<td>0.0078</td>
<td>*</td>
</tr>
<tr>
<td>X₂</td>
<td>0.12</td>
<td>1.00</td>
<td>0.12</td>
<td>8.91</td>
<td>0.0204</td>
<td>*</td>
</tr>
<tr>
<td>X₃</td>
<td>2.35</td>
<td>1.00</td>
<td>2.35</td>
<td>178.03</td>
<td>&lt; 0.0001</td>
<td>*</td>
</tr>
<tr>
<td>X₁X₂</td>
<td>5.30×10⁻⁴</td>
<td>1</td>
<td>5.30×10⁻⁴</td>
<td>0.04</td>
<td>0.847</td>
<td></td>
</tr>
<tr>
<td>X₁X₃</td>
<td>0.13</td>
<td>1</td>
<td>0.13</td>
<td>9.96</td>
<td>0.016</td>
<td>*</td>
</tr>
<tr>
<td>X₁X₅</td>
<td>1.08×10⁻³</td>
<td>1</td>
<td>1.08×10⁻³</td>
<td>0.82</td>
<td>0.783</td>
<td></td>
</tr>
<tr>
<td>X₁²</td>
<td>0.37</td>
<td>1</td>
<td>0.37</td>
<td>27.64</td>
<td>0.0012</td>
<td>*</td>
</tr>
<tr>
<td>X₂²</td>
<td>0.029</td>
<td>1</td>
<td>0.029</td>
<td>2.21</td>
<td>0.181</td>
<td></td>
</tr>
<tr>
<td>X₃²</td>
<td>3.72</td>
<td>1</td>
<td>3.72</td>
<td>281.7</td>
<td>&lt; 0.0001</td>
<td>*</td>
</tr>
<tr>
<td>Residual</td>
<td>0.093</td>
<td>7</td>
<td>0.013</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of fit</td>
<td>0.076</td>
<td>3</td>
<td>0.025</td>
<td>6.05</td>
<td>0.0573</td>
<td>Not significant</td>
</tr>
<tr>
<td>Pure error</td>
<td>0.017</td>
<td>4</td>
<td>4.18×10⁻³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected total</td>
<td>7.2</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-Squared</td>
<td>0.9871</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adj R-Squared</td>
<td>0.9706</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pred R-Squared</td>
<td>0.8278</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adep Precision</td>
<td>23.911</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant (p < 0.05)

Table 3: Flavonoids identified by UPLC-QTOF-MS/MS in EFA

<table>
<thead>
<tr>
<th>Molecular No</th>
<th>RT (min)</th>
<th>[M-H] Formula</th>
<th>Predicted Mass (Da)</th>
<th>Measured Mass (Da)</th>
<th>Error (ppm)</th>
<th>Fragment Ions (m/z)</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.083</td>
<td>C₇H₁₄O₃</td>
<td>609.1461</td>
<td>609.1486</td>
<td>4.1</td>
<td>301.0418, 300.0296</td>
<td>Isomer of rutin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>271.0258, 255.0324, 431.0320</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>755.2166, 301.0407, 300.0288</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>271.0247</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.174</td>
<td>C₇H₁₄O₃</td>
<td>755.2084</td>
<td>755.2081</td>
<td>5.3</td>
<td>301.0370, 300.0290</td>
<td>Mangustilin (da Silva et al., 2021)³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>271.0268, 255.0311</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6.417</td>
<td>C₇H₁₄O₃</td>
<td>609.1461</td>
<td>609.1496</td>
<td>5.7</td>
<td>301.0370, 300.0290</td>
<td>Isomer of rutin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>271.0268, 255.0311</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>271.2171, 691.1534, 463.0931, 01.0360, 300.0295</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.254</td>
<td>C₇H₁₄O₃</td>
<td>463.0882</td>
<td>463.0899</td>
<td>3.7</td>
<td>301.0366, 300.0291, 271.0263</td>
<td>Isomer of rutin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>255.0390, 243.0306, 151.0036</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>285.0412, 284.0323, 253.03, 151.0036</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7.457</td>
<td>C₇H₁₄O₃</td>
<td>609.1461</td>
<td>609.1478</td>
<td>2.8</td>
<td>301.0395, 300.039, 271.0289</td>
<td>Myricetin-J-galactosid (Chaudhary et al., 2011)³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>271.0264, 259.0263, 242.0229</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7.787</td>
<td>C₇H₁₄O₃</td>
<td>463.0882</td>
<td>463.0894</td>
<td>2.6</td>
<td>301.0395, 300.039, 271.0289</td>
<td>Hyperoside (Chaudhary et al., 2011)³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>255.0390, 243.0306, 150.0036</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7.931</td>
<td>C₇H₁₄O₃</td>
<td>593.1512</td>
<td>593.1537</td>
<td>4.2</td>
<td>301.0395, 300.039, 271.0289</td>
<td>Kaempferol-J-rutinoside (Yang et al., 2021)³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>255.0390, 243.0306, 150.0036</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>7.978</td>
<td>C₇H₁₄O₃</td>
<td>463.0882</td>
<td>463.0905</td>
<td>5</td>
<td>301.0395, 300.039, 271.0289</td>
<td>Kaempferol-J-rutinoside (Yang et al., 2021)³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>255.0390, 243.0306, 150.0036</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7.99</td>
<td>C₇H₁₄O₃</td>
<td>609.1461</td>
<td>609.1496</td>
<td>5.7</td>
<td>301.0395, 300.039, 271.0289</td>
<td>Isomer of rutin</td>
</tr>
<tr>
<td>11</td>
<td>8.157</td>
<td>C₇H₁₄O₃</td>
<td>579.1355</td>
<td>579.1384</td>
<td>4.9</td>
<td>301.0395, 300.039, 271.0289</td>
<td>Quercetin-J-O-xyleopyranosyl(1→2)-rhamnopyranoside] (Yang et al., 2021)³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>415.0661, 301.0389, 271.0289</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>8.355</td>
<td>C₇H₁₄O₃</td>
<td>433.0776</td>
<td>433.0797</td>
<td>4.8</td>
<td>301.0395, 300.039, 271.0289</td>
<td>Avicularin (Chaudhary et al., 2011)³</td>
</tr>
<tr>
<td>13</td>
<td>8.504</td>
<td>C₇H₁₄O₃</td>
<td>609.1461</td>
<td>609.1488</td>
<td>4.4</td>
<td>301.0395, 300.039, 271.0289</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>8.587</td>
<td>C₇H₁₄O₃</td>
<td>433.0776</td>
<td>433.0797</td>
<td>4.8</td>
<td>301.0395, 300.039, 271.0289</td>
<td>Reynoutrin (Chaudhary et al., 2011)³</td>
</tr>
</tbody>
</table>
Table 3: Continue

<table>
<thead>
<tr>
<th>Entry</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>9.16</td>
<td>C8H6O3</td>
<td>447.0933</td>
<td>447.0952</td>
<td>4.3</td>
</tr>
<tr>
<td>16</td>
<td>9.56</td>
<td>C8H6O3</td>
<td>447.0933</td>
<td>447.0955</td>
<td>5</td>
</tr>
<tr>
<td>17</td>
<td>9.56</td>
<td>C8H6O3</td>
<td>917.1841</td>
<td>917.1808</td>
<td>-3.6</td>
</tr>
<tr>
<td>18</td>
<td>9.827</td>
<td>C8H6O3</td>
<td>563.1406</td>
<td>563.1431</td>
<td>4.4</td>
</tr>
<tr>
<td>19</td>
<td>9.839</td>
<td>C8H6O3</td>
<td>593.1512</td>
<td>593.155</td>
<td>6.4</td>
</tr>
<tr>
<td>20</td>
<td>10.26</td>
<td>C8H6O3</td>
<td>417.0827</td>
<td>417.0848</td>
<td>5</td>
</tr>
<tr>
<td>21</td>
<td>10.92</td>
<td>C8H6O3</td>
<td>417.0827</td>
<td>417.0838</td>
<td>2.6</td>
</tr>
<tr>
<td>22</td>
<td>11.434</td>
<td>C8H6O3</td>
<td>431.0984</td>
<td>431.1</td>
<td>3.8</td>
</tr>
<tr>
<td>23</td>
<td>11.836</td>
<td>C8H6O3</td>
<td>301.0354</td>
<td>301.0359</td>
<td>1.7</td>
</tr>
<tr>
<td>24</td>
<td>12.26</td>
<td>C8H6O3</td>
<td>273.0758</td>
<td>273.076</td>
<td>0.9</td>
</tr>
<tr>
<td>25</td>
<td>15.462</td>
<td>C8H6O3</td>
<td>623.1406</td>
<td>623.1454</td>
<td>7.7</td>
</tr>
<tr>
<td>26</td>
<td>15.504</td>
<td>C8H6O3</td>
<td>593.1301</td>
<td>593.134</td>
<td>6.6</td>
</tr>
<tr>
<td>27</td>
<td>15.685</td>
<td>C8H6O3</td>
<td>593.1301</td>
<td>593.1329</td>
<td>4.8</td>
</tr>
<tr>
<td>28</td>
<td>15.805</td>
<td>C8H6O3</td>
<td>623.1406</td>
<td>623.1451</td>
<td>7.2</td>
</tr>
<tr>
<td>29</td>
<td>16.189</td>
<td>C8H6O3</td>
<td>593.1301</td>
<td>593.1332</td>
<td>5.3</td>
</tr>
<tr>
<td>30</td>
<td>16.452</td>
<td>C8H6O3</td>
<td>623.1406</td>
<td>623.1447</td>
<td>6.5</td>
</tr>
<tr>
<td>31</td>
<td>17.17</td>
<td>C8H6O3</td>
<td>577.1352</td>
<td>577.1374</td>
<td>3.9</td>
</tr>
<tr>
<td>32</td>
<td>17.81</td>
<td>C8H6O3</td>
<td>577.1352</td>
<td>577.1388</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Table 4: Antioxidative and tyrosinase inhibitory activities of EFA and Vc.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH (IC50) (μg/mL)</th>
<th>ABTS+ (IC50) (μg/mL)</th>
<th>Reducing power (EC50) (μg/mL)</th>
<th>Tyrosinase inhibitory activity (IC50) (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFA</td>
<td>90.91 ± 3.36*</td>
<td>15.09 ± 0.17*</td>
<td>67.69 ± 2.97*</td>
<td>1.32 ± 0.01*</td>
</tr>
<tr>
<td>Vc</td>
<td>43.08 ± 0.60</td>
<td>29.67 ± 0.05</td>
<td>58.09 ± 0.82</td>
<td>0.74 ± 0.02</td>
</tr>
</tbody>
</table>

*Significant (p<0.05)

Discussion

For characterization of chemical profile and assessment of biological activities, extraction method should be optimized for adequate extraction of target ingredients. In this study, the actual yield rate of total flavonoids from ‘He Huan Hua’ obtained by ultrasonic-assisted extraction is up to 3.31%, which is higher than that previous reported by cold soak (yield rate of total flavonoids was 2.53%) (Yuan et al., 2012). Xie et al. (2017) also found that ultrasonic-assisted extraction was more effective and convenient than other conventional extraction methods when used to extract flavonoids from plants. It is indicated that ultrasonic-assisted extraction is fit for total flavonoids extraction from ‘He Huan Hua’.

In single-factor experiments, the extraction yield of total flavonoids increased first and then decreased with continuous increase of ethanol concentration, extraction temperature and extraction time, which might be attributed to the change of extraction solvent polarity and the stability of flavonoids (Yuan et al., 2015). Afterwards, RSM was used to predict optimum experimental factors. Based on the graphical analysis of response surfaces and the results of ANOVA, it indicated that these three factors had different effects on the extraction rate of total flavonoids in EFA. The sequence was as follows: Ethanol concentration > extraction temperature > extraction time. Song et al. (2014) previously showed that the extraction yield of quercitin from ‘He Huan Hua’ was dependent on ethanol concentration and extraction temperature when performed by ultrasonic-assisted extraction method and the influence of ethanol concentration was most significant. It was similar with the present results. After illustrating the influence of the three extraction factors, the optimized process parameters were obtained according to the RSM regression equation. The actual extraction rate of total flavonoids by the predicted methods was similar with the predicted value with a deviation less than 3%. In summary, this condition suggests that the established response surface model could be effectively used to optimize the parameters for ultrasonic-assisted extraction of flavonoids from ‘He Huan Hua’.
To characterize the chemical profile of ‘He Huan Hua’, UHPLC-QTOF-MS/MS which own high efficiency and excellent accuracy was used (Aabideen et al., 2021; Aloo et al., 2021). In this research, a total of 32 different flavonoids were identified and the main components in EFA were flavonol glycosides, such as quercitin, rutin and kaempferol-3-O-glucopyranoside. Theses flavonol compounds contain more hydroxyl groups. As hydrogen from the hydroxyl groups of flavonoids could scavenge active oxygen free radical, the flavonol with more hydroxyl group exhibited better antioxidant activity (Islam et al., 2020; Ekaete and Saldaña, 2021; Shen et al., 2021). It had been reported that quercetin, kaempferol and their glycoside derivatives could reduce the damage of ROS in vivo and scavenge free radical in vitro exhibiting higher antioxidant capacity (Fuentes et al., 2021; de Lima Júnior et al., 2021). The results in this research also indicated that EFA owning good antioxidant activity. Therefore, it can be concluded that the good antioxidant activity of EFA may have a close relation with the presence of flavonols in ‘He Huan Hua’.

Flavonol are important natural products that widely distributed in plants. Previous studies have verified the importance of flavonol to control hyperpigmentation via the inhibition of tyrosinase enzyme (Lin et al., 2008). The chemical profile of EFA showed that ‘He Huan Hua’ was rich in flavonol glycosides and the extracts had good tyrosinase inhibitory activity. It is speculated that the flavonol compounds in EFA may be contributed to its tyrosinase inhibitory activity. The molecular docking analysis demonstrated that the of hydroxyl substitutions at C-7, C-3’ and C-4’ in structure of flavonol had crucial value for competitive inhibition of tyrosinase (Söhretoğlu et al., 2018). The presence of 3-O-rhamnosylmoiety in flavonol was also suggested to participate in tyrosinase inhibition (El-Nashar et al., 2021). As shown in Table 3, most flavonols identified in EFA owned hydroxyl substitution at C-7, C-3’ and/or C-4’. Quercitin, the principal component in EFA, has a 3-O-rhamnosylmoiety (Fig. 4). Previous studies have proved that kaempferol-3-O-rutinoside, rutin and quercetin possessed potent inhibitory activity on tyrosinase compared to the standard drug, kojic acid (Santi et al., 2019, Zhou et al., 2019). These three flavonols were also rich in EFA (shown in Table 3 and Fig. 4). Hence, it can be concluded that the tyrosinase inhibitory of EFA may be due to the rich presence of flavonols and ‘He Huan Hua’ has potent value for developing effective tyrosinase inhibitors. However, the effective and specific compounds in EFA for tyrosinase inhibitors have not been screened and clarified. It is worth to be further investigated in future studies.

Conclusion

The multi-response optimization of ultrasonic-assisted total flavonoids extraction from ‘He Huan Hua’ was successfully processed by RSM. The optimal extraction parameters were as follows: Ethanol concentration of 49%, extraction time of 43 min and extraction temperature of 40℃. There was no significant difference between actual extraction rate under optimized condition and the result predicted by model. The extraction yield of total flavonoids from ‘He Huan Hua’ under optimized method was higher than that by other conventional methods. Chemical profile of EFA was identified with totally 32 compounds which were mainly flavonols. The EFA also showed good antioxidant and tyrosinase inhibitory activities, probably due to high content of flavonols. In summary, the present study not only established an effective and convenient method for total flavonoids extraction from ‘He Huan Hua’, but also definitively characterized the chemical profile of ‘He Huan Hua’. More importantly, it also revealed the antioxidant and tyrosinase inhibitory activities of extracts of ‘He Huan Hua’. The flavonoids in ‘He Huan Hua’ had great application values in development of effective antioxidants and tyrosinase inhibitors for prevention of premature skin aging.

Nomenclature and Abbreviation Section

‘He Huan Hua’: The flower of Albizia julibrissin
Flavonol: A class of flavonoid compounds
EFA: Extracts of the flower of Albizia julibrissin
HPLC-QTOF-MS/MS: High performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry. An instrument widely used for characterization of chemical profile
RSM: Response surface methodology
ROS: Reactive oxygen species
DPPH: Diphenyl picryl hydrazinyl radical
ABTS: 2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)
IC₅₀: The half maximal inhibitory concentration
EC₅₀: Concentration for 50% of maximal effect
Acknowledgement

We thank professor Minjian Qin in China Pharmaceutical University for plant identification.

Funding Information

This study was supported by the National Natural Science Foundation of China [Grant number 82003885], Foreign academician workstation of Suzhou city [Grant number SWY2020001] and Jiangsu Pharmaceutical Association-TianQing hospital pharmacy research [Grant number Q202127].

Author’s Contributions

Yijie Cheng and Yuwen Zhang: Optimized the extraction method and wrote the study.

Hanzhang Sun and Dongting Huang: Performed the antioxidant assessment and tyrosinase inhibitory assay.

Bo Jiang: Characterized the chemical profile of EFA.

Jingyuan Xu: Conceived the project, designed the experiments, revised and polished the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References


Sunday, N. F. (2020). Chemometrics of solvent extraction of Mn (II) and Fe (III) bis (salicylidene) ethylenediamine complexes in acid medium. Journal of Human, Earth and Future, 1(2), 71-77. doi.org/10.28991/HEF-2020-01-02-03


Yahagi, T., Daikonya, A., & Kitanaka, S. (2012). Flavonol acylglycosides from flower of Albizia julibrissin and their inhibitory effects on lipid accumulation in 3T3-L1 cells. Chemical and Pharmaceutical Bulletin, 60(1), 129-136. doi.org/10.1248/cpb.60.129


