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*Full Length Article*

# Response Surface Optimized Extraction of Flavonoids from *Ginkgo biloba* Leaves by Ionic Liquid

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## Abstract

To explore the relationship between ionic liquids and flavonoids in *Ginko biloba* leaves, and to screen ionic liquids which can significantly increase the extraction yield of flavonoids from *G. biloba* leaves, and extract gingko by using this ionic liquid combined with alcohol extraction and microwave-ultrasonic extraction. The total flavonoids in the *G. biloba* leaves were investigated by microwave time, ultrasonic time and ultrasonic power as the single factor, and the experimental conditions were optimized by the response surface methodology (RSM). The optimal experimental conditions were: ultrasonic power 66 W, ultrasonic time 6 min and microwave time 2 min. Under the optimal conditions, the total extraction yield of flavonoids was 49.8854 mg/g, the difference with response surface and actual value is 0.33%. The total flavonoids yield was about five times of the traditional method. Moreover, the extraction time is shortened to one tenth of the traditional extraction method, and the operation is convenient, fast and reliable. It provides strong support for the development of flavonoids in *G. biloba* leaves. 

**Key words:** *Ginkgo biloba*; Ionic liquid; Microwave; Total flavonoids; Ultrasound

## Introduction

*Ginkgo biloba L.* is an economic tree species of East Asian countries. Jurassic ginkgo is one of the most prosperous plants for more than 250 million years ago. After hundreds of millions of years of earth life changes, especially after the glaciers in the fourth century, only ginkgo still maintains its most primitive appearance, and is called "living fossil" in the history of biological chemistry. China is the hometown of *G. biloba*, with more than 70% of its total distribution in the world. In China, *G. biloba* has a wide cultivation area, mainly distributed in temperate and subtropical climate zones, spanning 21°30′~41°46′ north latitude and 97°~125° east longitude, covering 22 provinces (autonomous regions) and 3 municipalities. *G. biloba* trees with good growth have been found in the eastern plains of several meters to 3,000 meters above sea level (Liu *et al.*, 2016). Therefore, ginkgo leaves are cheap in China, at the same time, ginkgo leaves have a long history of medicinal use in China. Their medicinal value mainly includes anti-cancer and anti-inflammation, anti-oxidation, anti-tumor and protection of cardiovascular and cerebrovascular disease and so on. In recent years, the medicinal value of ginkgo leaves has also received much attention (Chen *et al.*, 2017a, b).

Ionic liquids are a new kind of solvents (Li *et al.*, 2016). Compared with traditional organic solvents, ionic liquids have many advantages, such as good solubility, chemical stability, controllability, environmentally more safe, as non-flammable, non-explosive and so on. It is expected to replace the traditional organic solvents. In recent years, the ionic liquids have been used more and more in the extraction of natural products, and have good separation efficiency in this respect (Chen *et al.,* 2016a, b).

So far, the traditional method of extracting flavonoids from *G. biloba* leaves is time-consuming and inefficient, which limits the medicinal value of total flavonoids from *G. biloba* leaves (Ameer *et al.*, 2017; Liang *et al.*, 2017). In this paper, total flavonoids in *G. biloba* leaves were extracted by ionic liquid assisted extraction, and microwave-ultrasonic combined extraction method was used to replace the traditional extraction method (Ahmad *et al.,* 2017). The extraction efficiency was 5 times as high as the traditional method, and the extraction time was shortened to one tenth of the traditional extraction method (Gu *et al.,* 2016). The extraction time of flavonoids in *G. biloba* leaves was greatly shortened and the extraction time was improved. The total extraction yield of flavonoids is very important and far-reaching, which provides a strong support for the wider application of flavonoids in *G. biloba* leaves (Ma *et al.*, 2012; Gereniu *et al.,* 2018).

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The objective of the present study was to investigate how to use ionic liquids combined with alcohol extraction and microwave-ultrasonic extraction to maximize the extraction efficiency of flavonoids from *G. biloba* leaves and to explore and optimize experimental conditions using RSM. The development of Ginkgo flavonoids will make more and better contributions to human health.

## Materials and Methods

### Reagents and Materials

The leaves of *G. biloba* used were bought from Xinglong Seedling Planting Cooperative. The source of *G. biloba was* certified by Mr. Xiaoming Peng and their species were identified by Professor Zhangji of National Institute for Food and Drug Control. Samples of *G. biloba* leaves have been retained and they are available from the authors. The material we used belonged to Ginkgo Family, Ginkgo Genus and Ginkgo Species. *G. biloba* leaves were naturally dried as complete shape and broken and passed through 60 mesh sieves. The seedling height was 1–1.5 m, and the seedling diameter was 4–5 cm. The experimental ginkgo leaves were collected in April 2017 in Ginkgo tree base of Xinglong Seedling Planting Cooperative of Pizhou City, Jiangsu Province. The planting area is about 453334 square kilometers, about 86,000 *G. biloba* are planted. This base is located in the subtropical zone and the warm temperate zone. The climate is mild. The details were shown in Table 1.

The standard product of rutin (≥98%) was purchased from Beijing Century Aoke Biotechnology Co., Ltd. Absolute ethano (AR) was purchased from Beijing Chemical Factory. Analytically pure $NaNO_2$, $Al(NO)_3$ and NaOH were used at related experiments. The ionic liquids were all supplied by Shanghai Chengjie Chemical Co., Ltd and their purity was 99%. The details of experimental equipment and reagents used in the experiment are shown in Table 2 and Table 3.

**Table 1:** The information of *G. biloba* leaves used in this study

| Type of information | Information |
|---|---|
| Sapling height(cm) | 100-150 |
| Sapling diameter (cm) | 4-5 |
| Collection location | Pizhou City, Jiangsu Province, Xinglong Seedling Planting Professional Cooperative |
| Planting area ($km^2$) | 45334 |
| Amout of plants (tree) | 86000 |
| Collect time | April, 2017 |
| Average temperature(°C) | 13-16 |
| Sunshine time (h) | >8 |
| Shading rate (%) | 40-60 |

### Extraction Method and Content Measurement and Calculation of Total Flavonoids

**Extraction method:** *G. biloba* leaves were ground into powder, passed through a 60 mesh, sealed, and stored in a dark place. Took 0.5 g of sieved *G. biloba* powder and put into 10 mL of 70% pre-formed ethanol and ionic liquid mixture solution, so that the liquid to material ratio was 20:1 mL/g. Then transferred the extract solution into 25 mL conical bottle and then put it into the microwave catalytic synthesis extractor. Set the microwave extraction parameters to: microwave power 100 W, microwave time 2.0 min. When the microwave extraction was completed, the solution was stirred evenly, the extract solution poured into a 15 mL centrifuge tube, placed in an ultrasonic cell smasher for ultrasonic extraction, set ultrasonic extraction parameters: ultrasonic clearance and ultrasonic time 2 s/2 s, ultrasonic power 66 W, ultrasonic time 6 min. After the ultrasonic extraction was completed, the solution was placed in a low-speed centrifuge, and the centrifuge parameters were set: the centrifugal speed was 1500 pm, and the centrifugation time was 5 min. After the centrifugation was completed, the supernatant was taken for use, and the precipitate was taken for secondary extraction. After the secondary extraction and centrifugation, the supernatant was mixed with the first extraction supernatant, which was the extract solution, and the volume of the extract was recorded.

**Tissue concentration and calculation of total flavonoids:** To prepare the standard rutin curve solution, 5.0 mg of standard rutin was diluted to 50 mL with deionized water and a standard solution of rutin at a concentration of 0.1 mg/mL was prepared. Then 0.00, 0.10, 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.6 mL of 0.1 mg/mL rutin solution were taken into 10 mL brown volumetric flask, and then put 70% ethanol to make the solution volume up to 2 mL, and put 0.3 mL of 5% $NaNO_2$ was added, shaken and placed for 6 min; then 0.3 mL of 10% $Al(NO_3)_3$ was added, shaken and placed for 6 min. Then added 3.0 mL of 10% NaOH, shook and placed for 15 min. The rutin contents of these solutions were 0, 0.0018, 0.0036, 0.0071, 0.0107, 0.0143, 0.0179, 0.025, 0.0226 mg/mL. UV spectrophotometer was used for these measurements and construction of standard curve.

**Measurement of the total flavonoid content of the extract:** Took 1 mL of the extract solution into a 10 mL volumetric flask, and the deleted the concertrations of flavonoids according to the standard curve method. The UV absorbance value was taken into the equation of standard curve to calculate the total flavonoid contents of *G. biloba* leaves samples, and the following formula was used:

$$Extraction\,yield(mg/g)=\left\{\left[\left(\frac{A-0.00396}{12.2567}\right)*B\right]*V\right\}/m \quad (1)$$

In equation (1), A represents the UV spectrophotometer detection data, B represents the dilution factor, and V represents the total volume of the extract.

### Selection of Ionic Liquid

**Selection of ionic liquid cations and anion types:** The traditional ionic liquid can be divided into four types of

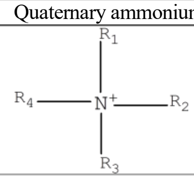
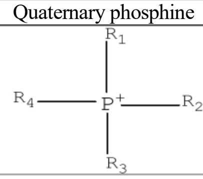
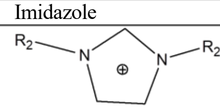
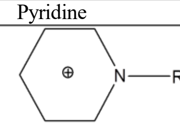
**Table 2:** Experimental equipment used in this paper

| Reagents | Factory | Model |
|---|---|---|
| Balance | Ohaus Instruments (Changzhou) Co., Ltd. | CP224C |
| Ultraviolet-visible spectrophotometer | Shanghai Haoyu Hengping Scientific Instrument Co., Ltd. | UV-2600 |
| Low speed centrifuge | Beijing Times Beili Centrifuge Co., Ltd. | DT5-4B |
| Ultrasonic cleaning machine | Kunshan Ultrasonic Instrument Co., Ltd. | KQ-300DB |
| Ultrasonic cell pulverizer | Ningbo Xinzhi Biotechnology Co., Ltd. | JY-IIDN |
| Crusher | Beijing Xingshi Lihe Technology Development Co., Ltd. | ZN-20L |
| Microwave catalytic synthesis extractor | Beijing Xiangyu Technology Development Technology Co., Ltd. | XH-100A |

**Table 3:** Experimental reagents used in this study

| Materials | Factory | Purity | NO. |
|---|---|---|---|
| Ginkgo Leaves | Shengsheng Biotechnology Co., Ltd. | Food level | 161008-1 |
| Standard Rutin | Beijing Century Aoke Biotechnology Co., Ltd. | ≥98% | 170712 |
| Ethanol | Beijing Chemical Factory | AR | 2080321 |
| $NaNO_2$ | Tianjin Yongda Chemical Reagent Co., Ltd. | AR | 20170425 |
| $Al(NO)_3$ | Beijing Chemical Factory | AR | 20170102 |
| NaOH | Beijing Chemical Factory | AR | 20180310 |
| [Bmim]Cl | Shanghai Chengjie Chemical Co., Ltd. | 99% | 20181005 |
| [Bmim]Br | Shanghai Chengjie Chemical Co., Ltd. | 99% | 20181005 |
| [Bmim]$NO_3$ | Shanghai Chengjie Chemical Co., Ltd. | 99% | 20181005 |
| [Bmim]$BF_4$ | Shanghai Chengjie Chemical Co., Ltd. | 99% | 20181005 |
| [Bmim]$PF_6$ | Shanghai Chengjie Chemical Co., Ltd. | 99% | 20181005 |
| [Bmim]$H_2PO_4$ | Shanghai Chengjie Chemical Co., Ltd. | 99% | 20180406 |
| [Emim] $H_2PO_4$ | Shanghai Chengjie Chemical Co., Ltd. | 99% | 20180406 |
| [Amim] $H_2PO_4$ | Shanghai Chengjie Chemical Co., Ltd. | 99% | 20180406 |
| [Hmim] $H_2PO_4$ | Shanghai Chengjie Chemical Co., Ltd. | 99% | 20180406 |

**Table 4:** Ionic liquid cation types

| Cation types | Quaternary ammonium | Quaternary phosphine | Imidazole | Pyridine |
|---|---|---|---|---|
| Structural formula |  |  |  |  |

ionic liquids, such as quaternary ammonium, quaternary phosphine, imidazole and pyridine (Table 4).

According to the results of quantitative calculation, fluorine atoms, nitrate ions, halogen anions, and phosphate ions can be strongly interacted with flavonoids, so these four types of anions were selected, and the preselected ionic liquids are shown in Table 5.

The ionic liquid was added to a 70% aqueous solution of ethanol at a molar concentration of 0.75 mol/L to extract flavonoid, according to the extraction method, the flavonoid was recorded.

**Selection of the alkyl chain length of the cation**: The ionic liquids with different carbon chain lengths were placed in a 70% aqueous solution of ethanol at a molar concentration of 0.75 mol/L, and the extracts of flavonoids containing ionic liquids were prepared and extracted according to experimental method, the flavonoid was recorded.

**Selection of ionic liquid concentration**: The experimental parameters were based on: microwave power of 100 W, ultrasonic power of 67.5 W, ultrasonic time of 6 min, and microwave time of 2 min. The ionic liquids were respectively 0.25, 0.50, 0.75, 1.00, and 1.25 mol/L, the ionic liquid was added to 70% ethanol aqueous solution to prepare flavonoid extracts with different molar concentrations. The flavonoids in *G. biloba* leaves were extracted according to the extraction method to explore different ionic liquids. The flavonoid yield was measured.

**Selection of Single Factor**

**Selection of ultrasonic power**: The experimental parameters were based on: ionic liquid molar concentration of 0.75 mol/L, microwave power of 100 W, ultrasonic time of 6 min, microwave time of 2 min, and ultrasonic power of 52.5, 60, 67.5, 75 and 82.5 W. The flavonoids in *G. biloba* leaves were extracted according to the extraction method, and the effects of different ultrasonic power on the extraction yield were recorded.

**Selection of ultrasonic time**: The experimental parameters were based on: ionic liquid molar concentration of 0.75 mol/L, microwave power of 100 W, microwave time of 2 min, ultrasonic power of 67.5 W, and ultrasonic time of 2, 4, 6, 8 and 10 min the flavonoids in G, biloba leaves were extracted according to the extraction method, and the effects

**Table 5:** Preselected ionic liquids

| Cation types | Anion types | Anion | Ionic liquid |
|---|---|---|---|
| Imidazole | Fluorine atom | $PF_6^-$ | [Bmim] $PF_6$ |
| | | $BF_4^-$ | [Bmim] $BF_4$ |
| | Nitrate | $NO_3^-$ | [Bmim] $NO_3$ |
| | Halogen | $Cl^-$ | [Bmim] Cl |
| | | $Br^-$ | [Bmim] Br |
| | Phosphate | $H_2PO_4^-$ | [Bmim] $H_2PO_4$ |

**Table 6:** Experimental factors level and the coding

| Factors | Code | Level | | |
|---|---|---|---|---|
| | | -1 | 0 | 1 |
| Ultrasound power(W) | $X_1$ | 60 | 67.5 | 75 |
| Ultrasonic time(min) | $X_2$ | 4 | 6 | 8 |
| Microwave time(min) | $X_3$ | 1 | 2 | 3 |

of different ultrasonic time on the extraction yield were investigated. The flavonoid yields were measured.

**Selection of microwave time**: The experimental parameters were based on: ionic liquid molar concentration of 0.75 mol/L, microwave power of 100 W, ultrasonic power of 67.5 W, ultrasonic time of 4 min, and microwave time of 0, 1, 2, 3 and 4 min, and the effects of different ultrasonic time on the extraction yield were estimated.

### Response Surface Methodology

Multiple linear regression analysis was performed based on the results in Table 1 using the following second-order polynomial equation (2):

$$Y = \gamma_0 + \sum_{i=1}^{3} a_i X_i + \sum_{i=1}^{3} a_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} a_{ij} X_i X_j \tag{2}$$

Where $Y$ is the predicted response, $\gamma_0$ is a constant, $a_i$, $a_{ii}$ and $a_{ij}$ are respectively the linear, quadratic and interactive coefficients of the model. Accordingly, $X_i$ and $X_j$ represent the levels of the independent variables, respectively.

Using the design expert 8.0, the obtained data were analyzed and fitted to obtain the quadratic multiple regression equations of the flavonoids encoding the independent variables $X_1$, $X_2$, $X_3$. The experimental factor level coding was shown in Table 6. The flavonoids were subjected to the 3-factor 3 level response surface experiment, the experiments comprised 17 groups.

## Results

### Standard Curves of Rutin

The standard curve is shown in Fig. 1. The rutin standard curve is: y=12.2567x+0.00396 $R^2$=0.99904 indicating a high accuracy.

### Selection of Ionic Liquid

**Effect of ionic liquid cations and anion types**: It can be seen from Fig. 2 that the dissolution of [Bmim]$H_2PO_4$ into 70% ethanol has the greatest influence on the extraction efficiency, and the 24.3995 mg/g of the unionized liquid can be raised to 49.8854 mg/g, within 49.8703–49.8854 mg/g, the same in the follows. It also can be found that among the selected ionic liquids other than [Bmim] $H_2PO_4$ have little effect on the extraction of flavonoids from *G. biloba* leaves or reduce the amount of flavonoids extracted from *G. biloba* leaves.

**Effect of the alkyl chain length of the cation**: The experimental results are shown in Fig. 3. as the length of the carbon chain increases, the extraction amount of flavonoids from *G. biloba* leaves increases first and then decreases. When the carbon chain length is butyl, the extraction efficiency was the highest, reaching 49.8854 mg/g.

**Effect of ionic liquid concentration**: The experimental results are shown in Fig. 4. As the concentration of ionic liquid increases, the extraction amount of flavonoids from *G. biloba* leaves increases rapidly before 0.75 mol/L, when the concentration of [Bmim] $H_2PO_4$ was more than 0.75 mol/L, the amount of flavonoids extracted slowly decreased. The molar concentration of [Bmim] $H_2PO_4$ is 0.75 mol/L, the extract yield of flavonoids was the highest, reaching 49.8854 mg/g.

### Selection of Single Factor

**Effect of ultrasonic power**: The experimental results are shown in Fig. 5. With the increase of ultrasonic power, the extraction amount of flavonoids from *G. biloba* leaves increased first and then decreased, and the rate of extraction decreases significantly faster than the rate of rise. When the ultrasonic power was 67.5 W, the extraction efficiency was the highest, reaching 49.8854 mg/g.

**Effect of ultrasonic time**: The experimental results are shown in Fig. 6. With the increase of ultrasonic time, the extraction amount of flavonoids from *G. biloba* leaves first increased and then decreased. The ultrasonic extraction time was 6 min, the extraction efficiency of flavonoids was the highest, reaching 49.8854 mg/g.

**Effect of microwave time**: The experimental results are shown in Fig. 7. With the increase of microwave time, the extraction amount of flavonoids from *G. biloba* leaves increased first and then decreased. The rise and fall of the extraction volume was obvious and the speed was very fast. When the microwave time was 2 min, the extraction efficiency was the highest, reaching 49.8854 mg/g.

### Response Surface Methodology

The influencing factors were ultrasound power ($X_1$), ultrasonic time ($X_2$), microwave time ($X_3$), flavonoids were the response value, and the response surface optimization experiments were carried out on the influencing factors. The horizontal coding of experimental factors is shown in Table 4. The response variable and the independent variables are related by the following second-order polynomial Eq. 3.

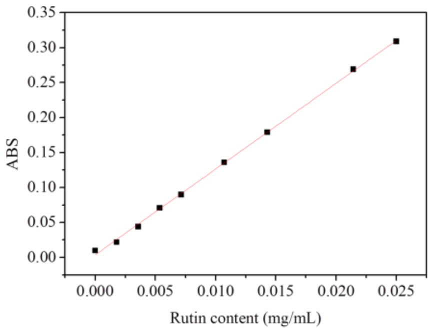



**Fig. 1:** Rutin standard curve

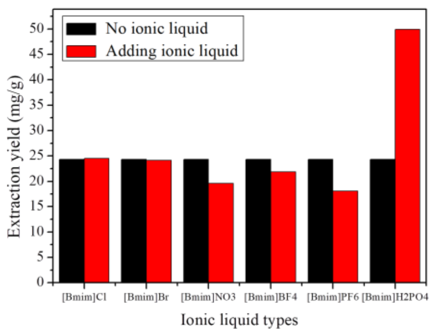



**Fig. 2:** Effect of imidazole ionic liquids on the extraction amount of flavonoids

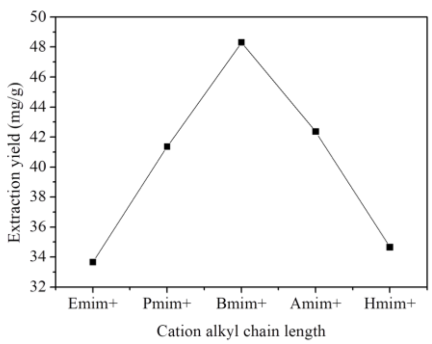



**Fig. 3:** Effect of cationic carbon chain length on the extraction amount of flavonoids

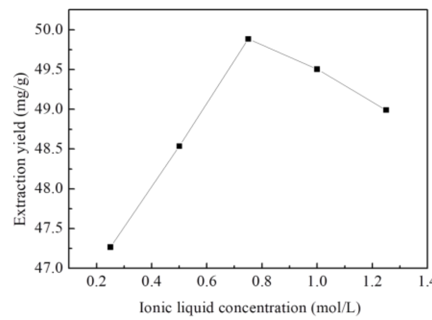



**Fig. 4:** Effect of microwave time on the extraction amount of flavonoids

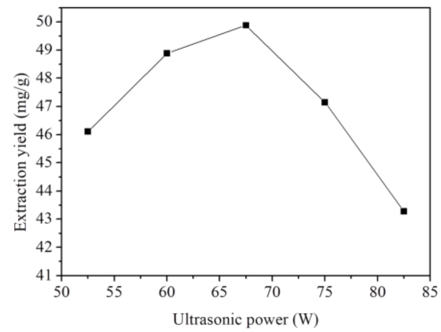



**Fig. 5:** Effect of ultrasonic power on extraction amount of flavonoids

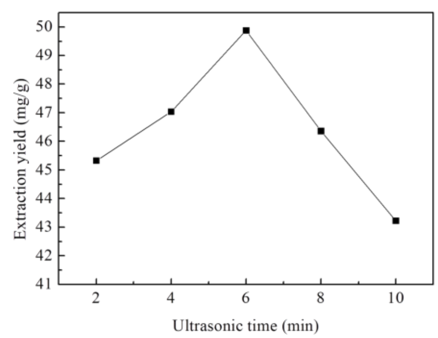



**Fig. 6:** Effect of ultrasonic time on the extraction amount of flavonoids

$$Y=49.88540-0.72\times A+0.68\times B-1.22\times C-0.34A\times B-0.49\times A\times C+0.88\times B\times C-0.74A^2-3.16\times B^2-3.51\times C^2 \quad (3)$$

Response surface methodology (RSM) was used to study flavonoids at 3 factors and 3 levels. Seventeen groups of RSM experiments were carried out and the results were shown in Table 7. The salient parameters of the regression equation obtained from the response surface are shown in Table 8. The response surface contour plot and 3D plot of the effects of various factors on the extraction efficiency of flavonoids in the experiment are shown in Fig. 8.

## Discussion

As can be seen from Fig. 2, the dissolution of [Bmim] $H_2PO_4$ into 70% ethanol has the greatest influence on the extraction efficiency, the reason for this may be that the two hydrogen atoms on the ionic liquid anion can form a strong hydrogen bonding force with the hydrogen atom on the flavonoid compound, and the π-π formed by the imidazole ionic liquid and the flavonoid compound. The interaction with n-π is an attraction that attracts flavonoids to ionic liquids and increases the extraction of flavonoids to 49.8854 mg/g. At the same time, nitrate (Wang *et al.*, 2018), tetrafluoroborate and hexafluorophosphate significantly reduce the extraction yield of flavonoids, probably because the viscosity of the ionic liquid is relatively large. So that the viscosity of the extract increases, which hinders the attraction of flavonoids and ionic liquids. So that the ginkgo leaf powder cannot be well dispersed in the extract,

**Table 7:** The data of experimental design

| Run | A:Ultrasonic power(W) | B: Ultrasonic time(min) | C:Microwave time(min) | Extraction yield(mg/g) |
|---|---|---|---|---|
| 1 | 0 | -1 | -1 | 45.1236 |
| 2 | 0 | -1 | 1 | 40.5000 |
| 3 | 0 | 0 | 0 | 49.8854 |
| 4 | 0 | 0 | 0 | 49.8854 |
| 5 | 1 | 0 | 1 | 44.1239 |
| 6 | -1 | 1 | 0 | 48.7248 |
| 7 | -1 | 0 | 1 | 45.1207 |
| 8 | -1 | 0 | -1 | 46.1527 |
| 9 | 0 | 0 | 0 | 49.8854 |
| 10 | 0 | 0 | 0 | 49.8854 |
| 11 | 1 | 1 | 0 | 45.1602 |
| 12 | 0 | 1 | 1 | 43.0547 |
| 13 | 1 | -1 | 0 | 43.9134 |
| 14 | -1 | -1 | 0 | 46.1239 |
| 15 | 0 | 1 | -1 | 44.1678 |
| 16 | 0 | 0 | 0 | 49.8854 |
| 17 | 1 | 0 | -1 | 47.1269 |

**Table 8:** Regression coefficients and ANOVA results

| Source | Sum of squares | DF | Mean square | F-value | P-value | $R^2$ |
|---|---|---|---|---|---|---|
| Model | 128.83 | 9 | 14.31 | 19.53 | 0.0004 | 0.9617 |
| A | 4.20 | 1 | 4.20 | 5.73 | 0.0479 | |
| B | 3.71 | 1 | 3.71 | 5.06 | 0.0593 | |
| C | 11.94 | 1 | 11.94 | 16.28 | 0.0050 | |
| AB | 0.46 | 1 | 0.46 | 0.63 | 0.4550 | |
| AC | 0.97 | 1 | 0.97 | 1.33 | 0.2875 | |
| BC | 3.08 | 1 | 3.08 | 4.20 | 0.0795 | |
| $A^2$ | 2.32 | 1 | 2.32 | 3.17 | 0.1183 | |
| $B^2$ | 42.10 | 1 | 42.10 | 57.44 | 0.0001 | |
| $C^2$ | 51.92 | 1 | 51.92 | 70.84 | <0.0001 | |
| Residual | 5.13 | 7 | 0.73 | | | |
| Lack of Fit | 5.13 | 3 | 1.71 | | | |
| Pure Error | 0.000 | 4 | 0.000 | | | |
| Cor Total | 133.96 | 16 | | | | |

thereby making the flavonoids. The amount of extraction is reduced (Thakker *et al.,* 2018).

As shown in Fig. 3, when the carbon chain length is butyl, the extraction efficiency is the highest. The alkyl chain increases from ethyl to butyl, the amount of extraction gradually increases because the flavonoids are non-polar substances. When the ionic liquid carbon chain grows, the polarity of the ionic liquid is weakened. According to the principle of similar compatibility, the extraction phase rate increases in turn as the carbon chain grows. When the length of the carbon chain is increased by butyl, the amount of extraction does not increase, which indicates that the alkyl chain is too long, and the prominent steric hindrance weakens the mutual attraction between the ionic liquid imidazole ring and the flavonoid. In addition, the increase in the length of the carbon chain causes the viscosity of the ionic liquid to rise sharply. The excessive viscosity of the ionic liquid hinders the attraction of the flavonoids and the ionic liquid, so that the ginkgo leaf powder cannot be well dispersed in the extract. Thereby, the extraction amount of the flavonoid compound is lowered.

It can be seen from Fig. 4 that when the molar concentration of [Bmim] $H_2PO_4$ was 0.75 mol/L, the extract yield of flavonoids was the highest. When the concentration of ionic liquid was lower than 0.75 mol/L. The extraction of flavonoids is incomplete. When the concentration of ionic liquid was higher than 0.75 mol/L, the viscosity of the extract was increased so high that the ginkgo leaf powder was unevenly dispersed in the extract, and the flavonoids cannot be fully extracted. So the optimal molar concentration of the ionic liquid was obtained. Therefore, the ionic liquid concentration was set at 0.75 mol/L (Yang *et al*., 2016).

From the Fig. 5 we can see that when the ultrasonic power is 67.5 W, the extraction efficiency was the highest. When the ultrasonic power was lower than 67.5 W, the flavonoids could not be completely extracted, when the ultrasonic power is higher than 67.5 W, the flavonoids will be damaged and the extraction yield of flavonoids extracted was reduced. So the optimal ultrasonic power was set at 67.5 W.

It can be seen from Fig. 6 that the ultrasonic extraction time was 6 min (Fig. 6), the extraction efficiency of flavonoids was the highest. While the ultrasonic time was shorter than 6 min, the content of flavonoids is incomplete. While the ultrasonic time is longer than 6 min, excessive cavitation will destroy the structure of flavonoids (Meenu *et al.,* 2017; Vo Dinh *et al*., 2018), resulting in a decrease in the extraction of flavonoids. Therefore, the optimal ultrasound time was set at 6 min.

From Fig. 7 it was seen that when the microwave time was 2 min, the extraction efficiency was the highest. When the microwave time was shorter than 2 min, the content of flavonoids was imperfect. When the microwave time was longer than 2 min, the temperature of the extract was too high to wreck the structure of the flavonoids, so the optimum microwave time was determined to be 2 min.

The results showed that the P-value of this model is 0.0004, which is far less than 0.01, and the correlation coefficient of the regression equation is 0.9617 (Table 8),

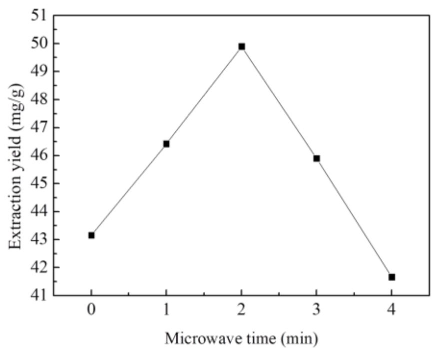



**Fig. 7:** Effect of microwave time on the extraction amount of flavonoids

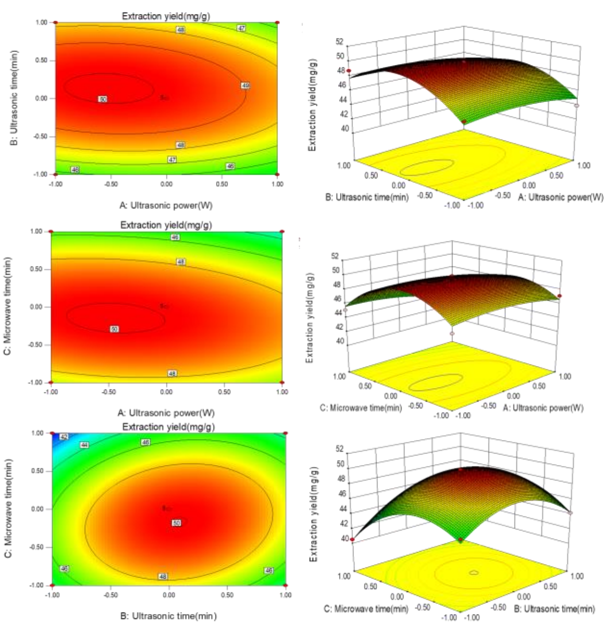



**Fig. 8:** Contour map and 3D plot of flavonoid response surface

which proves that the great significance of this model. The fitting of model was good, and the design of the experiment was reasonable. The regression equation can reflect the influence of various factors on the extraction amount yield of flavonoids better. The optimal process conditions obtained by using this model are reasonable. The effects of various factors on flavonoids were: microwave time > ultrasonic time > ultrasonic power, interaction of kinetics on flavonoids by microwave time and ultrasonic time > ultrasonic power and microwave time > ultrasonic power and ultrasonic time, quadratic term the order of influence on flavonoids was: microwave time > ultrasonic time > ultrasonic power.

The contour map and 3D map obtained from the response surface are shown in Fig. 8. It is well known that the trend line obtained by the contour line is more elliptical, and the interaction of the two factors becomes more obvious (Irfan *et al*., 2016; Feng *et al*., 2017). Therefore, the ultrasonic time and the microwave had strong effects (Fig. 8). The interaction of time is most pronounced, and the interaction between ultrasound power and ultrasound time is the weakest (Liu *et al*., 2016; Chen *et al.,* 2017a). At the same time, the 3D graph obtained by various factors showed that the highest point of the response surface was the best experimental condition we wanted. The ordinate of the highest point was the highest flavonoid we wanted (Chen *et al.,* 2017b).

The optimal experimental conditions for flavonoids based on response surface analysis were: ultrasonic power 66 W, ultrasonic time 5.952 min and microwave time 1.916 min. In order to facilitate the experimental operation, the optimal conditions were set as: ultrasonic power: 66 W, ultrasonic time: 6 min, microwave time 2 min. In order to test the reliability of the best experimental conditions obtained above, the flavonoids were extracted by the above optimal conditions, and the extraction yield of flavonoids was 49.8854 mg/g. Theoretically, the extraction yield of flavonoids was 50.0522 mg/ g, the relative error is 0.33%.

## Conclusion

In this paper, the effects of ionic liquid cation, anion species and cationic carbon chain length on the extraction of flavonoids from *G. biloba* leaves were investigated. Finally, ionic liquid [Bmim] $H_2PO_4$ was used to extract flavonoids from *G. biloba* leaves and combined with alcohol extraction. Combined with microwave-ultrasonic extraction method, the response surface method was used to optimize the experimental conditions. The optimal extraction conditions were as follows: ultrasonic power 66 W, ultrasonic time 6 min, microwave time 2 min, ionic liquid molar concentration 0.75 mol/L, Finally, the extraction yield of flavonoids in *G. biloba* leaves was increased to 49.8854 mg/g, which was 5 times that of the traditional extraction method, while using one tenth of traditional extraction time. Relevant research will have far-reaching significance for the development and utilization of flavonoids in *Ginkgo biloba* leaves.

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