



Subcritical ethanol extraction of flavonoids from *Moringa oleifera* leaf and evaluation of antioxidant activity



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ARTICLE INFO

Article history:

Received 27 November 2015

Received in revised form 24 August 2016

Accepted 8 September 2016

Available online 13 September 2016

Keywords:

Response surface methodology

Flavonoids

Moringa oleifera leaf

Subcritical ethanol extraction

Antioxidant property

ABSTRACT

A large-scale process to extract flavonoids from *Moringa oleifera* leaf by subcritical ethanol was developed and HPLC–MS analysis was conducted to qualitatively identify the compounds in the extracts. To optimize the effects of process parameters on the yield of flavonoids, a Box-Behnken design combined with response surface methodology was conducted in the present work. The results indicated that the highest extraction yield of flavonoids by subcritical ethanol extraction could reach 2.60% using 70% ethanol at 126.6 °C for 2.05 h extraction. Under the optimized conditions, flavonoids yield was substantially improved by 26.7% compared with the traditional ethanol reflux method while the extraction time was only 2 h, and obvious energy saving was observed. FRAP and DPPH[•] assays showed that the extracts had strong antioxidant and free radical scavenging activities.

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1. Introduction

Moringa oleifera (*M. oleifera*) is a widely growing tree in India, and also being cultivated in Niger, Haiti, Mexico, China, etc (Morton, 1991). Recent years, *M. oleifera* has attracted great attention among researchers because of the potential use in many fields. Almost every part of the tree can be eaten, and more important thing is that all parts of the tree have great potential as medicines (Abdulkarim, Long, Lai, Muhammad, & Ghazali, 2005). Therefore, many research workers paid great attention to the medicinal and nutritional uses of *M. oleifera* (Anwar, Latif, Ashraf, & Gilani, 2007). Meanwhile, environmental uses of *M. oleifera* seed such as treatment of waste water also aroused scholarly interest (Kansal & Kumari, 2014). *M. oleifera* leaf contains lots of nutrients which can be absorbed into human body, such as vitamins, minerals, and fatty acids (Moyo, Masika, Hugo, & Muchenje, 2011). Additionally, the leaf has been certified to contain various compounds like flavonoids, phenolics, and carotenoids which can be used as antioxidant (Alhakmani, Kumar, & Khan, 2013; Vongsak, Sithisarn, & Critsanapan, 2014).

Flavonoids are widely distributed in plants and have many roles and functions. The wide distribution compared with other

bioactive compounds makes the animals and humans ingest significant amounts of flavonoids in their diet (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). In human bodies, oxidation is an essential process to product energy, but sometimes the production of oxygen-derived free radicals is uncontrolled and damaging to human cells. Antioxidants such as flavonoids have ability to scavenge these free radicals and reduce the risk of death from coronary heart disease (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993). Therefore, it is essential to develop and utilize antioxidants to protect human body from free radicals (Singh & Rajini, 2004).

Many new extraction technologies have been developed to separate flavonoids from plants (Dai & Mumper, 2010), such as ultrasound-assisted extraction (UAE) (Albu, Joyce, Paniwnyk, Lorimer, & Mason, 2004; Zhang, Yang, Li, & Wang, 2008), subcritical water extraction (SWE) (Jo et al., 2013; Matshediso, Cukrowska, & Chimuka, 2015), supercritical fluid extraction (SFE) (Maran, Manikandan, Priya, & Gurumoorthi, 2015), microwave-assisted extraction (MAE) (Rostagno, Palma, & Barroso, 2007). Subcritical extraction does not require an alternative energy source such as microwave and ultrasound. In addition, subcritical water extraction (SWE) is a new and 'green' method to extract bioactive compounds from plants, and the dielectric constant of subcritical water is different under different conditions. But SWE needs high temperature to reach the subcritical condition which may destroy the bioactive compounds (Dai & Mumper, 2010), and extraction

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efficiency of subcritical water still needs more investigation. Ethanol is a widely used solvent for bioactive compounds extraction and relatively safe for human (Shi et al., 2005), and supercritical or subcritical temperature of ethanol is much lower than water. However, few studies have been done on subcritical ethanol extraction of flavonoids from *M. oleifera* leaf.

There are many factors influencing the subcritical extraction process, and the purpose of the present study was to find an optimal condition to extract flavonoids by large-scale subcritical ethanol extraction and investigate antioxidant ability of the extracts. Response surface methodology (RSM) was used to optimize process conditions. The antioxidant property of the extracts was investigated by FRAP and DPPH[•] assay.

2. Experimental procedures

2.1. Materials

Moringa oleifera leaf was obtained from Guangxi province, China. Rutin and oligomeric proantho cyanidins (OPC) standards were purchased from J&K Scientific Co. (Beijing, China), and the purity was ≥98% and 99% respectively according to the manufacturer. 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]) were purchased from J&K Scientific Co. (Beijing, China). Iron (III) chloride hexahydrate (FeCl₃·6H₂O) and ferrous sulfate (FeSO₄) were obtained from Bodi Chemical Reagents Co. (Tianjin, China). All other solvents and chemicals were obtained from Jiangtian Chemical Reagents Co. (Tianjin, China) and were analytical grade.

2.2. HPLC–MS conditions for identification of flavonoids

Before the HPLC–MS analysis, a purification process was conducted by the following procedure to enrich the antioxidant compounds. A column (30 mm × 500 mm) was packed with 150 g D101 macroporous adsorbent resin, and washed with 300 mL ethanol, 5% HCl, 2% NaOH respectively. After each wash, water was used to eliminate the residual ethanol, HCl or NaOH. An extract tank containing 600 mL extracts was fixed on the top of the column to prepare the purification process. When the purification was performed, a pump was used to supply the extracts to the tank and keep the volume of extracts fixed in the tank, and the flow rate of extracts was 1.5 mL/min. The purification process was lasted for 8 h, and then the purified extracts were analyzed by the HPLC–MS analysis.

The compounds were separated on a C18 column (150 mm × 4.60 mm) operated at 25 °C with the elution solvents A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). In addition, a flow-rate of 1.5 mL/min for the following gradient: 10–30% B in 20 min and 30% B in 20–40 min was performed to conduct the HPLC separation.

To identify the separated compounds, an electrospray ion mass spectrometer (ESI-MS) was used under positive ion mode and scanned from *m/z* 100 to 1000. Detailed conditions were as follows, needle voltage at 3.5 kV, capillary temperature at 350 °C, nitrogen as the drying gas at 12 L/min and 350 °C and nebulizer pressure at 40 psi.

2.3. Reflux extraction of flavonoids

A round-bottom flask and an attached reflux condenser were used to conduct this experiment. 10.0 g dried powder of *M. oleifera* leaf was extracted with 200 mL of 90% ethanol for 3 h at a controlled temperature, and heating capacity was fixed at 500 W to maintain the temperature. Then the extracts were filtered, the

solvent was evaporated at 50 °C under vacuum, and the residue was extracted again under the same conditions. The sample was extracted for 3 times in total. This whole experiment was conducted for 3 times for accuracy. Finally the products were analyzed by UV–vis spectrophotometry.

2.4. Subcritical extraction of flavonoids

60.0 g dried powder of *M. oleifera* leaf was dissolved by 1.5 L ethanol. Then the mixture was introduced into a gas-cooled fast (GCF) reactor (shown in Fig. S1) to extract flavonoids, and a vacuum pump was used to evacuate the air in the reactor. Heating capacity was fixed at 2000 W to heat up the mixture at the beginning, and then turned to 200 W to maintain the temperature. And the pressure was approximately equaled to the saturated vapor pressure of over-heated ethanol solution due to the high vacuum at the beginning. The extracts were filtered, and the solvent was evaporated at 50 °C under vacuum. UV–vis spectrophotometry was used to analyze the products. Three factors were considered to be the most important in this extraction process. Therefore we conducted a set of single factor analysis. Flavonoids were extracted with different concentrations of ethanol (55%, 70%, 85% and 100%) for a given time ranging from 1 to 2.5 h, while the extraction temperature ranged from 110 to 140 °C.

2.5. UV–vis spectrophotometry analysis

A standard solution (60 µg/mL) of rutin was prepared. The solvent used in this process was ethanol–water (60:40, v/v). And then 1, 2, 3, 4 and 5 mL rutin solutions were removed in five volumetric flasks (10 mL) respectively. Next, we added 2 mL of AlCl₃ (0.1 mol/L) solution and 3 mL of CH₃COONa (1 mol/L) solution, waiting for 5 min, followed by adding ethanol–water (60:40, v/v) solvent to the scale. The sample solution without coloration was used as a reference. Determination wavelength of 420 nm was used to analyze the samples. Results were used to draw the rutin standard curve.

1 mL of the extracts were removed and diluted to 10 mL in a volumetric flask (10 mL) by ethanol–water (60:40, v/v) solvent. Then 1 mL of this solution was colorated and analyzed, using the method stated above. The extraction yield *Y*₁ (mg RE/g) which meant milligrams of rutin equivalent from 1 g *M. oleifera* leaf was calculated as the following Eq. (1), where *C* (µg/mL) was the flavonoid concentration calculated by rutin standard curve, *V* (mL) was the volume of the extracts, *M* (g) was the mass of *M. oleifera* leaf used in extraction process. And the extraction yield *Y* (%) could be calculated as the following Eq. (2). For simplicity, we used *Y* as the extraction yield in the following text.

$$Y_1 = (100 \times C \times V \times 10^{-3}) / M \quad (1)$$

$$Y = Y_1 \times 10^{-3} \times 100\% \quad (2)$$

2.6. Experimental design and statistical analysis

In the present study, we used Design Expert Version 8.0 software as a design and analysis tool to conduct experiments. Regression coefficients, significance of the process variables, conformity of the experimental data to models and optimal response variables can be obtained by using this software. Response variable was predicted by a quadratic model shown as the following Eq. (3)

$$Y = A + \sum_{i=1}^3 B_i X_i + \sum_{i=1}^3 C_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 C_{ij} X_i X_j \quad (3)$$

where Y was the predicted dependent variable, X_i were the independent variables, A was the constant coefficient, B_i were the linear regression coefficients, C_{ij} were the interaction effect terms, and C_{ii} were the quadratic effect terms, respectively.

A three-factor RSM was conducted in this study to investigate the relationship between the response variables and process variables, and optimize the extraction process conditions. Concentration of ethanol (X_1 : 55%–85%), extraction temperature (X_2 : 120–140 °C) and extraction time (X_3 : 1.5–2.5 h) were chosen as independent or process variables, while response variable was the extraction yield (Y) of flavonoids. After optimization, each variable was coded at three levels of $-1, 0, +1$ as shown in Table S1.

The accuracy of the model was investigated by the regression analysis (R^2). And F -test was conducted to analyze the significance of the model terms. The response surface plots and contour plots were used in combination to show how the process variables affect the response variables.

2.7. Antioxidant assay

The total antioxidant activity of the extracts and standards was determined by ferric reducing antioxidant power (FRAP) assay, and FeSO_4 solution was used as a standard to compare with the extracts. The antioxidant activity was also determined by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH \cdot) assay to investigate the free radical scavenging activity of the extracts, and the oligomeric proantho cyanidins (OPC) was used as a standard to compare with the extracts.

2.7.1. FRAP assay

FRAP reagent included 50 mmol/L acetate buffer which contained 20.4 g $\text{C}_2\text{H}_3\text{NaO}_2$ and 80 mL $\text{C}_2\text{H}_4\text{O}_2$ per liter; 10 mmol/L TPTZ (2,4,6-tripyridyl- s -triazine) solution in which 40 mmol/L HCl solution was used as the solvent; 20 mmol/L $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. FRAP reagent was obtained by mixing 100 mL acetate buffer, 10 mL TPTZ solution, and 10 mL $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution (Benzie & Strain, 1996).

0.1 mL different amounts of FeSO_4 solutions or extracts were added into 6 mL FRAP reagent, and then put in a 37 °C water bath for 30 min. Determination wavelength of 593 nm was used to monitor the absorbance of samples.

2.7.2. DPPH \cdot assay

60 μM DPPH \cdot was dissolved in 3 mL ethanol, and then 0.5 mL different amounts of extracts or OPC were added. Blank experiments which contained 0.5 mL of 99% ethanol instead of the extract were also conducted. The absorbances were monitored at 517 nm (Cervato et al., 2000). The inhibition rate of DPPH free radical (IR) was calculated by Eq. (4) (Guil-Guerrero, Martínez-Guirado, del Mar Reboloso-Fuentes, & Carrique-Pérez, 2006), where A_0 was the absorbance of the blank experiments and A_s was the absorbance in the presence of the samples.

$$\text{IR} = (A_0 - A_s) / A_0 \times 100\% \quad (4)$$

When the IR is 50%, the corresponding concentration is called IC_{50} . Therefore, the value of IC_{50} was obtained by fitting the sample concentration and the inhibition rate (Song, Zhang, Zhang, & Wang, 2010).

3. Results and discussion

3.1. Standard curve of rutin and results of reflux extraction

The rutin standard curve was drawn by plotting the concentration of rutin standard solution versus the corresponding absorbency, as shown in Fig. S2. The regression equation was Eq. (5),

where A was the absorbance of the sample, C ($\mu\text{g/mL}$) was the concentration of rutin. And R^2 of this equation was 0.9997.

$$A = 33.6574 \times C - 0.45632 \quad (5)$$

The extraction yield was calculated by Eq. (1), and the volume of extracts was about 100 mL in the reflux extractions while 10.67 g *M. oleifera* leaf was used. And the absorbances of the three experiments were 0.666, 0.667 and 0.664. According to the Eqs. (5) and (1), the average extraction yield which was 20.6 mg RE/g (2.06%) could be obtained.

3.2. Single factor analysis

There are many factors affecting the extraction yield, among which the ethanol concentration, extraction time, and extraction temperature are the main factors. Single factor analysis was performed with one factor changed and the others kept unvaried.

The extraction by different ethanol concentrations (55%, 70%, 85%, 100%) was investigated, while the other conditions were kept the same (extraction temperature was 140 °C, and extraction time was 2 h). And the extraction by extraction time of 1, 1.5, 2 and 2.5 h was investigated, while the other conditions were kept the same (extraction temperature was 130 °C, and ethanol concentration was 100%). Finally, the extraction by extraction temperature of 110, 120, 130 and 140 °C was investigated, while the other conditions were kept the same (extraction time was 2 h, and ethanol concentration was 100%). The detail experimental conditions and results were shown in Table. S2.

As shown in Fig. 1, the extraction efficiency was the highest when the extraction temperature was 130 °C. When the temperature was higher, the extraction efficiency decreased. It could speculate that some of the heat-sensitive components in *M. oleifera* leaf decomposed at higher temperature. The extraction efficiency increased following the increase of extraction time and reached a peak at 2 h, and then significantly decreased. A long treatment time was also adverse to this process. We found that there was also a best ethanol concentration existing in this extraction process, which was 70%. Therefore, center point (all variables were coded as zero) of RSM was 130 °C (extraction temperature), 2 h (extraction time), 70% (ethanol concentration).

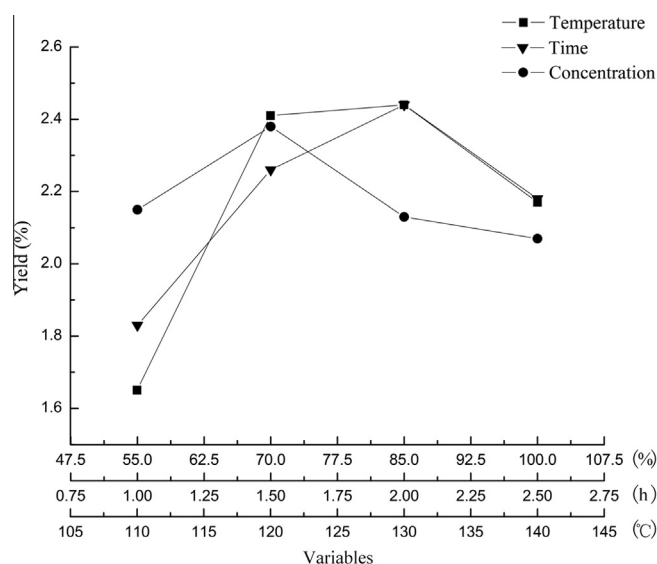


Fig. 1. Effects of extraction temperature, extraction time and ethanol concentration on yield of flavonoids.

Table 1
Analysis of variance (ANOVA) for the developed second order polynomial models.

Source	Sum of squares	Mean square	F-value	P-value
Model	1.06	0.12	26.23	0.0004
X_1	0.018	0.018	4.03	0.0915
X_2	0.12	0.12	27.06	0.0020
X_3	0.037	0.037	8.19	0.0288
X_1X_2	0.11	0.11	25.27	0.0024
X_1X_3	0.034	0.034	7.66	0.0325
X_2X_3	0.0094	0.0094	2.09	0.1982
X_1^2	0.16	0.16	34.77	0.0011
X_2^2	0.33	0.33	74.26	0.0001
X_3^2	0.24	0.24	52.73	0.0003

3.3. Extraction model and statistical analysis

Box-Behnken design (BBD) used in the present work was a three factorial design with three levels that consisted of 16 runs to obtain an optimal process condition. The experimental conditions and flavonoids extraction yield results were shown in Table S3. Additionally, an analysis of variance (ANOVA) was conducted and the regression model was summarized in Table 1. By multiple regression analysis, the following second-order polynomial could be obtained.

$$Y = 2.59 - 0.048X_1 - 0.12X_2 + 0.068X_3 - 0.17X_1X_2 + 0.093X_1X_3 + 0.049X_2X_3 - 0.2X_1^2 - 0.29X_2^2 - 0.24X_3^2 \quad (6)$$

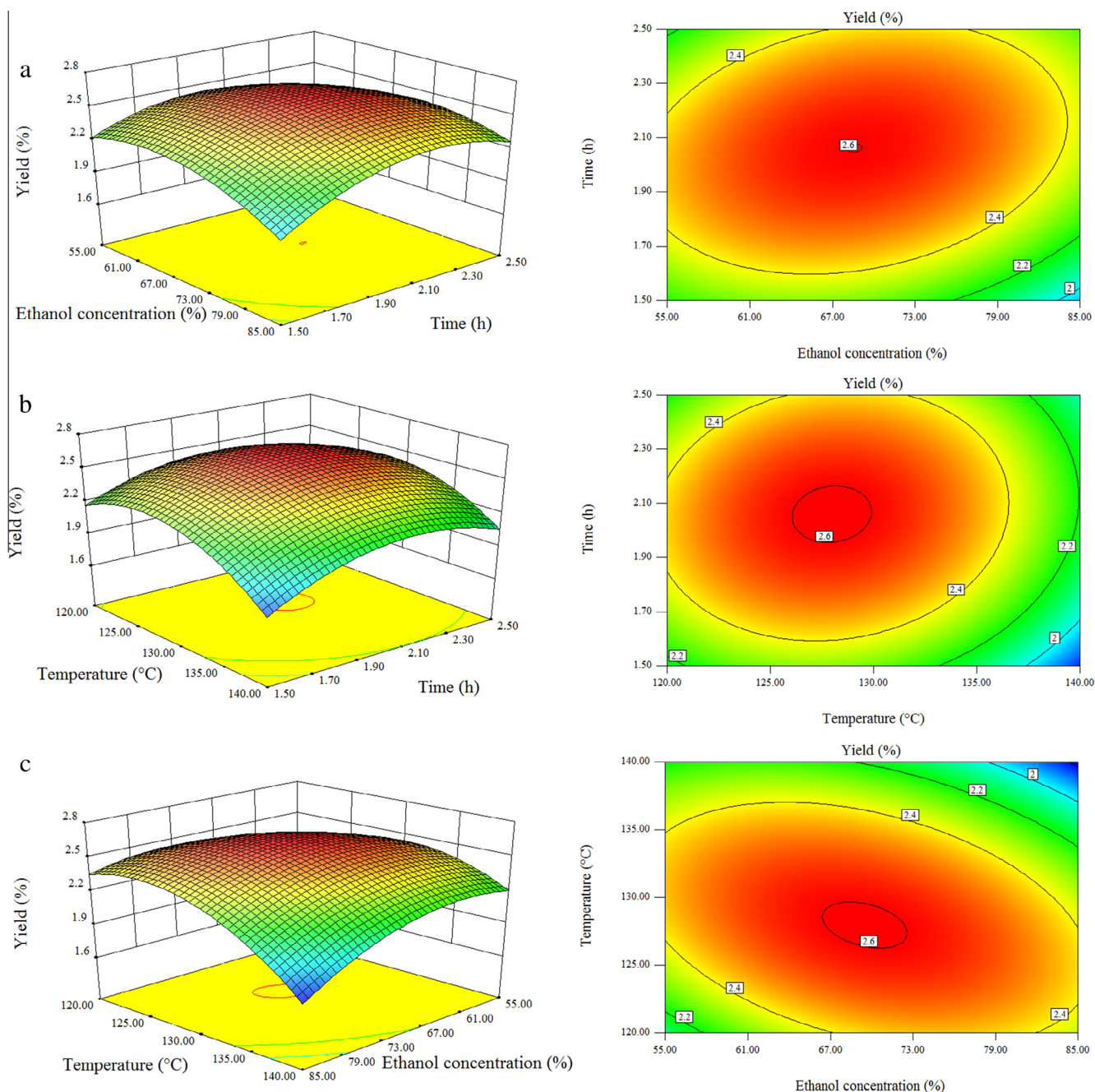


Fig. 2. Three-dimensional (3D) response surface and contour plot curve illustrating combined effects of (a) extraction time and ethanol concentration (b) extraction time and extraction temperature (c) extraction temperature and ethanol concentration on extraction yield.

As we can see in Table 1, F value of the model was 26.23 while the P value was only 0.0004, which showed a high significance of the model. For a good accuracy of a model, R^2 must be more than 75% (Chauhan & Gupta, 2004). Thus the model stated above with a relatively high coefficient of determination value ($R^2 = 0.9752$) illustrated that almost all extraction data could be explained by this model. Therefore, using this model to predict the influence of the process variables on the extraction yield was reasonable and reliable. The quadratic variable X_2^2 was statistically very significant because the P value was lower than 0.0001; two-variable interaction X_1X_2 , linear variable X_2 , quadratic variables X_1^2 and X_2^2 had significant influences ($P < 0.01$) on the extraction yield of flavonoids; linear variable X_3 and two-variable interaction X_1X_3 had influences ($P < 0.05$) on the extraction process, whereas the linear variable X_1 and two-variable interaction X_2X_3 had no significant influence ($P > 0.1$) on the extraction yield of flavonoids. The linear and quadratic coefficients of each process variable indicated that extraction temperature had more influence on the extraction yield than extraction time, while extraction time was more significant than ethanol concentration.

3.4. Response surface analysis

The effects of the process variables and their mutual interactions on the extraction yield can be investigated by the response surface plots and their contour plots. And the interactions between the process variables are significant while the shape of contour plots is elliptical (Muralidhar, Chirumamila, Marchant, & Nigam, 2001).

The effects of extraction time and ethanol concentration on extraction yield were shown in Fig. 2(a) while extraction temperature was set at 130 °C. Fig. 2(a) indicated that the interaction between extraction time and ethanol concentration had a great impact on the yield of flavonoids, and extraction time had a more significant influence than ethanol concentration. The flavonoids yield increased as the extraction time increased from 1.5 to approximately 2.1 h, and then dropped as the extraction time increased from about 2.1–2.5 h, when ethanol concentration was at a certain value (70%). It was not significant that the increase of the ethanol concentration affected the extraction yield at a certain extraction time, but there was still a peak of the extraction efficiency while ethanol concentration was about 70%.

As shown in Fig. 2(b), in which ethanol concentration was 70%, both extraction time and extraction temperature had obvious impact on extraction efficiency, but the interaction had no significant impact. When extraction time was 2 h, Fig. 2(c) showed extraction yield obviously increased with temperature raised while ethanol concentration had no significant effect on the yield. In addition, the interaction between ethanol concentration and extraction temperature had a significant impact on the yield of flavonoids. All the results were in good agreement with our findings in the ANOVA.

3.5. Optimization of extraction and identification of flavonoids

According to the analysis of response surface, the optimum condition was obtained: ethanol concentration, 69.9%; extraction temperature, 126.6 °C; extraction time, 2.06 h. Under the above condition, the estimated value for Y was obtained, which was 2.61%. The extracts obtained from the optimum condition were purified by D101 macroporous adsorbent resin as stated in the chapter 2.2, and analyzed by HPLC–MS. The chromatogram of HPLC–MS was shown in Fig. S3 while the MS spectrums were shown in Fig. S4. In Fig. S4, two main fragment ion peaks (287, 303) were obviously observed which represent kaempferol and quercetin glycosides respectively. Therefore, the main flavone

Table 2
Identified flavonoid compounds of *Moringa oleifera* leaf extracts.

Peak	t_R (min)	MS (m/z)	MS fragment ion (m/z)	Identities
a	13.4	595.2	303	Quercetin-diR
b	18.8	465.1	303	Quercetin-G
c	21.0	449.1	287	Kaempferol-G
d	21.6	507.1	303	Quercetin-G-Ac
e	23.4	507.1	303	Quercetin-G-Ac
f	24.4	491.1	287	Kaempferol-G-Ac
g	27.1	533.1	303	Quercetin-Xyl/Api-S
h	27.8	517.1	287	Kaempferol-Xyl/Api-S

diR: dirhamnosyl, G: Glucosyl/Galactosyl moiety, Ac: Acetyl, Xyl: Xylosyl, Api: Apiosyl, S: Succinoyl.

glycosides in the *Moringa oleifera* leaf extracts are quercetin and kaempferol. According to Figs. S3 and S4, eight flavonoid compounds were observed in the extracts, and the details of the compounds were listed in Table 2.

Experiment under the predicted optimal conditions was conducted for three times to obtain a mean value of extraction yield. By comparing the experimental and predicted extraction yield, the model could be verified. Adjusted extraction conditions were 70% of ethanol concentration, 126.6 °C of extraction temperature and 2.05 h of extraction time. The obtained result (2.60%) was closed to the above prediction and showed that the experimental values had a great agreement with the predictive values. Therefore, the optimal extraction conditions obtained by RSM were accurate,

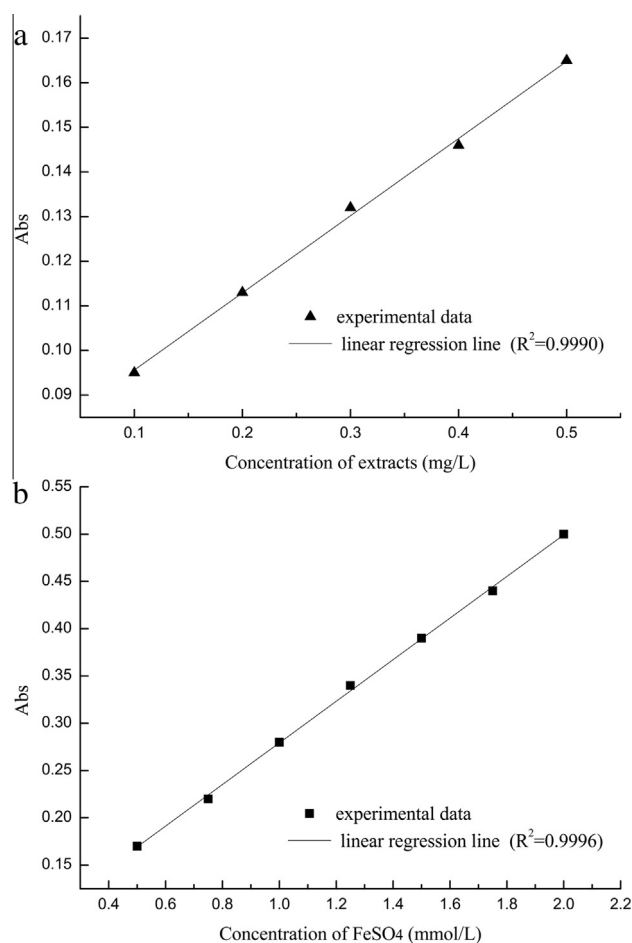


Fig. 3. Total antioxidant activity of (a) extracts and (b) FeSO₄ solutions.

reliable, and efficient. Extraction yield of subcritical extraction increased about 26.70% compared with the traditional ethanol reflux method. Most importantly, this subcritical extraction process only needed 4 h (including heating time) to reach the optimal yield which was 2.60% while the traditional method needed nearly 11 h to reach a yield of 2.06%. And we also observed the power consumption of traditional method totaled 4.6 kW·h while 2.4 kW·h in the subcritical ethanol extraction process.

3.6. Antioxidant activity

3.6.1. FRAP assay

As shown in Fig. 3, FeSO_4 solution was used as a standard to evaluate the total antioxidant activity of extracts. The figure showed a linear correlation between the concentration of FeSO_4 solution or extracts and the absorbance, and the concentration of extracts was expressed as milligrams of *M. oleifera* leaf per liter. Furthermore, the total antioxidant activity of extracts from 1 mg *M. oleifera* leaf approximately equaled the total antioxidant activity of 0.95–1.35 mmol FeSO_4 .

3.6.2. DPPH \cdot assay

As a stable and well-characterized solid radical source, DPPH \cdot is a traditional and perhaps the most popular free radical used for free radical scavenging activity assay (Arabshahi-Delouee & Urooj, 2007). OPC, with the strong ability to scavenge free radical, was used as the standard to evaluate the free radical scavenging activity of extracts. As shown in Fig. 4, we used inhibition rate

(IR%) and IC_{50} to characterize the free radical scavenging activity of extracts. The value of IR increased with the concentration added. For extracts from *M. oleifera* leaf, the IC_{50} value was 0.7440 mg/L, while the IC_{50} value of OPC was 0.0195 mg/L. Therefore, the free radical scavenging activity of extracts from 1 mg *M. oleifera* leaf was approximately equivalent to that of 0.026 mg OPC.

4. Conclusions

In the present study, RSM in combination with three-factor and three-level BBD was successfully applied to study and optimize the process variables for the subcritical ethanol extraction of flavonoids from *M. oleifera* leaf. According to the HPLC–MS analysis, quercetin and kaempferol glycosides were found in the extracts. The experiment results showed that, extraction time and extraction temperature had significant effects on the extraction yield. Analysis of variance (ANOVA) showed a high coefficient of determination value ($R^2 > 0.95$). Therefore, the mathematical model developed by Box-Behnken design can be used to predict the extraction efficiency of flavonoids. Under the optimal conditions (extraction temperature: 126.6 °C, extraction time: 2.05 h, ethanol concentration: 70%), the experimental result was 2.60% and shown to be in agreement with the predicted one. And the subcritical extraction used only 4 h to reach the optimal result, while the traditional ethanol reflux method needed nearly half a day to obtain a yield of 2.06%. The traditional method spent twice as much energy as subcritical extraction did. Subcritical ethanol extraction will have great potential use in industry. In addition, antioxidant assays showed that the extracts had strong antioxidant ability, and extracts from 1 mg *M. oleifera* leaf approximately equaled 0.95–1.35 mmol FeSO_4 or 0.026 mg OPC.

Acknowledgement

This work was financially supported by the National Natural Science Foundation of China (Grant No. 21376166).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2016.09.058>.

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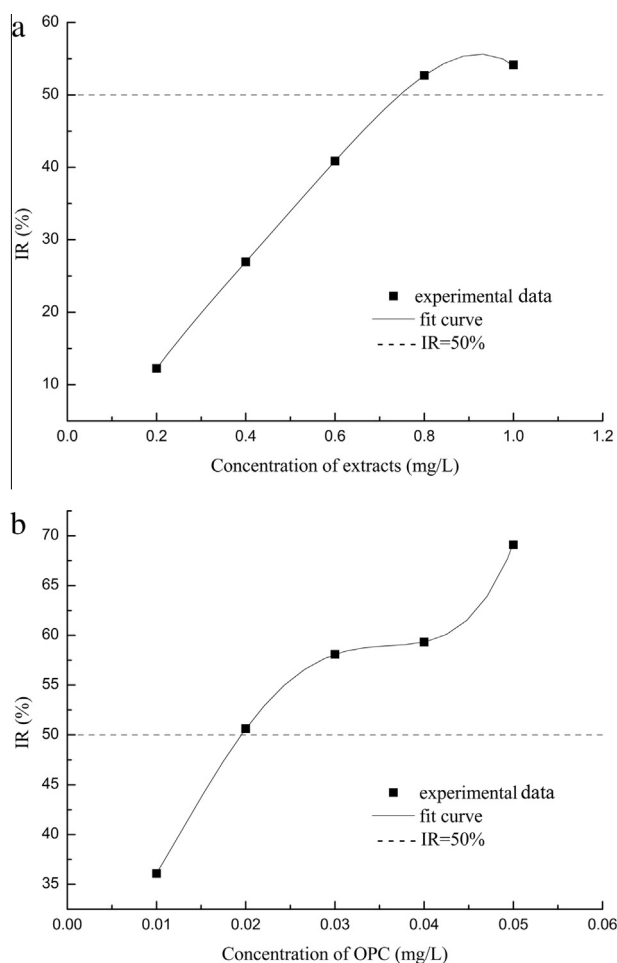


Fig. 4. Scavenging activity of (a) extracts and (b) OPC on DPPH free radical.

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