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The optimization of ultrasonic-microwave assisted synergistic extraction of *Lotus plumule* extract rich in flavonoids and its hypoglycemic activity

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Abstract

Lotus (*Nelumbo nucifera* Gaertn), a kind of perennial aquatic plant, is widely cultivated and consumed by people in Asian countries. *Lotus plumule* flavonoids (LPF) have been recognized as a hypoglycemic agent. LPF was optimally obtained using novel ultrasonic-microwave assisted synergistic extraction (UMSE) method by response surface methodology (RSM) on the basis of the results of single-factor experiments. Furthermore, the hypoglycemic activity of LPF was investigated by measuring the body weight, fasting blood glucose (FBG) level, and oral glucose tolerance test (OGTT) and analyzing the physiological indexes in streptozotocin-diabetic mice model. The optimum extraction conditions consisted of microwave power 355 W, ultrasonic power 423 W, extraction time 15 min, solid-liquid ratio 1:40, ultrasound/interval time 1/0, and ethanol concentration 70% with the maximum LPF yield of 2.62%. LPF supplementation significantly decreased the body weight, FBG, OGTT, serum total cholesterol (TC), serum total triglycerides (TG), and insulin levels, indicating the antidiabetic activity of LPF. This research verified that the UMSE technique was highly efficient to extract LPF to the maximum extent and the flavonoids from *L. plumule* exhibited hypoglycemic activity, which showed broad development and application prospects.

Keywords: *Lotus plumule*, Flavonoids, Ultrasonic-microwave assisted synergistic extraction, Hypoglycemic

Introduction

Diabetes mellitus (DM), a chronic metabolic disorder, is characterized by hyperglycemia syndrome and subsequently caused co-morbidities syndrome including hypertension, hyperlipidemia, and cardiovascular diseases (Rubio et al. 2008; Wan et al. 2020). DM has become a global public health issue with rapidly rising incidence. Besides, the type 2 diabetes mellitus (T2DM), also named non-insulin-dependent diabetes, takes up 90% of the diabetes (Zhao et al. 2019). The International Diabetes Federation reported that the diabetes is expected to reach 642 million by the year 2040 (Chan et al. 2017). At present, a variety of oral anti-diabetic agents such as biguanides, sulfonylureas, α-glucosidase inhibitors, and dietary glucose regulators could be used in clinical treatment, controlling blood glucose within in normal range (Dall et al. 2014; Natalia & Montori, n.d.). However, these medicines might cause toxicity and serious side effects in a long-time cure (Vasconcelos et al. 2011). Therefore, the research for finding effective and natural hypoglycemic compounds which have less or no side effects is of great significance. In recent years, several studies have demonstrated that the medicinal plant extract presented antidiabetic activities (Soares et al. 2017; Testa et al. 2016), among which the flavonoids in the extract played an important part (Singab et al. 2005).

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Lotus (*Nelumbo nucifera* Gaertn) is a kind of perennial aquatic plant which were widely cultivated and consumed by people in Asian countries (Xiong et al. 2016). Almost every parts of lotus could be eaten while its green seed embryo, named *Lotus plumule*, was generally removed because it tastes bitter. In fact, *L. plumule* has been officially recognized as pharmaceutical and food resources in Chinese Pharmacopoeia for a long time (Ono et al. 2006). *L. plumule* exerted multiple pharmacological effects with high nutritional values, including anti-hypertensive, anti-arrhythmic, anti-inflammatory, and anti-psychotic (Jun et al., 2016; Mukherjee et al. 2009; Sharma et al. 2017). Meanwhile, various bioactive ingredients and dietary nutrients were identified in lotus plumule, such as alkaloids, polysaccharides, flavonoids, tannins, and proteins. Limwachiranon et al. (2018) have reported that the total polyphenol and flavonoids content of *lotus plumule* were 29.0 mg/g and 18.9 mg/g, respectively, which is consistent with Feng et al. (2016) and Liu et al. (2017) reported that the flavonoids content was 9.3–17.9 mg/g. UPLC elution profile showed that the flavonoids content in crude 70% ethanol extract of *Lotus plumule* was higher than 50% (Feng et al. 2016). Flavonoids were reported as the main bioactive component in *L. plumule* (Chen et al. 2012; Jiang et al. 2018a, 2018b). Although the bioactive effects including antioxidant and anti-inflammatory properties from *L. plumule* flavonoids (LPF) were well reported (Chen et al. 2019), systematic studies on their hypoglycemic activity remain elusive.

The traditional extraction methods of flavonoids commonly included reflux extraction (Liao et al. 2011), sonication extraction (Chen et al., 2019), microwave assisted extraction (Alara et al. 2018), and dynamic high pressure microfluidization-assisted extraction (Huang et al. 2013), which were time-consuming, solvent wasting, laborious, and finally with low extraction efficiency. Ultrasonic-microwave synergistic extraction (UMSE) is a novel extraction technique which is fast, efficient, and economic. It combines with the ultrasonic and microwave, which takes the full advantages in the penetrating heating effect of microwave and cavitation of ultrasonic (Chemat & Khan 2011; Mustapa et al. 2015). UMSE method is commonly selected for extracting flavonoids from medicinal plants, such as in sweet potato leaves (Liu et al. 2019), *Cassia alata* (Lin, Fun, Yeop, Yusoff, Gimbun 2019), and *Spatholobus suberectus* (Cheng et al. 2011). Response surface methodology (RSM) is an effective statistical technique which optimizes the multiple parameters. It is used for designing experimental experiments, modeling the extraction variables, verifying the statistical significance, and finally obtaining the optimum conditions of extracting LPF (Alara et al. 2017). Meanwhile, Box-Behnken design (BBD), a type of RSM independent design, is easy to establish and decode examination with highest adept than other designs (Li et al. 2013).

In this study, total free and bound flavonoids were extracted from *L. plumule* by UMSE. The extraction efficiency of LPF was assessed with RSM coupled with BBD, determining the optimum processing parameters. The single-factor experiments, including ethanol concentration, solid-liquid ratio, ultrasound time/interval time, microwave power, ultrasonic power, and extraction time, were conducted in advance. In addition, the hypoglycemic activity of LPF was investigated by measuring the body weight, FBG level, and OGTT and analyzing the physiological indexes.

Materials and methods

Materials and chemicals

L. plumule was obtained from Jianning, Fujian, China. The samples were dried, ground into fine powder, and passed through 60 mesh sieves. Metformin hydrochloride sustained-release tablets were purchased from Yuekang Pharmaceutical Group Co., Ltd. (Beijing, China). Streptozotocin was purchased from Sigma Aldrich Co. (St. Louis, MO, USA). All chemical reagents were of analytical grade.

Single factor for extraction efficiency of LPF by UMSE

L. plumule powder (5 g) was extracted by Ultrasonic-microwave device (XH-300B, Cheung Wing Technology Development Co., Ltd., China), which was set the microwave power of 300, 350, 400, 450, 500 W with the ultrasonic power of 200, 400, 600, 800, 1000 W, immersed in ethanol solution of 10, 30, 50, 70, 90% at different solid-liquid ratio (g/ml) of 1:10, 1:20, 1:30, 1:40, 1:50, using three types of ultrasound time/interval time of 1/2, 2/1, 1/0, and continued for 5, 10, 15, 20, 25 min. There were six parameters of ethanol concentration, microwave, ultrasonic power, solid-liquid ratio, ultrasound/interval time, and extraction time, respectively. While one factor was changed, the others were kept constant in single factor experiment. For example, the ethanol concentration, microwave, ultrasonic power, solid-liquid ratio, and extraction time were set at 70%, 350 W, 400 W, 1:40, and 15 min when the ultrasound/interval time was measured. The extraction solution was filtered and centrifuged at 4000 rpm for 20 min, and 30% ethanol was added into the supernatant to the volume of 500 mL. The content of the LPF was quantified by HPLC with rutin was a standard using the method of Feng et al. (2016) referred. The extraction efficiency (%) of LPF was calculated by the following equation:

$$\text{Extraction efficiency}\ (\%) = \frac{\mathrm{m}}{\mathrm{M}} \times 100\%$$

Where ***m*** presented the *L. plumule* flavonoids content of

extraction (g) and M was the actual mass of *L. plumule* (g).

Experiment design of RSM

Since 1:40 and 1:50 of solid-liquid ratio (g/ml) showed no obvious difference, 70% concentration of ethanol and 1/0 ultrasound time/interval time had the best extraction efficiency of *L. plumule* flavonoids, considering saving the solvent, thus the solid-liquid ratio, ethanol concentration, and ultrasound time/interval time were set to 1:40, 70%, and 1/0, respectively. Three other parameters that had more significant effects on extraction rate were optimized by RSM. It designed with three factors and three levels: microwave power (A), ultrasonic power (B), and extraction time (C). The three single-factor levels were adopted to explore and optimize their independent and interactive impacts, which were settled down as A (300, 350, 400 W), B (200, 400, 600 W), and C (10, 15, 20 min). Total seventeen combinations containing five replicates were produced by the Box-Behnken design as shown in Table 1. Each of them was repeated three times.

Hypoglycemic activity of LPF

Experiment animals

A total of 60 male ICR Kunming healthy mice (20 ± 2 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). They were acclimatized for one week under standard ambient conditions (a 12/12 h light-dark cycle, temperature maintained at 25 ± 3 °C, and a suitable humidity of 50% ± 10%) with free access to normal diet and water. All animals were treated strictly in accordance with the guidelines for laboratory animal welfare ethics and daily animal care. The procedures were approved by the Ethics Review Committee of College of Food Science, Fujian Agriculture and Forestry University, China (No. FS-2017-002).

Establishment of diabetic mice model

Ten mice were assigned to feed normal diet, the other 50 mice were fed high-fat high-sucrose diet (HFHS, 63.6% basic forage, 15% sucrose, 10% egg yolk powder, 10% lard, 1.2% cholesterol, and 0.2% sodium cholate). After 30 days on feeding HFHS, 50 mice were fasted for 15 h with free access to water only, and then induced by intraperitoneal injections of freshly prepared streptozotocin (STZ, 100 mg/kg in 0.1 M trisodium citrate-citric acid buffer, pH 4.2). The normal diet mice were given buffer only. Two weeks after injection, blood was taken from the tip of the mice tail vein to measure fasting blood glucose (FBG). The mice whose FBG levels were higher than 11.1 mmol/L were considered as T2DM mice (Chen et al. 2018). All mice in six groups were given accordingly treatment as specified below. The amount of intragastric administration was 0.5 mL per day for 3 weeks. The amount of 10 mice feeding normal diet was treated as normal group (NG), the other 50 mice which were fed HFHS were randomly divided into

Table 1 Box-Behnken design and predicted values of flavonoids from *L. plumule*

Run	Factor			Extraction efficiency of LPF (%)
	A: Microwave power (W)	B: Ultrasonic power (W)	C: Extraction time (min)	
1	400	600	15	2.33
2	350	400	15	2.64
3	300	600	15	2.24
4	400	200	15	2.07
5	350	600	20	2.01
6	400	400	10	2.30
7	350	400	15	2.65
8	350	600	10	2.15
9	350	400	15	2.66
10	300	400	20	2.20
11	350	400	15	2.65
12	350	200	10	2.00
13	350	200	20	1.93
14	300	200	15	2.18
15	300	400	10	2.21
16	350	400	15	2.68
17	400	400	20	2.14

five groups (10 mice per group), the dosage regimens were designed as follows:

- Group 1: Normal group (NG) with physiological saline
- Group 2: Model group (MG) with physiological saline
- Group 3: Control group (CG) with 100 mg/(kg·d) metformin hydrochloride
- Group 4: Low dose of LPF (LPF-L) with 200 mg/(kg·d) *L. plumule* flavonoid extracts
- Group 5: Middle dose of LPF (LPF-M) with 400 mg/(kg·d) *L. plumule* flavonoid extracts
- Group 6: High dose of LPF (LPF-H) with 800 mg/(kg·d) *L. plumule* flavonoid extracts

Clinical symptoms, body weight, and FBG measurement

General daily observations were monitoring their food intake, drinking and urination consumption in each group. The body mass and fasting blood glucose of mice were measured and recorded on the 0th, 3rd, 7th, and 21st days, of which mice were fasted for 12 h before the determination of FBG. The bed material should be changed and alcohol disinfected after FBG analysis for preventing tail wound infection (Zhu et al. 2020).

Oral glucose tolerance test

On the 20th day of different diets treatment, mice in each group were given fasted for 5 h and gavage again subsequently. After 20 min, all mice were administered glucose solution (2 g/kg BW). Blood samples were withdrawn from the tail for measuring FBG at 0 h, 0.5 h, and 2 h. The area under the curve (AUC) of glucose tolerance was determined as the equation follows:

$$\mathrm{AUC\ (mmol/L)} = \frac{0.5(A+B)}{2} + \frac{1.5(B+C)}{2}$$

Where A, B, C presented the BG levels at 0 h, 0.5 h, and 2 h respectively after administering glucose.

Physiological index analysis

On the 21st day of different diets treatment, mice in each group were fasted but can drink water freely for 8 h. The blood samples were collected from the orbital sinus and centrifugated (12,000 r/min, 4 °C) for 15 min to separate and transfer serum samples into microcentrifuge tube. Serum TG and TC were measured through their respective ELISA kits following the manufacturers' instructions (Jiancheng Bioengineering Institute, Nanjing, China). And serum insulin was determined using a corresponding ELISA kit (Xinyu Biotechnology Co., Ltd., Shanghai, China). Besides, the serum TG and TC levels were calculated as follows:

$$\mathrm{TG\ (mmol/L)} = \frac{\text{Sample OD value} - \text{Blank OD value}}{\text{Calibration OD value} - \text{Blank OD value}} \times \text{Calibrator concentration (2.26 mmol/L)}$$

$$\mathrm{TC\ (mmol/L)} = \frac{\text{Sample OD value} - \text{Blank OD value}}{\text{Calibration OD value} - \text{Blank OD value}} \times \text{Calibrator concentration (5.17 mmol/L)}$$

Statistical analysis

The Box-Behnken design and RSM values were processed used Design-Expert software (version 12.0.3.3, Stat-Ease, Inc.). Other experimental data were analyzed by SPSS version 22 software package for windows. Results were expressed as mean ± standard deviation (SD), and values were considered statistically significant when the *p*-values were less than 0.05.

Results and discussion

Analysis of extraction parameter influences

The parameters which influence the extraction yield of LPF include ethanol concentration, solid-liquid ratio, ultrasound time/interval time, microwave power, ultrasonic power, and extraction time, the effects of them were evaluated through single factor experiments (Fig. 1). Ethanol has been widely used to extract biologically active compounds in different plants because of its low toxicity and inexpensive cost. The effect of ethanol concentration on the extraction efficiency of LPF was shown in Fig. 1a. As seen, the extraction rate peaked at 2.62% at the concentration of 70%, and then decreased. The reason may be that excessive ethanol concentration could make impurities such as polysaccharides and pigments dissolve into the solvent easily. Moreover, the extract solution appeared bright green color and the LPF sample was light in color in the treatment of 90% ethanol concentration during the experiment, which may be caused by excessive dissolution of chlorophyII. This result was in good agreement with those found by Liu et al. (Liu et al. 2019) who studied polyphenols from sweet potato leaves, as they also reported 70% as an optimum ethanol concentration.

It is important to maximize the extraction yield and reduce extraction solvent consumption, thus the feed-to-solvent ratio has been a research hotspot in industrial processes (Spigno & Faveri 2009). The influence of solid-liquid ratio on the extraction efficiency was illustrated (Fig. 1b), and there was a significant improvement when the solid-liquid ratio increased from 1:10 to 1:40, but further increment was not obvious at 1:50. This might be due to the reason that flavonoids inside and outside the cell had reached the equilibrium of dissolution. In addition, larger amount of solvent needed more absorption of microwave power, which leads to insufficient energy diffusion to break the cell wall, thus slowing down the flavonoids leaching (Alara et al. 2018). Considering avoiding extra cost and inconvenient follow-up

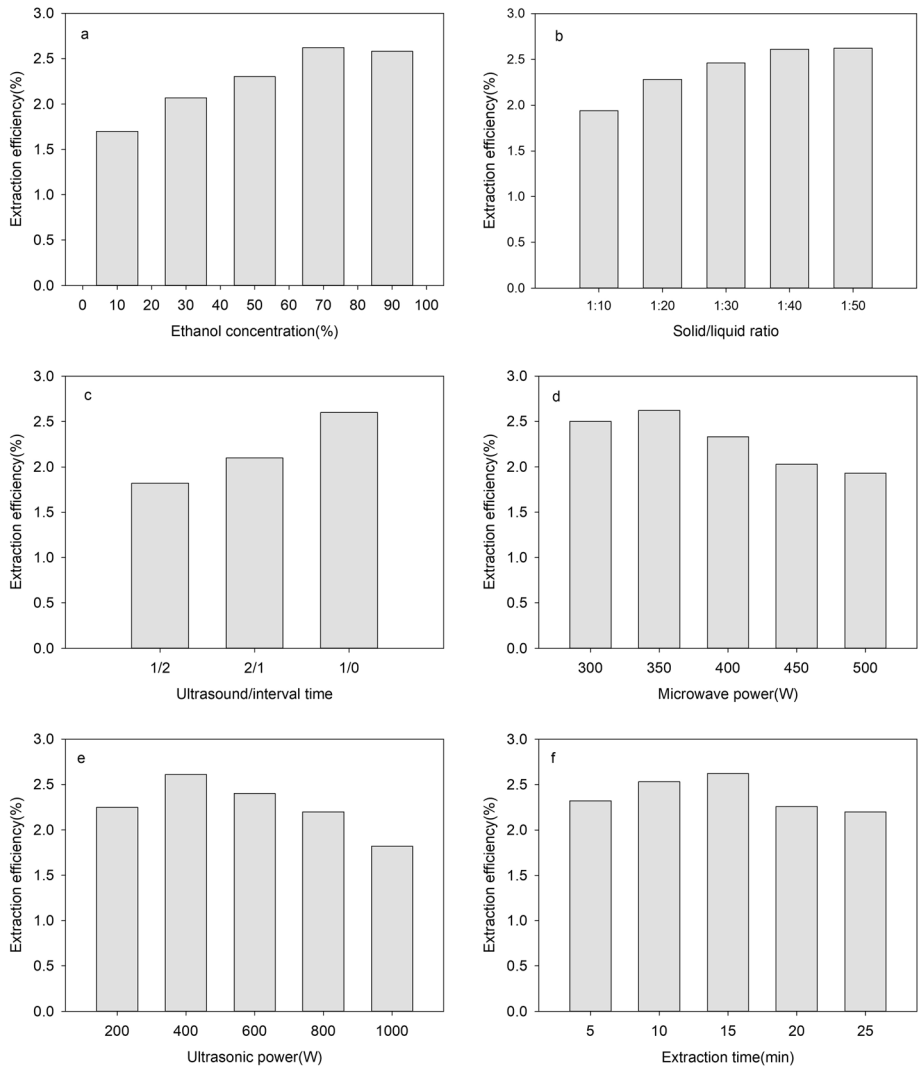

Fig. 1 (**a**) Influence of ethanol concentration on flavonoids extraction efficiency; (**b**) Influence of solid-liquid ratio on flavonoids extraction efficiency; (**c**) Influence of ultrasound/interval time on flavonoids extraction efficiency; (**d**) Influence of microwave power on flavonoids extraction efficiency; (**e**) Influence of ultrasonic power on flavonoids extraction efficiency; (**f**) Influence of extraction time on flavonoids extraction efficiency

concentration operation, the solid-liquid ratio of 1:40 revealed better.

Ultrasound/interval time represented the effect of different ultrasound methods (continuous or intermittent) on extraction rate (Fig. 1c). The yield of LPF gradually increased with an increase in ultrasound/interval time from 1/2 to 1/0, showing continuous ultrasound did better than intermittent ultrasound. This may be explained by continuous ultrasonication breaking down the cells more thoroughly, which was beneficial for the dissolution of flavonoids from *L. plumule*. Therefore, 1/0 of ultrasound/interval time was chosen for LPF extraction. The influences of microwave power on LPF extraction efficiency were investigated under different ranges (300–500 W). The extraction rate increased only when the microwave power level increased from 300 to 350 W (Fig. 1d). Beyond 350 W, the rate declined significantly. Excessive microwave power resulted in accelerating flavonoids oxidation, leading to the degradation of flavonoid content (Dahmoune et al. 2014; Spigno & Faveri 2009).

The ultrasonic power is one of the important parameters that affecting the extraction rate of LPF. As shown in Fig. 1e, the yield of LPF increased to the highest value of 2.62% at 400 W but then decreased sharply when the ultrasonic power was settled down higher. Suitable

ultrasonic power would produce the phenomenon of ultrasonic cavitation, leading to the decomposition of the plant cell wall (Jalili et al. 2017; Zhu et al. 2015). However, with the continuous increase of ultrasonic power, the fragmentation effect of ultrasonic wave on the cells was enhanced, resulting in dissolution of impurities. In addition, the heat generated would destroy certain flavonoids, reducing extraction efficiency. The duration of extraction time is a key factor of extraction efficiency because microwaveand ultrasonic power require time to transfer the energy into the matrix (Liu, Mu, Sun, & Fauconnier, 2019). The extraction yield had remarkable enhancement and then achieved to maximum when the time lasting to 15 min compared to 5 min and 10 min, but quickly decreased at 20 min (Fig. 1f). This result might be interpreted that the extraction time can break plant tissue structure thus enhancing the reactive site in the effective extraction process by ultrasonic cavitation and microwave irradiation (Arasi et al. 2016). However, extended period caused plant matrix excessive exposed to the microwave radiation, probably leading to the thermal degradation of flavonoid compounds (Dahmoune et al., 2014; Xu et al. 2012).

Statistical analysis and model fitting using RSM

In order to optimize the influences of single variables and experiment time, finally acquiring the maximum extraction efficiency, the response surface methodology analysis and Box-Behnken design was conducted. The analysis of variance was depicted in Table 2. Using the multiple regression analysis, the extraction rate was estimated by the second-order polynomial equation: $Y = 2.66 + 0.0012A + 0.0688B - 0.0475C + 0.050AB - 0.0375AC - 0.0175BC - 0.1305A^2 - 0.3205B^2 - 0.3130C^2$

where Y represents the yield of LPF and A, B, and C are factors for microwave power (W), ultrasonic power (W), and extraction time (min), respectively. F-test and *P*-value were used to check the statistical significance of each coefficient in the regression model. In this table, linear coefficients (B and C), interaction coefficients (AB and AC), and quadratic coefficients (A^2, B^2, and C^2) were found significantly affecting extraction rate ($p < 0.05$), indicating these factors were quite important to the extraction rate of *L. plumule* flavonoids. The *p*-value of the model was less than 0.0001, presenting the model was proximately to the reality. The determination coefficient ($R^2 = 0.9977$) showed that 99.77% of real extraction process could be explained, just 0.23% could not be clarified (Wai et al. 2010), indicating the model was able to make good prediction under the range of experimental variables. Likewise, the value of "the lack of fit" parameter was insignificant ($p = 0.2294 > 0.05$), which represented that the regression model was significant compared with the pure error. Therefore, these data clearly showed the reliability of the experimental value in the model.

Analysis of response surface plots

The 3D response surface reflected the relationship between two variables among microwave power, ultrasonic power, and extraction time (Fig. 2a-c). Ultrasonic power presented the most important factor (*p*-value< 0.0001), affecting the extraction efficiency of LPF effectively. At

Table 2 Analysis of variance of response surface quadratic model

Source	Sum of squares	df	Mean squares	F-Value	*p*-value Prob > F
Model	1.080943	9	0.1201048	343.8583	< 0.0001
A-Microwave power	0.0000125	1	0.0000125	0.035787	0.8553
B-Ultrasonic power	0.037813	1	0.0378125	108.2566	< 0.0001
C-Extraction time	0.01805	1	0.01805	51.67689	0.0002
AB	0.01	1	0.01	28.62986	0.0011
AC	0.005625	1	0.005625	16.10429	0.0051
BC	0.001225	1	0.001225	3.507157	0.1033
A^2	0.071706	1	0.0717063	205.2942	< 0.0001
B^2	0.432506	1	0.4325063	1238.259	< 0.0001
C^2	0.412501	1	0.4125011	1180.985	< 0.0001
Residual	0.002445	7	0.0003493		
Lack of fit	0.001525	3	0.0005083	2.210145	0.2294
Pure error	0.00092	4	0.00023		
Cor total	1.083388	16			
R^2	0.9977				

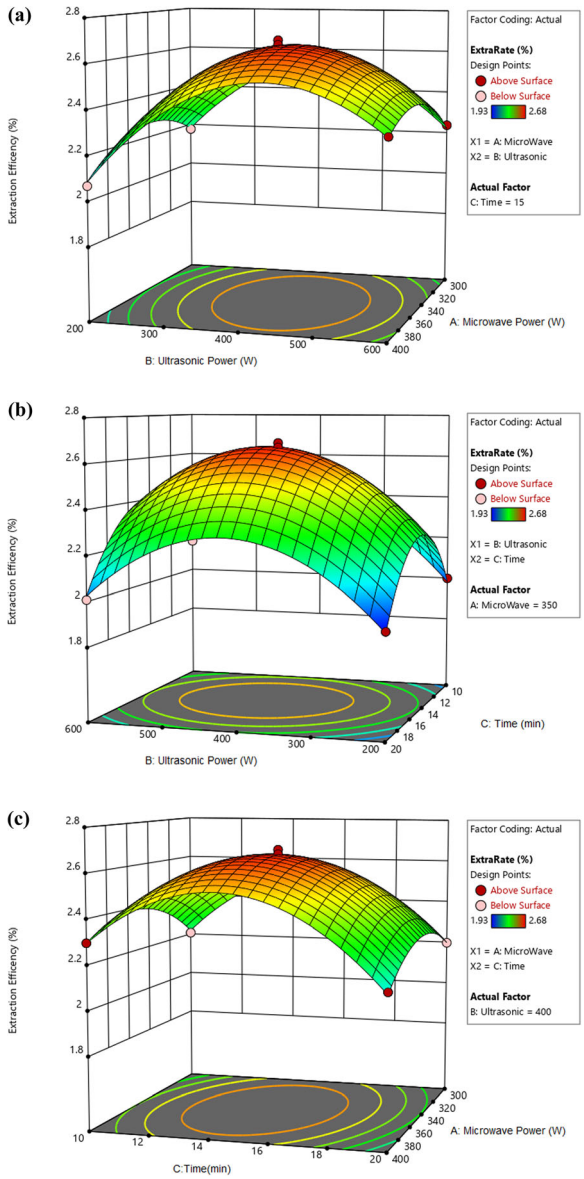

Fig. 2 (**a**) Interactive effects of microwave power and ultrasonic power; (**b**) Interactive effects of ultrasonic power and time; (**c**) Interactive effects of microwave power and time

fixed extraction time of 15 min, with the enhancing of ultrasonic power or microwave power, the extraction yield of LPF was appeared first rising but then falling tendency (Fig. 2a). The influence of both parameters on the extraction rate showed a certain degree of similarity. The extraction yield of LPF increased linearly with the increase of ultrasonic power and extraction time in a certain range (Fig. 2b). And the peak yield was obtained when ultrasonic power and extraction time were 400 W and 25 min, respectively, which the microwave power was set to 350 W. The interaction of microwave power and extraction time on the yield of LPF were shown (Fig. 2c). The extraction rate climbed to highest when microwave power was 350 W and extraction time was 15 min.

Model verification

The model equation was verified by the results of response surface analysis. The optimal extraction conditions were predicted as: 352 W of microwave power, 423 W of ultrasonic power, 14.6 min of extraction time, 1:40 of solid-liquid ratio, 1/0 of ultrasound/interval time, and 70% ethanol concentration, which the predicted extract efficiency was 2.68%. In order to further explain the deviation between the actual value and the predicted value and consider the practical operation, the experiment rechecking was conducted using following modified conditions: microwave power 355 W, ultrasonic power 423 W, extraction time 15 min, solid-liquid ratio 1:40, ultrasound/interval time 1/0, and ethanol concentration 70%. The actual extraction rate reached 2.62%, which represented not significant to the predicted value, clarified the validation of RSM model, and finally indicated that the predicted model was adequate and accurate for this research.

Hypoglycemic effect analysis of LPF

Daily status of T2DM mice

The number of 50 T2DM mice had greater food and water consumption compared to the normal group. Besides, significant differences were observed on cage padding and defecated situation between T2DM mice and normal mice, where T2DM mice showed moist cage padding and thin stools. These behaviors were consistent with the typical diabetic symptoms as “polyphagia, polydipsia, urorrhagia, and loss of body weight”, which called “three more and one less”. It was observed that the “ three more and one less “ symptoms of mice in control group, LPF-L group, LPF-M group, and LPF-H group were all alleviated during the progress of administration, while the model group got worsened with time, indicating the potential of improving anti-diabetic effect from *L. plumule* flavonoids.

Effect of LPF on body weight in T2DM mice

Overweight is one of the characteristics on type 2 diabetes, but it would be relieved in the clinically treatment. The variation of the mice body weight was measured on the day 0, 7, 14, and 21 during administration (Table 3). Before flavonoids administration, the body weights of MG, CG, LPF-L, LPF-M, and LPF-H groups were obvious higher than that of the normal group ($p < 0.01$), indicating that T2DM mice were successfully established and were appropriate for subsequent experiments. Minor increases were observed in the normal and model groups, but the body weights of the control group and different dose of the LFP groups were decreased during

Table 3 Effect of flavonoids from *L. plumule* on body weight of diabetic mice

Group	Body weight (g)			
	Day 0	Day 7	Day 14	Day 21
Normal	37.76 ± 0.94 ^{BD}	39.57 ± 0.99 ^{BD}	40.84 ± 0.76 ^{BD}	41.61 ± 0.66 ^{B}
Model	49.06 ± 1.49 ^{A}	50.57 ± 1.53 ^{AD}	50.98 ± 1.36 ^{AD}	51.28 ± 1.13 ^{AD}
Control	48.58 ± 2.74 ^{A}	47.60 ± 2.50 ^{AB}	45.77 ± 2.16 ^{AB}	43.33 ± 2.97 ^{B}
LPF-L	47.94 ± 2.65 ^{A}	47.31 ± 2.24 ^{AB}	46.97 ± 2.70 ^{AB}	46.62 ± 2.00 ^{ABd}
LPF-M	48.53 ± 2.40 ^{A}	47.23 ± 2.69 ^{AB}	45.03 ± 2.59 ^{AB}	44.06 ± 2.11 ^{aB}
LPF-H	48.12 ± 2.85 ^{A}	46.84 ± 2.59 ^{AB}	45.88 ± 2.26 ^{AB}	45.47 ± 2.22 ^{AB}

The results were expressed as mean ± SD (n = 8/group). ^{A} $p < 0.01$, ^{a} $p < 0.05$, compared with normal group; ^{B} $p < 0.01$, ^{b} $p < 0.05$, compared with model group; ^{D} $p < 0.01$, ^{d} $p < 0.05$, compared with control group

21 days of treatment. The control and LPF groups represented huge reductions in body weight ($p < 0.01$) than the model group, of which the control group and the LPF-M group showed the largest decline. The LPF-L, LPF-M, and LPF-H groups showed insignificant difference compared with control group, demonstrating that *L. plumule* flavonoids could effectively control the body weight of T2DM mice. In addition, the body weights of mice in three LPF groups were still higher than the normal group in the 21th day, which indicated that *L. plumule* flavonoids were unable to completely decrease the body weight back to normal level. It might because *L. plumule* flavonoids could not completely repair the islet cell damage induced by STZ (Zhu et al. 2020).

FBG levels in T2DM mice

The FBG levels in the normal group mice were maintained at a stable normal level during the 21-day administration (Table 4), suggesting experimental environment and eating diet did not affect normal glucose metabolism. After 7 days, three doses of LPF all exhibited significant hypoglycemic effects ($p < 0.01$, $p < 0.05$, and $p < 0.01$) of streptozotocin-diabetic mice compared with the model group. Moreover, there was no significant difference between the control group and LPF-M group as well as LPF-H group, of which the anti-diabetic effect of middle dose was equivalent with 100 mg/(kg·d) metformin hydrochloride. Therefore, *L. plumule* flavonoids treatment had notable hypoglycemic effect.

Oral glucose tolerance test in T2DM mice

The oral glucose tolerance test (OGTT) could measure the ability to regulate blood glucose after taking glucose, which is the major method for diabetes diagnosis (Zhu et al. 2020). The blood glucose levels exerted remarkable increases in T2DM mice after glucose administration (Table 5), while the LPF-group of three doses saw modest rises compared to the model group, suggesting *L. plumule* flavonoids could suppress the sharp increase in blood glucose level. The LPF-M group and LPF-H group exerted no significant difference to control group, which indicated the excellent modulating effect on OGTT. Besides, the AUC of the control, LPF-L, LPF-M, and LPF-H groups showed significant reduction ($p < 0.05$) compared with the model group (Fig. 3). Thus, flavonoids from *L. plumule* could utilize the glucose well and significantly improve glucose tolerance in T2DM mice.

Effects of LPF on serum TG, TC, and insulin levels of T2DM mice

Dyslipidemia is one representative symptom of T2DM patients. And the T2DM-related hyperlipidemia would also cause cardiovascular disease (Yan et al. 2019). In comparison with the model group, the levels of serum TG and TC revealed considerable diminution after the

Table 4 Effect of flavonoids from *L. plumule* on serum glucose of diabetic mice

Group	FBG (mmol/L)			
	Day 0	Day 7	Day 14	Day 21
Normal	5.47 ± 0.31 ^{BD}	5.64 ± 0.75^{BD}	5.35 ± 0.79^{BD}	5.5 ± 0.46^{Bd}
Model	12.79 ± 2.16 ^{A}	13.16 ± 1.92 ^{AD}	12.85 ± 1.83 ^{AD}	12.59 ± 1.54 ^{AD}
Control	11.96 ± 1.22 ^{A}	9.43 ± 1.95 ^{AB}	8.18 ± 1.97 ^{AB}	6.83 ± 1.68 ^{aB}
LPF-L	12.52 ± 1.57 ^{A}	10.97 ± 2.05 ^{Ab}	9.87 ± 1.47 ^{ABd}	9.25 ± 1.05 ^{ABD}
LPF-M	12.60 ± 1.45 ^{A}	10.24 ± 2.19 ^{AB}	8.68 ± 1.87 ^{AB}	7.18 ± 1.50 ^{aB}
LPF-H	12.95 ± 2.03 ^{A}	11.17 ± 1.63 ^{Ab}	9.49 ± 1.56 ^{AB}	8.11 ± 1.27 ^{AB}

The results were expressed as mean ± SD (n = 8/group). ^{A} $p < 0.01$, ^{a} $p < 0.05$, compared with normal group; ^{B} $p < 0.01$, ^{b} $p < 0.05$, compared with model group; ^{D} $p < 0.01$, ^{d} $p < 0.05$, compared with control group

Table 5 Effect of flavonoids from *L. plumule* on glucose tolerance of diabetic mice

Group	Blood glucose level (mg/dl)		
	0 h	0.5 h	2 h
Normal	5.5 ± 0.29^{Bd}	10.15 ± 1.38^{B}	5.98 ± 1.02^{Bd}
Model	12.56 ± 1.58^{AD}	20.16 ± 2.43^{AD}	15.71 ± 3.13^{AD}
Control	7.07 ± 1.46^{aB}	11.23 ± 1.52^{B}	7.94 ± 1.23^{aB}
LPF-L	9.21 ± 1.05^{ABD}	16.78 ± 1.85^{ABD}	11.65 ± 2.16^{ABD}
LPF-M	7.36 ± 1.62^{AB}	13.58 ± 2.40^{ABd}	7.75 ± 1.34^{aB}
LPF-H	7.98 ± 1.67^{AB}	14.82 ± 2.56^{ABD}	9.34 ± 1.26^{AB}

The results were expressed as mean ± SD (n = 8/group). $^{A}p < 0.01$, $^{a}p < 0.05$, compared with normal group; $^{B}p < 0.01$, $^{b}p < 0.05$, compared with model group; $^{D}p < 0.01$, $^{d}p < 0.05$, compared with control group

treatment of *L. plumule* flavonoids or metformin hydrochloride ($p < 0.05$) (Fig. 4). Additionally, LPF-M and LPF-H groups represented inapparent differences when compared with the control group, verifying that the supplementation of *L. plumule* flavonoids can regulate the lipid metabolism in diabetic mice.

The metabolism of lipid, glycogen, and protein are closely related to insulin regulation. Meanwhile, insulin can control the blood glucose homeostasis (Zhu et al. 2020). As shown in Fig. 5, the serum insulin levels were remarkably decrease ($p < 0.05$) in STZ-induced diabetic mice compared with the normal one, which implied the islet β cells of diabetic mice were damaged by STZ (Li et al. 2014). However, considerable ($p < 0.05$) increases were observed in serum insulin levels of LPF and metformin hydrochloride treatments in comparison with the model group. Thus, flavonoids from *L. plumule* were able to lower serum insulin levels, improving islet β cells function and insulin resistance.

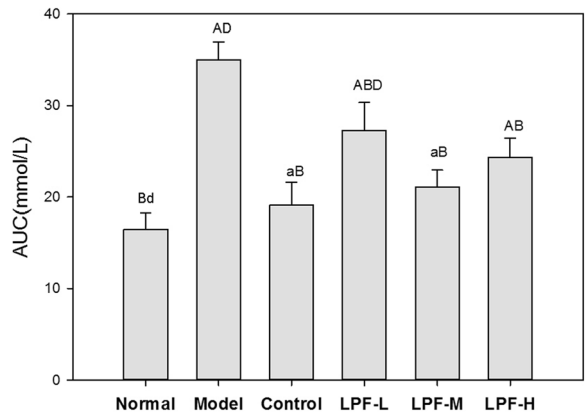

Fig. 3 Effects of flavonoids from *Lotus plumule* on AUC level of T2DM mice. The results were expressed as mean ± SD ($n = 8$/group). $^{A}p < 0.01$, $^{a}p < 0.05$, compared with normal group; $^{B}p < 0.01$, $^{b}p < 0.05$, compared with model group; $^{D}p < 0.01$, $^{d}p < 0.05$, compared with control group

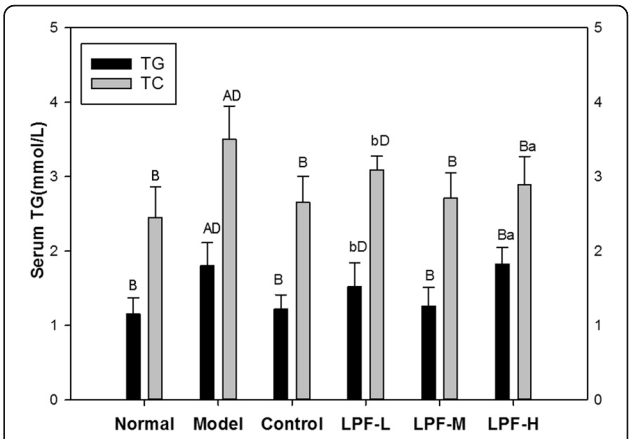

Fig. 4 Effects of LPF on serum TG and TC. The results were expressed as mean ± SD (n = 8/group). $^{A}p < 0.01$, $^{a}p < 0.05$, compared with normal group; $^{B}p < 0.01$, $^{b}p < 0.05$, compared with model group; $^{D}p < 0.01$, $^{d}p < 0.05$, compared with control group

Conclusions

In this study, the optimum UMSE parameters and hypoglycemic activity of *L. plumule* extract rich in flavonoids were investigated. The extraction variables were optimized by RSM on the basis of the single factor test. The highest extraction efficiency of LPF was 2.62% at the optimal conditions as follows: microwave power 355 W, ultrasonic power 423 W, extraction time 15 min, solid-liquid ratio 1:40, ultrasound/interval time 1/0, and ethanol concentration 70%, which was confirmed to well agreed with the predicted computed value. Moreover, *L. plumule* ethanol extract exhibited hypoglycemic effect in STZ-induced diabetic mice, showing reductions in parameters like body weight, FBG, OGTT, serum TC, TG, and insulin levels. And these antidiabetic activities might be related to the improvement of the islet β cell

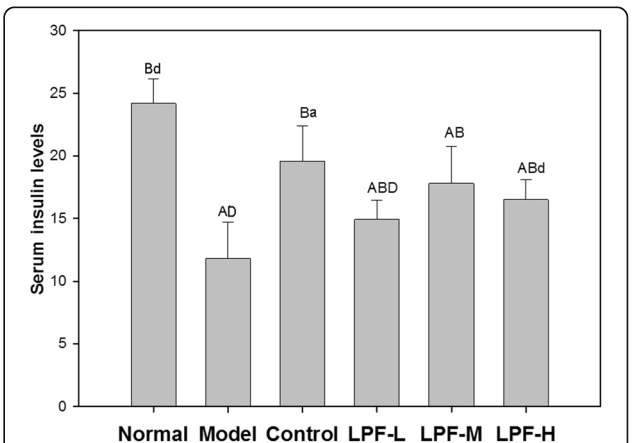

Fig. 5 Effects of LPF on insulin levels. The results were expressed as mean ± SD (n = 8/group). $^{A}p < 0.01$, $^{a}p < 0.05$, compared with normal group; $^{B}p < 0.01$, $^{b}p < 0.05$, compared with model group; $^{D}p < 0.01$, $^{d}p < 0.05$, compared with control group

function. Further experimental and clinical investigations are necessary to elucidate the regulation mechanisms of action underlying the hypoglycemic effect from *L. plumule* ethanol extract and look for their potential clinical applications.

Abbreviations

LPF: *Lotus plumule* flavonoids; FBG: fasting blood glucose; OGTT: oral glucose tolerance test; TC: serum total cholesterol; TG: serum total triglycerides; T2DM: type 2 diabetes mellitus; UMSE: ultrasonic-microwave synergistic extraction; RSM: response surface methodology; BBD: Box-Behnken design; HFHS: high-fat high-sucrose diet

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Not applicable.

Authors' contributions

Qiuzhe Li and Xiaoqing Li performed the experiment and wrote the article. Baodong Zheng and Chao Zhao designed the study. All authors read and approved the final manuscript.

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Availability of data and materials

All data needed to evaluate the conclusions are present in the paper. Additional data related to this paper may be available upon request.

Declarations

Ethical approval and consent to participate

The animal procedures were approved by the Ethics Review Committee of College of Food Science, Fujian Agriculture and Forestry University, China (No. FS-2017-002).

Consent for publication

All authors consent to the publication.

Competing interests

The authors declare that they have no competing interest.

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