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Research Article

Flavonoids Extraction from *Taraxacum officinale* (Dandelion): Optimisation Using Response Surface Methodology and Antioxidant Activity

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The Box-Behnken design combined with response surface method was employed to optimize ultrasonic-assisted extraction of flavonoids from *Taraxacum officinale*. The optimized results showed that the highest extraction yield with ultrasonic-assisted extraction could reach 2.62% using 39.6% (v/v) ethanol and 59.5 : 1 (mL/g) liquid-solid ratio for 43.8 min. The crude extract was then purified by HPD-100 macroporous adsorption resin, and the flavonoids content in the purified extract increased to 54.7%. The antioxidant activity of the purified flavonoids was evaluated *in vitro* by scavenging capacity of ABTS or DPPH, β-carotene bleaching, and FTC test. The knowledge obtained from this study should be useful to further develop and apply this plant resource.

1. Introduction

Taraxacum officinale (family Asteraceae), commonly known as dandelion, is a perennial plant widely distributed in the northern hemisphere, which mainly contains flavonoids, triterpenes, coumarins, and phytosterols [1]. *T. officinale* has been traditionally used as a folk medicine for removing boils, reducing fever, lactating, and relieving sore throat [2]. Pharmacological investigations have revealed that the extracts of this plant possess antioxidant, antifertility, hepatoprotective, anti-inflammatory, and antitumor activities [3–6]. Previous studies showed that the flavonoids contained in *T. officinale* have been associated with these efficacies [7–9].

At present, various extraction methods have been used for the extraction of flavonoids from *T. officinale* such as conventional extraction [10, 11], ultrasound-assisted extraction (UAE) [12], and microwave-assisted extraction [13]. The UAE technique is an inexpensive, rapid, simple, and efficient method [14], which is due to the effects of acoustic cavitation generated in the medium by passage of an ultrasound wave [15]. Further, there is no chemical involvement in the UAE, which can avoid possible structural changes and degradation of target compounds [16].

Response surface methodology (RSM), an effective statistical technique for optimizing complex processes, has been extensively used to optimize processing parameters due to more efficient and easier arrangement and interpretation of experiments compared to others [17–19]. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions [20]. To the best of our knowledge, there are no studies about the use of RSM on UAE optimisation for the extraction of flavonoids from *T. officinale* (TOFs). In this study, UAE for the flavonoids-enriched extract from *T. officinale* was investigated and the operational parameters were optimized using RSM. The antioxidant activity of flavonoids was also evaluated with several established *in vitro* systems. The findings obtained from this work will be useful to further develop and utilize this abundant resource.

2. Experimental

2.1. Reagents. Rutin, DPPH, and ABTS were purchased from Sigma-Aldrich Co., Ltd. (Sigma Chemical Co., St. Louis, MO, USA). All other chemical reagents used in this study were of analytical grade from Shanghai Chemical Reagent

Company (Shanghai, China) and doubly distilled water was used throughout the experiment.

2.2. Preparation of the T. officinale Sample. *T. officinale* was collected in September 2012 from Linyi City of Shandong Province, China, and authenticated by the corresponding author. The plant was allowed to dry naturally and pulverized by a disintegrator and sifted through a 40-mesh sieve. The powdered sample was kept in sealed polyethylene bags at 4°C until use.

2.3. Ultrasound-Assisted Extraction of Flavonoids. About 0.5 g sample was exactly weighted into a 150 mL conical flask and mixed with ethanol. The extraction process was performed using an ultrasonic device (KQ5200DB, 40 kHz, 160 W, Kunshan Ultrasonic Instrument Co., Jiangsu, China) equipped with a digital timer and a temperature controller. The extract was filtered to collect supernatant. UV-2401 spectrophotometer (Shimadzu Corporation, Japan) was used for total flavonoids analysis of sample, R-501 rotavapor (Shanghai Shenshun Bio-Tech Co., Ltd, Shanghai, China) for concentration of sample, and LGJ-10D freeze drier (Beijing Four-Ring Science Instrument Plant Co., Ltd., Beijing, China) for dryness of the concentrated sample.

2.4. Determination of Total Flavonoids Content. Total flavonoids content was determined by the colorimetric-based method assay [21]. Briefly, 0.5 mL sample solution was mixed with 4.0 mL distilled water and 0.4 mL $NaNO_2$ (5%, w/v) was added. After 6 min, 0.4 mL $Al(NO_3)_3$ (10%, w/v) and 4 mL NaOH (1 M) were added. The solution was mixed thoroughly and incubated for 15 min. The absorbance was measured at 512 nm against the control. The standard curve regression equations were $C = 68.89A - 0.462$ and $R^2 = 0.9995$ (where C is the rutin concentration in μg/mL and A is absorbance). The flavonoids content was calculated from the calibration curve and expressed as rutin equivalents (RE).

2.5. Experimental Design. Box-Behnken design (BBD) was employed to determine the best combination of extraction variables for the flavonoids based on the results of preliminary single-factor-test. Ethanol concentration (v/v, X_1), liquid-to-solid ratio (mL/g, X_2), and extraction time (min, X_3) were the independent variables and their coded and uncoded (actual) levels of the independent variables are given in Table 1. The temperature was not considered in this study because the extraction process was maintained at room temperature to avoid the degradation of temperature-sensitive ingredients. It is generally believed that higher ultrasonic power increases the extraction yield [19]; the ultrasound power of 160 W was chosen as the optimal parameter. Extraction yield (Y, %) taken as the response of the design experiment was presented in Table 2. Experimental data were fitted to a quadratic polynomial model and the model was explained by the following quadratic equation:

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} A_{ij} X_{ij}, \quad (1)$$

Table 1: Code and level of factors chosen for the trials.

Symbols	Independent variables	−1	0	+1
X_1	Ethanol concentration (%, v/v)	30	40	50
X_2	Liquid-solid ratio (mL/g)	50	60	70
X_3	Extraction time (min)	30	40	50

Table 2: BBD for independent variables and extraction yield.

Run	X_1 (%, v/v)	X_2 (mL/g)	X_3 (min)	Y (%)
1	+1	+1	0	2.38
2	0	+1	−1	2.43
3	−1	+1	0	2.41
4	−1	0	−1	2.44
5	−1	0	+1	2.49
6	−1	−1	0	2.43
7	0	0	0	2.60
8	+1	0	+1	2.49
9	0	−1	+1	2.52
10	+1	0	−1	2.42
11	+1	−1	0	2.40
12	0	0	0	2.63
13	0	+1	+1	2.48
14	0	0	0	2.65
15	0	0	0	2.58
16	0	0	0	2.61
17	0	−1	−1	2.36

where Y represents the response variables, A_0 is a constant, and A_i, A_{ii}, and A_{ij} are the linear, quadratic, and interactive coefficients, respectively. X_i and X_j are the levels of the independent variables.

2.6. Purification of Flavonoids by Macroporous Resin Adsorption. The crude flavonoids-enriched extract obtained under the optimized condition was purified using a column (40 × 2.0 cm) packed with HPD 100 macroporous adsorption resin. The optimized conditions for separating and purifying the flavonoids were injecting concentration 3.3 mg/mL, pH = 3.5, injecting velocity 1.0 mL/min, 60% (v/v) ethanol as desorption solvent, and desorption velocity of flow 2.0 mL/min. The purified extract of flavonoids was collected and evaporated at 70°C and was then freeze-dried for determination of bioactivity.

2.7. Evaluation of Flavonoids Antioxidant Activities

2.7.1. ABTS Radical Scavenging Activity. The ABTS radical scavenging assay was conducted in accordance with the method described by Re et al. [22]. The ABTS radical was produced by a reaction of 7 mM ABTS with 2.45 mM potassium persulfate. The reaction mixture was kept in the dark at room temperature for 16 h before use. The solution was prepared by diluting with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. Sample solution (0.3 mL) at different concentrations was mixed with diluted ABTS solution;

Table 3: ANOVA for fitted quadratic polynomial model of extraction of flavonoids.

Source	Sum of squares	Degree of freedom	Mean square	F value	P value
Model	0.13	9	0.015	21.99	<0.001
Residual	4.74×10^{-3}	7	6.78×10^{-4}		
Lack of fit	1.82×10^{-3}	3	6.08×10^{-3}	0.83	0.5413
Pure error	2.92×10^{-3}	4	7.30×10^{-4}		
Cor. total	0.14	16			

Note: $R^2 = 0.9658$; $R^2_{adj} = 0.9219$; CV = 1.05.

then absorbance was determined at room temperature after 7 min. BHT and vitamin C with the same concentration served as positive control. The radical scavenging activity of the samples was calculated by the following formula: scavenging activity (%) = $[(A_s - A_i)/A_s] \times 100$, where A_s and A_i are the absorbance of control (without sample) and sample, respectively.

2.7.2. DPPH Radical Scavenging Activity. The free radical scavenging activity of DPPH was determined using a modified protocol based on He et al. [23]. Briefly, 2.7 mL of DPPH solution (0.2 mM) was mixed with 0.3 mL of the samples at different concentrations. After shaking vigorously, the reaction mixture was incubated at room temperature in the dark for 20 min. The absorbance was measured at 517 nm against a blank. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. BHT and vitamin C were used for positive controls. The level of radical scavenging was calculated using the equation described above for ABTS.

2.7.3. β-Carotene Bleaching Assay. The β-carotene bleaching test was determined according to the method of Shakirin et al. [24] with some modifications. A β-carotene solution (4 mL) with a concentration of 0.3 mg/mL dissolved in chloroform was transferred into a flask containing 80 mg linoleic acid and 800 mg Tween-80. The chloroform was removed using a rotary evaporator at 40°C; then 200 mL of distilled water was added slowly to the mixture with vigorous agitation to form an emulsion. 3 mL aliquots of the emulsion were removed into a series of test tubes containing 0.2 mL of the samples at 400 μg/mL and incubated in a water bath at 50°C. BHT and vitamin C were used as standards for comparison. The absorbance reading was measured at 30 min intervals for 120 min at 470 nm. The antioxidant activity was calculated in terms of the successful bleaching of β-carotene using the formula: antioxidant activity (%) = $[1 - (A_0 - A_t)/(A_0^0 - A_t^0)] \times 100$, where A_0 and A_0^0 are the absorbance values measured at the zero incubation time for the samples and control, respectively, and A_t and A_t^0 are the absorbance values measured in the test sample and control at $t = 120$ min.

2.7.4. Antioxidant Activity in a Linoleic Acid System Using Ferrothiocyanate (FTC). The FTC method was adopted from Zhu et al. [25]. The samples (400 μg) in ethanol (4 mL) were mixed with 2.5% linolenic acid in ethanol (4 mL), phosphate buffer (8 mL, 50 mM, and pH 7.0), and distilled water (4 mL). The mixture was placed in a dark oven at 40°C. Aliquots (0.1 mL) were withdrawn and mixed with 75% ethanol (9.7 mL) and 30% ammonium thiocyanate (0.1 mL). After 3 min, 20 mM ferrous chloride in 3.5% hydrochloric acid (0.1 mL) was added to the reaction mixture. The absorbance of the mixture was recorded at 500 nm at 24 h intervals until a constant maximum was reached. Controls without sample and standard containing BHT in place of sample were subjected to the same procedure.

3. Results and Discussion

3.1. Optimization of Ultrasonic-Assisted Operational Parameters for Flavonoids

3.1.1. Fitting the Model. In this study, UAE was employed for flavonoids extraction from *T. officinale*. The operational parameters were optimized using BBD combined with RSM. Table 1 shows the experiment design and the corresponding response data for the TOFs. The percentage yield ranges from 2.36% to 2.65%. By applying multiple regression analysis on the experimental data, the model for the response variable could be expressed using the following quadratic polynomial equation in the form of coded values:

$$Y = +2.61 + 0.01X_1 - 0.00125X_2 + 0.041X_3 + 0.005X_1X_3 - 0.028X_2X_3 - 0.098X_1^2 - 0.11X_2^2 - 0.056X_3^2, \quad (2)$$

where Y is the yield of TOFs and X_1, X_2, and X_3 are the coded variables for ethanol concentration, liquid-solid ratio, and extraction time, respectively.

The analysis of variance (ANOVA) for the model is shown in Table 3. The determination coefficient ($R^2 = 0.9658$) indicated that only 3.42% of the total variations was not explained by the model. The value of lack of fit test was insignificant ($P > 0.05$) thereby confirming the validity of the model. The value of the adjusted determination coefficient ($R^2_{adj} = 0.9219$) also confirmed that the model was highly significant. Furthermore, a relatively low value (1.05) of the coefficient of variation (CV) indicates the high degree of precision and reliability of the experimental values. The model P value was very low ($P < 0.001$), which suggested that the model was significant.

The model was found to be adequate for prediction in the range of experimental variables. The regression coefficient values were listed in Table 4. Smaller P value and greater

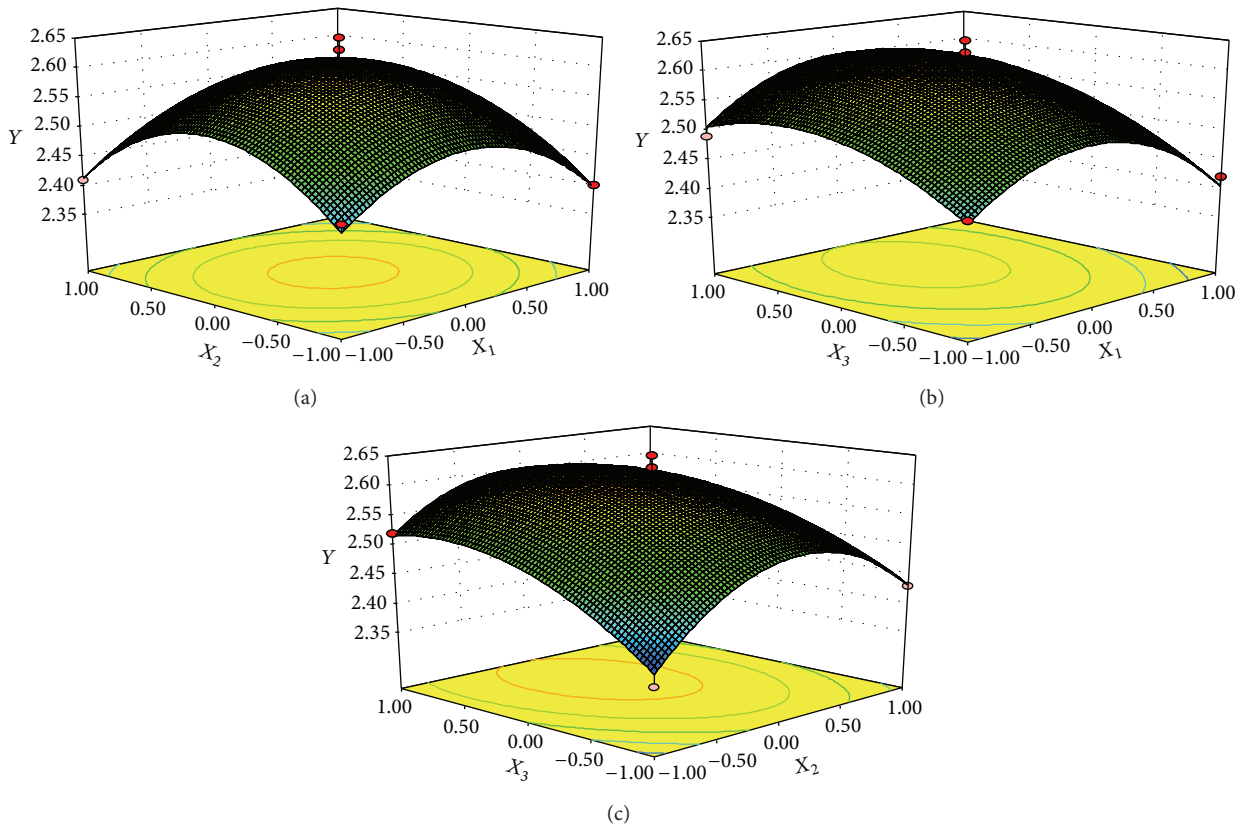

Figure 1: Response surface and contour plots for effect of independent parameters on extraction yield of the total flavonoids.

Table 4: Regression coefficients and significance test for quadratic model.

Source	Degree of freedom	Mean square	F value	P value
X_1	1	8×10^{-4}	1.18	0.3133
X_2	1	1.25×10^{-5}	0.018	0.8958
X_3	1	0.014	20.08	0.0029
X_1^2	1	0.041	59.96	0.0001
X_2^2	1	0.052	76.19	<0.0001
X_3^2	1	0.013	19.31	0.0032
X_1X_2	1	0	0	1
X_1X_3	1	1×10^{-4}	0.15	0.7123
X_2X_3	1	3.025×10^{-3}	4.46	0.0725

F value mean the corresponding variables would be more significant. The independent variable (X_3), along with three quadratic terms (X_1^2, X_2^2, and X_2^3), was significant with P values ($P < 0.05$). The other term coefficients were not significant ($P > 0.05$). Meanwhile, extraction time is the most significant factor affecting the extracting yield.

3.1.2. Analysis of Response Surfaces. The relationship between independent and dependent variables is illustrated by a three-dimensional representation of the response surfaces and by two-dimensional contours generated by the model. The shapes of the contour plots, elliptical or circular, indicate whether the interactions between the corresponding variables are significant or not. An elliptical contour plot means the interactions between the variables are significant while a circular contour plot means otherwise. In these three variables, when two variables within the experimental range are depicted in three-dimensional surface plots, the third variable is kept constant at its 0 level (center value of the testing ranges). As can be seen in Figure 1, the extraction yields are not affected significantly by alterations of test variables through three independent response surface plots and their respective contour plots in the experimental range. From Figure 1(a), the extraction yields increased with the increase of ethanol concentration (X_1) from 30 to 39.6%, but, beyond 39.6%, extraction yield decreased with the increasing ethanol concentration. When the ethanol concentration was fixed, extraction yield was also found to increase with the increase of liquid-solid ratio from 50 to 59.5 and then decreased with the extension of extraction time. Figure 1(b) depicted that the extraction yield increased when the extraction time (X_3) increased from 30 to 43.8 min and began to slightly decrease. In Figure 1(c), the maximum extraction yield (2.62%) was achieved when liquid-solid ratio and extraction time were 69.5 and 43.8 min, respectively.

It could be concluded that the optimal extraction conditions of TOFs were ethanol concentration of 39.6, liquid-solid ratio of 59.5, and extraction time of 43.8 min. Under these conditions, the experimental yield is 2.61 ± 0.02% (n = 3) and is well matched with the predicted yield (2.62%),

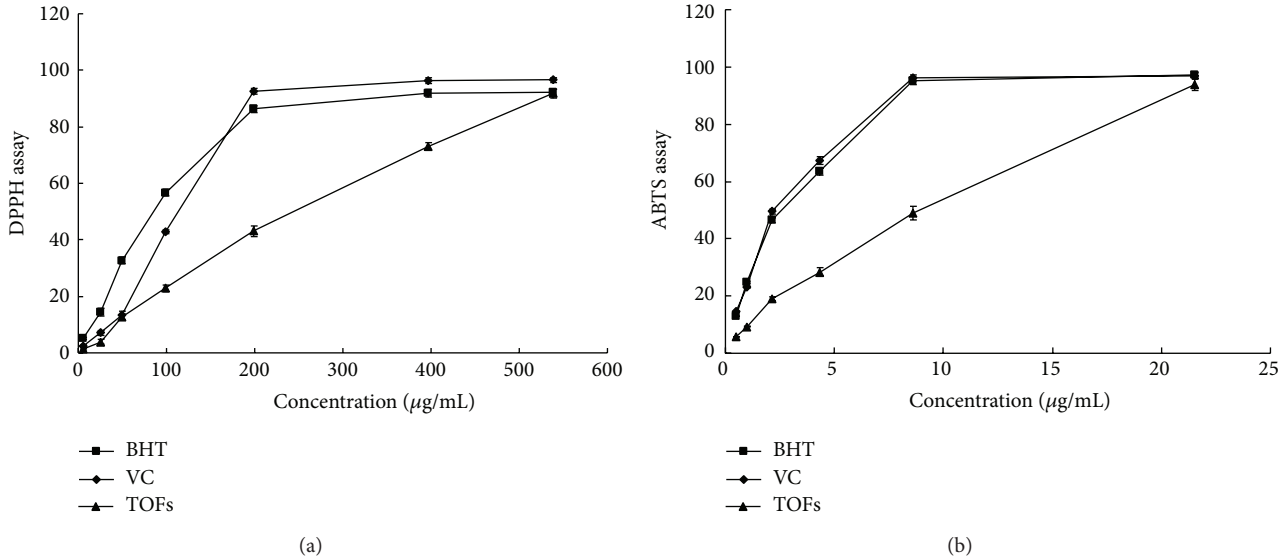

Figure 2: DPPH and ABTS radical scavenging activities of sample and control standard.

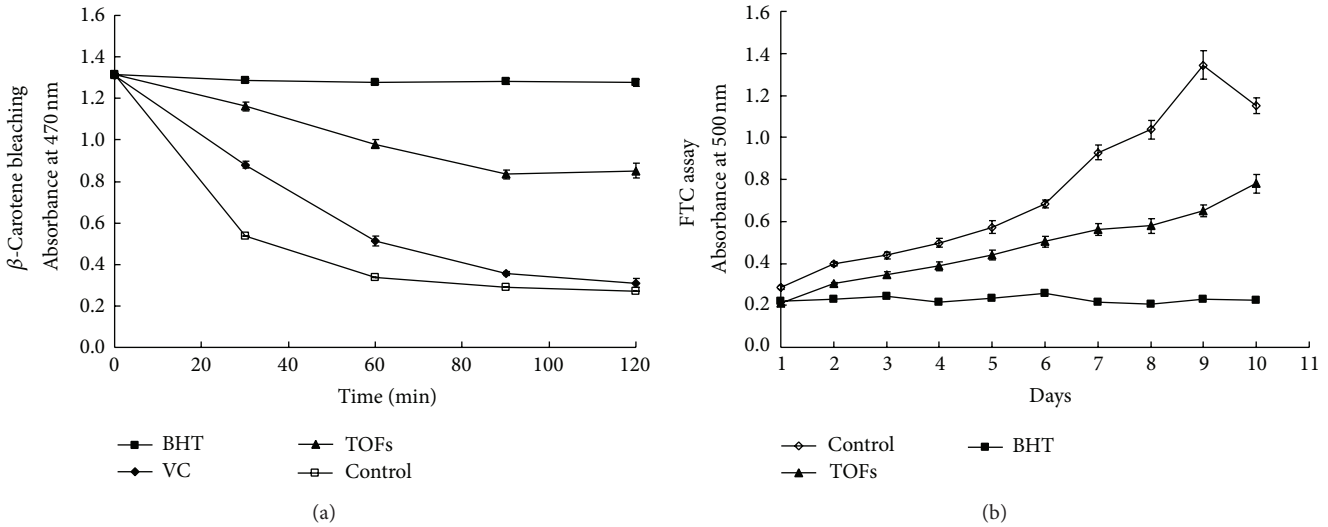

Figure 3: Antioxidant activity determined using β-carotene bleaching and FTC.

consequently indicating the RSM model is satisfactory and accurate.

To evaluate antioxidant activity of TOFs, macroporous adsorption resin was used to purify the flavonoids-enriched extract of UAE. After the purification, the flavonoids content could increase to 54.7%. The purified extract was used for the next bioactivity analysis.

3.2. Antioxidant Activity Analysis

3.2.1. DPPH and ABTS Free Radical Scavenging Activity. Antioxidants have been considered to exhibit protective effects against oxidative damage and are associated with reduced risk of chronic diseases [26]. Recently, the capacity of antioxidants for scavenging free radicals has been evaluated more often and extensively by competitive methods using a reference compound as a probe and a conventional UV-visible absorption spectrophotometer for detection. The DPPH and ABTS are commercially available, stable, and easy to handle, which have been broadly accepted to estimate the free radical scavenging activity of antioxidant from plant extracts [27]. As shown in Figure 2, for the DPPH assay, the IC_{50} (the concentration required to scavenge 50% of radicals) values of TOFs, BHT, and vitamin C were 180.11 ± 7.85, 69.13±4.32, and 77.98±3.68 μg/mL, respectively. In the ABTS assay, the IC_{50} values of TOFs, BHT, and vitamin C were 10.18 ± 1.07, 2.02 ± 0.18, and 1.92 ± 0.04 μg/mL, respectively.

3.2.2. β-Carotene Bleaching Assay. Antioxidants extracted from herbs can hinder β-carotene bleaching by neutralizing free radicals [23]. The β-carotene bleaching activities of the samples are presented in Figure 3 which shows that

the absorbance of the control and vitamin C declined rapidly over 2 hours when TOFs and BHT were much slower. The values showed that TOFs was as effective as BHT and was much more effective than vitamin C. The LPO inhibitions of TOFs, BHT, and vitamin C were 55.78%, 96.37%, and 4.30%, respectively. The TOFs presented excellent antioxidant capacity.

3.2.3. Antioxidant Activity in a Linoleic Acid System Using Ferrothiocyanate (FTC). The FTC method was used to determine the amount of peroxide at the initial phase of lipid peroxidation. Peroxides resulting from linoleic acid can convert Fe^{2+} to Fe^{3+}, and the presence of Fe^{3+} ions is detected by absorbance at 500 nm after a complex with SCN^- is formed [28]. High absorbance indicates high concentration of peroxide formed during the incubation. In Figure 3, the absorbance of the control showed rapid increase and reached a maximum level on day 9. BHT exhibited the lowest absorbance, which indicates the lowest peroxide concentrations and the highest level of antioxidant activity. The absorbance of TOFs increased slower than that of the control but faster than that of BHT. Hence, the TOFs presented a strong antioxidant activity, but it was less efficient than BHT.

4. Conclusion

In this study, we have investigated a UAE method to extract TOFs using RSM. The optimum extraction conditions were obtained and a maximum flavonoids yield of 2.62% was achieved. Moreover, the antioxidant activities of flavonoids *in vitro* including ABTS or DPPH radical scavenging capacity, Fe^{2+} chelating activity, and FTC method were evaluated. Based on our finding, it is revealed that the ultrasonic extraction is an effective method for TOFs extraction and the purified flavonoids exhibited strong antioxidant activities. More research should be carried out to illustrate the bioactivity through animal experiments with the purpose of developing its application in food ingredients or raw materials resulting in greater use of this abundant resource.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Zongxi Sun and Ruiqiang Su contributed equally to this study.

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