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*Research Article*

# Antiradical Activity Studies of Ultrasound-Assisted Extraction of Total Flavonoids from *Sanghuangporus vaninii* Fruit Bodies

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Flavonoids are a class of secondary metabolites present in many plant species. Flavonoids are important active components in *Sanghuangporus vaninii*. It has attracted widely attention because of its unique antitumor, antioxidation, immunomodulatory, bacteriostatic, and other biological activities. In this study, the *Sanghuangporus vaninii* fruit bodies are used as raw materials to study the optimal extraction technology of the *Sanghuangporus* flavonoids. In addition, the antioxidant and bacteriostatic abilities of *Sanghuangporus vaninii* are analyzed through antioxidant and bacteriostatic experiments. Ultrasound-assisted ethanol extraction is used to extract *Sanghuangporus* flavonoids. The optimum extraction process was determined by single factor test and response surface analysis, namely, ratio of material to liquid 1 : 60, ethanol concentration 80%, ultrasonic temperature 70°C, and ultrasonic time 40 min. Under these conditions, the flavonoids content is 90.24 ± 0.89 mg/g (on dry weight basis). Using $V_C$ as the reference substance, the total flavonoid concentration was tested for its ability to scavenge free radicals such as DPPH, $ABTS^+$, OH, and $O_2^-$. The reducing power of total crude flavonoids, the scavenging ability of $NO_2^-$, and the antibacterial and anti-inflammatory abilities are also used as evaluation indicators for this experiment. The results showed that the best scavenging rates of *Sanghuangporus vaninii* flavonoids for DPPH, $ABTS^+$,· OH, and $O_2^-$were, respectively, 92.17 ± 1.33%, 92.49 ± 1.55%, 94.85 ± 1.39%, and 86.01 ± 1.21%. In terms of reducibility, the absorbance of *Sanghuangporus vaninii* flavonoids at 0.008 mg/ml was 2.65 ± 0.04, which was higher than that of positive control $V_C$ at 0.90 ± 0.05. *Sanghuangporus vaninii* flavonoids at 200 μg/mL could effectively inhibit the $NO_2^-$ content in vitro to 9.38 ± 0.23 pg/ml. The inhibitory rates of *Sanghuangporus vaninii* flavonoids on *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* were 79.48 ± 1.57%, 85.64 ± 1.22%, and 80.29 ± 0.04%. When the concentration of *Sanghuangporus vaninii* flavonoids was 200 μg/mL, the concentration of NO was 9.38 ± 0.23 pg/ml.

## 1. Introduction

*Sanghuangporus vaninii* (*S. vaninii*), also known as SangHuangGu in China, is the fruiting body of the *Polyporales* family. It has served as a functional food and an exemplary source of natural medicines. As a medicinal mushroom, a large number of chemical constituents have been isolated and obtained from *Phellinus igniarius*. The main chemical components include polysaccharides [1], flavonoids [2], protein [3], amino acids [4], neuraminidase inhibitors [5], anticholinesterase, protocatechuic acid, protocatechuic aldehyde, caffeic acid, ellagic acid, histidine, divaricate, bacteriocin A, and phosphinic acid B [6]. It has many pharmacological effects, including antitumor [7], anticancer [8], immunomodulatory [9], antidiabetic [10], hypoglycemic [11], anti-inflammatory [12], and antioxidative effects [13]. Flavonoid is a secondary metabolite of plant polyphenol, most of which exist in the form of glycosides in plants, and a few of which exist in the form of free aglycones (oxyglycosides and carbon glycosides). Flavonoids are natural products with significant biological activities, including antiviral [14], antiallergic [15], lipid-lowering [16], antibacterial [17], and anti-inflammatory effects [18] as well as prevention of cardiovascular and cerebrovascular diseases [19]. At present, there is no systematic study on the biological activity of flavonoids from *S. vaninii* fruit bodies, so this study evaluates the biological activity of flavonoids from *S. vaninii* fruit bodies *in vitro*.

At present, the method of extracting plant flavonoids includes conventional solvent extraction, Soxhlet extraction, heat reflux extraction, continuous-flow microextraction, and

enzyme-assisted extraction which have already been reported in the literature [20–22]. Conventional extraction methods for the solvent extraction of plant materials have been proved to have the drawbacks of longer extraction time, higher temperature, and lower extraction efficiency; especially in the extraction process, the amount of organic solvent is large, the energy consumption is high, and the natural molecular structure is easily destroyed [23], and many organic solvents cause ionization, hydrolysis, oxidation and deactivation of flavonoids [24]. It should be noted that some organic solvents cause serious environmental problems and are hazardous to human health. Compared with ultrasound-assisted extraction technology, supercritical carbon dioxide extraction has high cost and is not commonly used. Ultrasound-assisted extraction can quickly induce acoustic cavitation of plant cell wall so as to enhance the extraction of bioactive constituents from plant matrices [25]. These technologies are quick, simple, ecofriendly, and low solvent consumption, which can improve the quality and quantity of natural plant-derived extracts [26]. High-power sonication technology is based on mechanical waves at high frequencies that lead to the cavitation phenomenon. Cavitation is the phenomenon that occurs when ultrasonic waves pass through a liquid and generate cavities that then collapse under specific conditions. This phenomenon can inactivate enzymes, disrupt cell walls and cause an increased contact between the solvent and the cell contents, providing better extraction of compound [27, 28].

Ultrasound-assisted extraction of *S. vaninii* fruit bodies flavonoids improves the extraction amount of *S. vaninii* fruit bodies flavonoids, which lays a good foundation for the subsequent application of *S. vaninii* fruit bodies flavonoids. Moreover, it has the characteristics of scavenging free radicals, anti-inflammatory, and antibacterial and can be used as a natural antioxidant and antibacterial agent to add to cosmetics and foods and can also be used as an additive for health care products.

## 2. Materials and Methods

*2.1. Reagents and Strains.* Standard of rutin, 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and ascorbic acid (Vc) were purchased from Shanghai Macklin Biochemical Co., Ltd. $AlCl_3$, NaOH, $NaNO_2$, absolute ethyl alcohol, and $FeSO_4$ were purchased from China National Pharmaceutical Group Corporation. All other chemicals and reagents are of analytical grade.

*Staphyloccocus aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were obtained from American Type Culture Collection. Murine macrophage cell line RAW 264.7 was purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China).

*2.2. Methods*

*2.2.1. Determination of Total S. vaninii Fruit Bodies Flavonoids.* In this study, flavonoid content was measured by $AlCl_3$ method [29]. In short, powdered samples of 0.5 g in 20 mL ethanol and extracted by ultrasonic for 1 h. 0.1 mL of extracting solution was mixed with 4.9 mL of ethanol, 0.3 mL 5% of $NaNO_2$ was added and let it stand for 5 min. 0.3 mL 10% of $AlCl_3$ are added and let it stand for 6 min. Then, 2 mL 4% of NaOH was added and constant volume to 10 mL and let it stand for 15 min. The absorbance was determined by ultraviolet spectrophotometry at 354 nm. The rutin equivalents express flavonoid content of the rutin calibration curve. Extraction capacity of *S. vaninii* was calculated using the following equation:

$$\text{The extraction capacity } (T) = \frac{\text{CVf}}{m}, \quad (1)$$

where $T$ is the extraction capacity of flavonoids *S. vaninii* (mg/g); $C$ is the flavonoids concentration in from the calibration curve (mg/mL); $V$ is the volume of the solvent used in the extraction (mL); $f$ is multiple of dilution; and $m$ represents the weight of the sample used (g).

*2.2.2. Optimization of Extraction Technology of Flavonoids from S. vaninii Fruit Bodies.* Extracted flavonoid was carried out to examine the extraction capacity of *S. vaninii* fruit bodies with different ultrasonic times (20, 40, 60, 80, and 100 min), different extraction temperatures (40, 50, 60, 70, and 80°C), different solid-to-liquid ratios (1 : 30, 1 : 40, 1 : 50, 1 : 60, and 1 : 70 g/mL), and different ethanol concentrations (50, 60, 70, 80, and 90%). Extraction capacity of *S. vaninii* fruit bodies flavonoids was calculated using equation (1).

*2.2.3. Response Surface Methodology Experiment*

*Response Surface Optimization Design*. According to the results of single factor investigation, the extraction capacity was used as the response value, and the Box–Behnken design was used to conduct the 4-factor 3-level experiment.

*2.2.4. Maceration Extract (ME).* Flavonoid was extracted from *S. vaninii* fruit bodies at the optimal temperatures, solid-to-liquid ratio, and ethanol concentration without using ultrasonic in 3 h. The supernatant was acquiring by centrifugation at 8000 rpm for 10 min. Extraction capacity of *S. vaninii* fruit bodies flavonoids was calculated using equation (1).

*2.2.5. Percolation Extraction (PE).* Flavonoid was extracted from *S. vaninii* fruit bodies at the optimal temperatures, solid-to-liquid ratio, and ethanol concentration without using ultrasonic in 3 h. The sample was shifted to a percolator to extract with ethanol for 12 h. The supernatant was acquired by centrifugation at 8000 rpm for 10 min. Extraction capacity of *S. vaninii* fruit bodies was calculated using equation (1).

*2.2.6. Anti-Free Radical Activity*

*(1) DPPH Radical Scavenging Capacity Assay*. The radical scavenging capacity of the flavonoid of *S. vaninii* fruit bodies against DPPH was conducted using the method described by Feng et al. [30] with some modifications.

$$\text{DPPH radical scavenging rate} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\%, \quad (2)$$

where $A_0$ is the absorbance of ethanol and DPPH mixture, $A_1$ is the absorbance of sample and DPPH mixture, and $A_2$ is the absorbance of sample and ethanol mixture.

*(2) $ABTS^+$ Radical Cation Scavenging Capacity Assay*. The radical scavenging capacity of the flavonoid of *S. vaninii* fruit bodies against $ABTS^{\cdot+}$ was conducted using the method described by Xie et al. [31] with some modifications. 0.1 mL aliquot of the sample solution was mixed with 3.9 mL solution of $ABTS^{\cdot+}$ solution (absorbance of 0.7). The mixture was shaken vigorously and incubated for 6 min in the dark at 20°C. The absorbance was measured at 734 nm using a UV-vis spectrophotometer. $V_C$ was used as positive controls in this assay. The radical scavenging capacity assay was calculated using the following equation:

$$ABTS^+ \text{ radical scavenging rate} = \frac{A_0 - A_1}{A_0} \times 100\%, \quad (3)$$

where $A_0$ and $A_1$ are the absorbances of the blank sample at $t = 0$ and the reaction solution at $t = 6$ min, respectively.

*(3) Hydroxyl Radical (·OH) Scavenging Capacity Assay*. The radical scavenging capacity of the flavonoid of *S. vaninii* fruit bodies against hydroxyl was conducted using the method described by Xia et al. [32] with some modifications. A 0.5 mL aliquot of the sample solution was mixed with 1.5 mL of sodium phosphate buffer (150 mM, pH 7.4) containing 0.5 mL of 6 mM $FeSO_4$, 0.5 mL of 6 mM sodium salicylate, and 0.5 mL of 6 mM $H_2O_2$. The mixture was shaken vigorously and incubated for 1 h at 37°C. The absorbance was measured at 517 nm using a UV-vis spectrophotometer. $V_C$ was used as positive controls in this assay. The radical scavenging capacity assay was calculated using the following equation:

$$\cdot OH \text{ radical scavenging rate} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\%, \quad (4)$$

where $A_0$, $A_1$, and $A_2$ are the absorbances of the control, samples, and samples without $H_2O_2$, respectively.

*(4) Superoxide Radical ($O_2^-\cdot$) Scavenging Capacity Assay*. The radical scavenging capacity of the flavonoid of *S. vaninii* fruit bodies against superoxide anion radical was conducted using the method described by Zhu et al. [33] with some modifications. A 0.5 mL aliquot of the sample solution was mixed with 0.5 mL of a 4.5 mL of Tris–HCl buffer (50 mM, pH 8.2). The mixture was shaken vigorously and incubated for 20 min at 37°C and 0.5 mL of pyrogallol (3 mM) was then added. The absorbance was measured at 325 nm using a UV-vis spectrophotometer. $V_C$ was used as positive controls in this assay. The radical scavenging capacity assay was calculated using the following equation:

$$O_2^- \cdot \text{ radical scavenging rate} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\%, \quad (5)$$

where $A_0$, $A_1$, and $A_2$ are the absorbances of the control, samples, and samples without pyrogallol, respectively.

*2.2.7. Reducibility Assay*. The reducibility was determined according to the $Fe^{3+}$ reduction ("reducing power") method [34]. A 0.2-mL aliquot of the sample solution was mixed with a 0.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) containing 0.5 mL of 1% $K_3[Fe(CN)_6]$. The mixture was shaken vigorously and incubated for 20 min at 50°C and 0.2 mL of 10% $C_2HCl_3O_2$ was then added. A 0.2-mL aliquot was mixed with 0.2 mL of distilled water and 0.2 mL of 0.1% of $FeCl_3$. The absorbance was measured at 700 nm using a UV-vis spectrophotometer. $V_C$ was used as positive controls in this assay.

*2.2.8. Nitrite ($NO_2^-$) Scavenging Capacity Assay*. Scavenging capacity of the flavonoid of *S. vaninii* fruit bodies against nitrite was conducted using the method described by Kim et al. [35] with some modifications. A 2 mL aliquot of the sample solution was mixed with a 1 mL of 0.02 mg/mL nitrite solution. The mixture was shaken vigorously and incubated for 30 min at 30°C and 2 mL of 0.4% p-aminobenzene sulfonic acid solution and let it stand for 5 min. Then, 1 mL 0.2% of naphthalene ethylenediamine hydrochloride was added and constant volume to 25 mL and let it stand for 15 min. The absorbance was measured at 540 nm using a UV-vis spectrophotometer. $V_C$ was used as positive controls in this assay. The radical scavenging capacity assay was calculated using the following equation:

$$NO_2^- \text{ scavenging rate} = \left(1 - \frac{A_1}{A_0}\right) \times 100\%, \quad (6)$$

where $A_0$ and $A_1$ are the absorbances of the control and samples, respectively.

*2.2.9. Biological Activity*

*(1) Determination of Antibacterial Activity In Vitro*. The bacteriostasis of flavonoid was determined by the Filandr method [36]. The typical standard single bacterial colony was selected and inoculated in normal saline at 37°C until the absorbance value $OD_{600}$ about 0.5. The culture medium prepared flavonoid solutions with different concentrations, after sample solution and the bacterial solution coculturing at 37°C for 12 h, measured $OD_{600}$ value, the bacterial inhibition was recorded with bacteriostatic capacity assay.

*(2) Determination of Anti-Inflammatory Activity In Vitro*. The concentration of NO was quantified with the reported Griess reagent method [37]. The Raw264.7 mouse macrophages were planted in 96-well plates at a density of $2 \times 10^4$ cells/well. After the cells adhered to the wall, the culture medium was discarded and 100 $\mu$L of medium was added. The blank medium was added in the control group, and LPS (final concentration of 100 ng/mL) was added in the positive control and cultured in a $CO_2$ incubator at 37°C for

24 h. The cell supernatant was reacted with Griess reagent and measured at 540 nm.

*(3) Cell Viability*. MTT assay was used to detect the toxicity of Folium isatidis TFE to RAW 264.7 cells according to the report of Tian et al. [37]. Cells were incubated and grouped according to the method of the anti-inflammatory activity in vitro. Then, the supernatant was reacted with MTT reagent for 4 h. Finally, after the supernatant discarded, the residual was dissolved with 150 $\mu$l of dimethyl sulfoxide and detected by a microplate reader at 490 nm.

## 3. Results and Discussion

### 3.1. Optimization of Extraction Process by Response Surface Method

*3.1.1. Single Factor Investigation.* The effect of extraction temperature, ethanol concentration, solid-to-liquid ratio, and ultrasonic time on the flavonoid extraction capacity was investigated, and the results are shown in Figure 1. Extraction capacity of flavonoid increased significantly as the temperature increased from 40°C to 60°C and decreased significantly at 80°C (Figure 1(a)). The highest extraction capacity (77.07 ± 0.83 mg/g) was obtained at the temperature of 60°C. The trend is similar to that reported by Amiri et al. [38]. Heat treatment can effectively break the walls of cells, making it more convenient for effective substances to diffuse out of cells. At the same time, with the increase of the temperature of the solution system, the thermal motion of solute molecules is accelerated, and the diffusion coefficient is increased, while the viscosity and surface tension of the solution are reduced and the mass transfer resistance is reduced [39], which the extraction capacity of flavonoid is increased. On the other hand, excessive extraction temperature degrades flavonoid compounds and reduces the solvent density [40], which leads to the extraction capacity of flavonoid being decreased. Extraction capacity of flavonoid increased significantly as the ethanol concentration increased from 50% to 80% and decreased significantly at 90% (Figure 1(b)). The highest extraction capacity (82.71 ± 1.03 mg/g) was obtained at the ethanol concentration of 80%. Flavonoid is active and its hydroxyl can form hydrogen bonds with ethanol, modifying its density, viscosity, and dielectric constant, and the extraction capacity of flavonoid is increased. A higher water content in the solvent will increase the extraction of contaminant solutes at the expense of flavonoid compounds [41]. Extraction capacity of flavonoid increased significantly as the solid-to-liquid ratio decreased from 1 : 30 to 1 : 60 and decreased significantly at 1 : 70 (Figure 1(c)). The highest extraction capacity (87.83 ± 0.88 mg/g) was obtained at the solid-to-liquid ratio of 1 : 60. When a higher solvent-to-solid ratio is applied, the concentration gradient between the solid and the bulk of the liquid being the driving force for mass transfer is greater. It also attributed this positive effect on the improvement of the contact area between solvent and sample at greater solvent ratio, and as a result, the flavonoid can readily diffuse out from the plant cells [42]. Excessive water content in the solvent will increase the extraction of contaminant solutes at the expense of flavonoid, which leads to the extraction capacity of flavonoid being decreased. The trend is similar to that reported by Ji et al. [43]. Extraction capacity of flavonoid increased significantly as the ultrasonic time increased from 20 min to 40 min and decreased significantly at 60 min (Figure 1(d)). The highest extraction capacity (87.88 ± 0.83 mg/g) was obtained at the ultrasonic time of 40 min. Ultrasound can enhance mass transfer rate, destroy the plant tissues, and weaken forces between the solute and the matrix via a series of complex mechanisms, such as mechanical fluctuation, thermal effect, and acoustic cavitation [44]. Excessive exposure to ultrasound treatment for a longer sonication time resulted in the degradation of flavonoids in the extract [40, 45].

*3.1.2. Response Surface Model.* BBD, with four independent variables, at three levels (high, low, and middle) was chosen to investigate the extraction capacity of flavonoid, and the data generated used for model fitting (Table 1). Based on the results, the extraction capacity of flavonoid (Y) and the coded values of the study factors are shown in the following equation:

$$\begin{aligned} Y = {} & 94.85 - 1.71A + 2.44B - 0.64C + 1.32D \\ & + 1.8 \times 10^{-3} AB - 1.98AC - 3.19AD - 0.27BC \\ & - 1.90BD + 0.98CD - 7.58A^2 - 5.41B^2 - 7.58C_2 \\ & - 12.33D^2. \end{aligned} \tag{7}$$

The ANOVA results are summarized in Table 2. Based on the obtained $F$ value and $P$ value of the models, it was revealed that the models are significant at less than a 0.05% level. In the evaluation of variance analysis, the higher the value of $F$ and the lower the value of $P$, the more adaptable the model would be [46]. In Table 2, the model is extremely significant, AB is extremely significant in the primary term, $D$ is significant, AD is extremely significant in the interactive term, and AC BD is significant. Lack of Fit is not significant. $R$ square is 0.9727, which shows that the fitting degree of the model is also significant and the model could be well used to explain the temperature, solid-to-liquid ratio, ultrasonic min and ethanol concentration as the extraction capacity of the conditions of the selected factors. According to the $P$ value, A, B, $D$, AC, AD, BD, $A^2$, $B^2$, $C^2$, and $D^2$ variables are statistically significant ($P < 0.05$), indicating that the response value and the independent variable were not simply one time. The order of the effect of three factors on the extraction capacity of flavonoid is: $B > A > D > C$. The order of the effect of the mutually factors on the extraction capacity of flavonoid is AD > AC > BD > CD > BC > AB.

3D diagram of response surface methodology provided useful information about the interaction between two factors on flavonoid capacity with *S. vaninii* (Figure 2). 3D diagram shows an arch shape from one point to the highest point; flavonoid extraction capacity increases with temperature, solid-to-liquid ratio, ultrasonic time, and ethanol concentration and

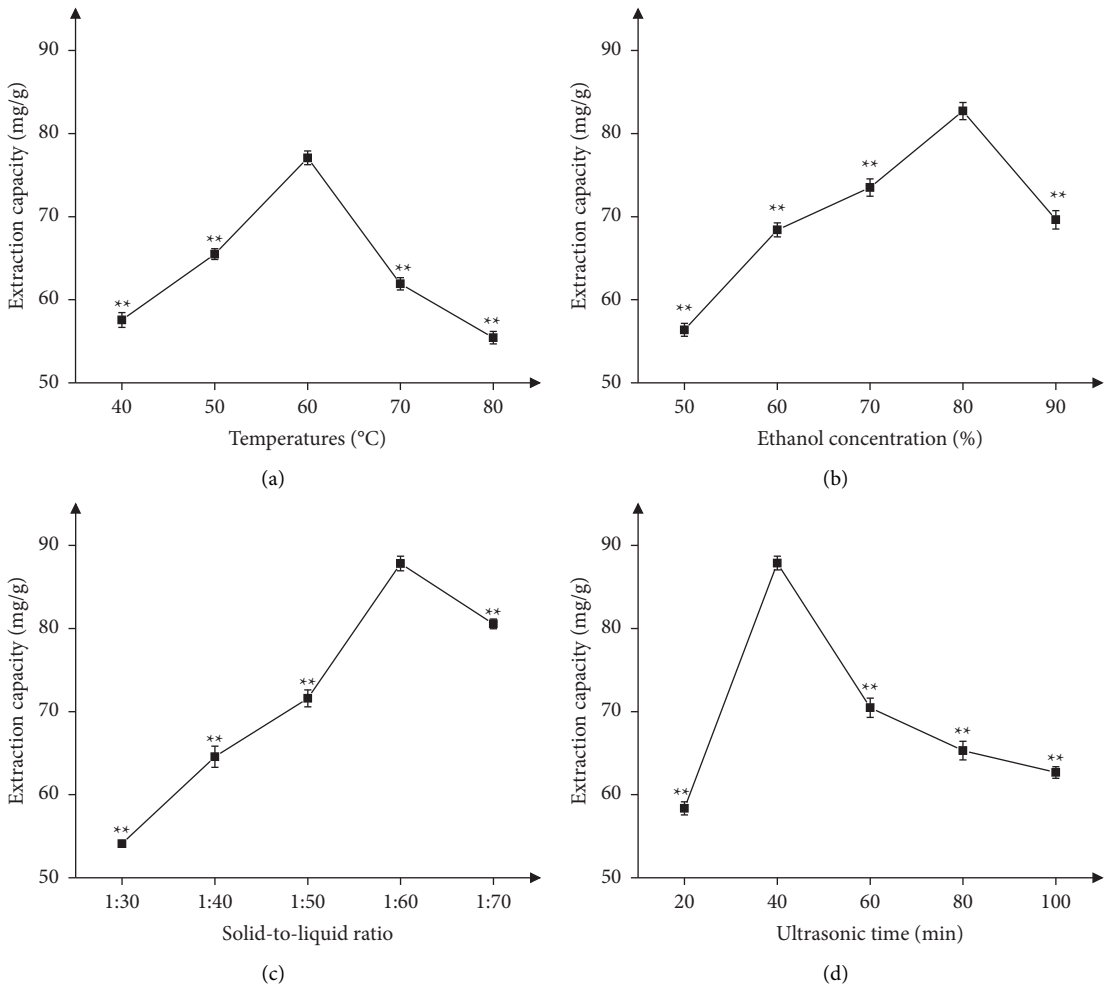


Figure 1: Effect of different factors on flavonoid capacity: (a) temperature, (b) ethanol concentration, (c) solid-to-liquid-ratio, and (d) ultrasonic time, where the significance in that figure is based on the highest value.

then decreases in parabolic manner [39]. The steeper the slope of the response surface, the more inclined, the more elliptical shape of the contour, and the more significant the interaction between the two factors [47, 48], which is consistent with Table 2. In evaluating the main factors and their interactions influence, economically optimal conditions can be achieved. The optimum technological conditions were determined as follows: temperature of 67°C, solid-to-liquid ratio of 1 : 62, ultrasonic min of 37 min, and ethanol concentration of 79%. Under the optimum reaction condition, RSM predicted the flavonoid maximum extraction capacity is $90.17 \pm 0.82$ mg/g. According to the actual conditions, modify the process parameters, the corrected coefficient follows: temperature of 70°C, solid-to-liquid ratio of 1 : 60, ultrasonic min of 40 min, and ethanol concentration of 80%. It was validated by experimental data generated ($90.24 \pm 0.89$ mg/g). The obtained results revealed an acceptable agreement between the experimental values and the predicted values; hence, the model can effectively predict the responses. The trend is similar to that reported by Chakraborty et al. [49].

*3.2. Comparison of Extraction Methods.* To objectively evaluate that ultrasonic extraction could significantly improve the flavonoid content in *S. vaninii*. Ultrasonic extraction approach was compared with those of the other approaches, including ME and PE. As shown in Figure 3, the extraction capacity of flavonoids from *S. vaninii* by ultrasonic extraction, ME, and PE was $90.24 \pm 0.89$ mg/g, $35.61 \pm 1.21$ mg/g, and $39.24 \pm 0.72$ mg/g, respectively. Compared with the ME and PE, ultrasonic was not only able to save time but also increased flavonoid content by nearly 2.5 times. The increased extraction capacity is attributed to the phenomenon called acoustic cavitation caused by the propagation of ultrasound pressure waves [21], which can disrupt and penetrate the cell wall and accelerate the release of compounds [26, 50], which facilitate and expedite the effect of mass transfer. According to the results, it could be concluded that ultrasonic extraction method was an efficient extraction method to extract flavonoids, and the trend is similar to that reported by Lei et al. [51].

Table 1: Experimental design and extraction results.

| Test number | A: temperature (°C) | B: solid-to liquid ratio | C: ultrasonic min (min) | D: ethanol concentration (%) | Extraction capacity (mg/g) |
|---|---|---|---|---|---|
| 1 | 70 | 1 : 60 | 20 | 80 | 80.36 |
| 2 | 60 | 1 : 50 | 40 | 90 | 76.30 |
| 3 | 60 | 1 : 60 | 60 | 70 | 71.81 |
| 4 | 60 | 1 : 70 | 40 | 70 | 79.30 |
| 5 | 60 | 1 : 60 | 40 | 80 | 95.59 |
| 6 | 50 | 1 : 60 | 60 | 80 | 80.59 |
| 7 | 50 | 1 : 60 | 40 | 90 | 82.65 |
| 8 | 60 | 1 : 60 | 40 | 80 | 96.03 |
| 9 | 60 | 1 : 70 | 40 | 90 | 78.64 |
| 10 | 60 | 1 : 60 | 40 | 80 | 94.83 |
| 11 | 50 | 1 : 50 | 40 | 80 | 81.79 |
| 12 | 70 | 1 : 60 | 40 | 70 | 74.66 |
| 13 | 60 | 1 : 50 | 20 | 80 | 79.30 |
| 14 | 70 | 1 : 60 | 40 | 90 | 70.42 |
| 15 | 60 | 1 : 70 | 60 | 80 | 84.94 |
| 16 | 60 | 1 : 50 | 40 | 70 | 69.36 |
| 17 | 60 | 1 : 60 | 20 | 70 | 76.78 |
| 18 | 50 | 1 : 60 | 40 | 70 | 74.13 |
| 19 | 50 | 1 : 60 | 20 | 80 | 78.42 |
| 20 | 60 | 1 : 50 | 60 | 80 | 80.79 |
| 21 | 60 | 1 : 60 | 40 | 80 | 94.74 |
| 22 | 60 | 1 : 60 | 20 | 90 | 77.42 |
| 23 | 60 | 1 : 70 | 20 | 80 | 84.53 |
| 24 | 50 | 1 : 70 | 40 | 80 | 85.60 |
| 25 | 60 | 1 : 60 | 40 | 80 | 93.03 |
| 26 | 70 | 1 : 50 | 40 | 80 | 79.43 |
| 27 | 60 | 1 : 60 | 60 | 90 | 76.40 |
| 28 | 70 | 1 : 60 | 60 | 80 | 74.60 |
| 29 | 70 | 1 : 70 | 40 | 80 | 83.25 |

Table 2: Analysis of variance of regression mode.

| Sources | Sum of squares | d$f$ | Mean square | $F$ value | $P$ value Prob > $F$ | |
|---|---|---|---|---|---|---|
| Model | 1532.38 | 14 | 109.46 | 35.69 | <0.0001 | ** |
| A | 34.99 | 1 | 34.99 | 11.41 | 0.0045 | ** |
| B | 71.55 | 1 | 71.55 | 23.33 | 0.0003 | ** |
| C | 4.93 | 1 | 4.93 | 1.61 | 0.2254 | |
| D | 20.79 | 1 | 20.79 | 6.78 | 0.0208 | * |
| AB | $1.29 \times 10^{-5}$ | 1 | $1.29 \times 10^{-5}$ | $4.23 \times 10^{-6}$ | 0.9984 | |
| AC | 15.73 | 1 | 15.73 | 5.13 | 0.0399 | * |
| AD | 40.69 | 1 | 40.69 | 13.27 | 0.0027 | ** |
| BC | 0.29 | 1 | 0.29 | 0.09 | 0.7638 | |
| BD | 14.45 | 1 | 14.45 | 4.71 | 0.0476 | * |
| CD | 3.88 | 1 | 3.88 | 1.27 | 0.2796 | |
| $A^2$ | 372.71 | 1 | 372.71 | 121.53 | <0.0001 | ** |
| $B^2$ | 190.10 | 1 | 190.10 | 61.99 | <0.0001 | ** |
| $C^2$ | 372.27 | 1 | 372.27 | 121.39 | <0.0001 | ** |
| $D^2$ | 986.89 | 1 | 986.89 | 321.81 | <0.0001 | ** |
| Residual | 42.93 | 14 | 3.07 | | | |
| Lack of fit | 37.67 | 10 | 3.77 | 2.86 | 0.1613 | |
| Pure error | 5.26 | 4 | 1.32 | | | |
| Cor total | 1575.32 | 28 | | | | |
| Coefficient | | | $R^2 = 0.9727$ | | | |

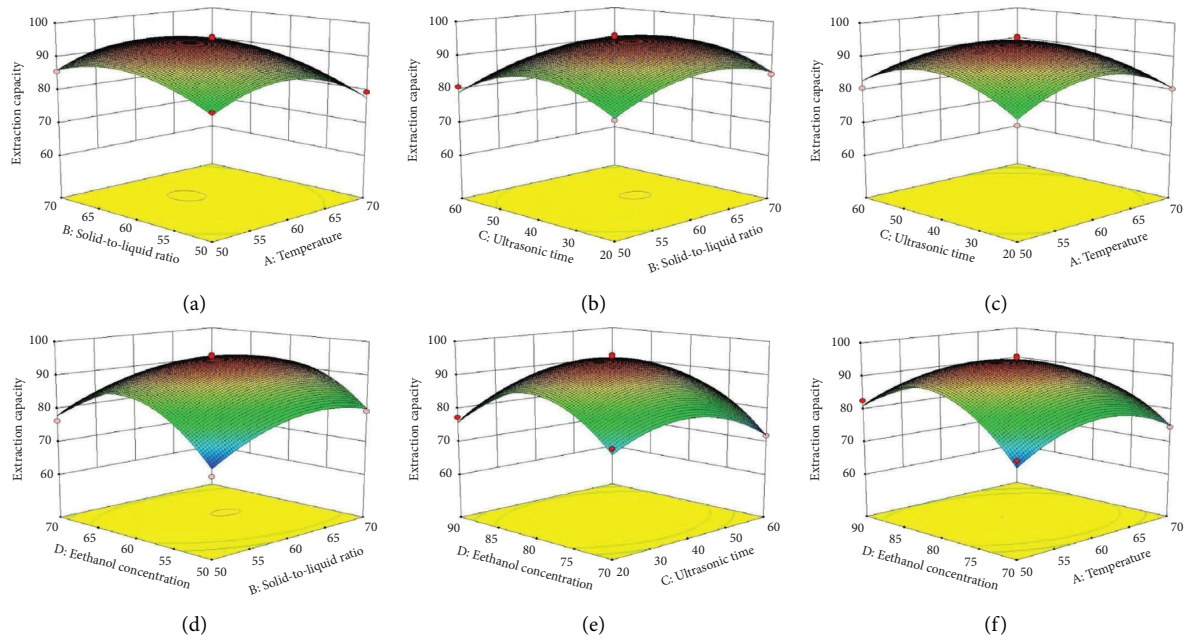


Figure 2: Response surface plots effects of different factors combination on flavonoid extraction capacity.

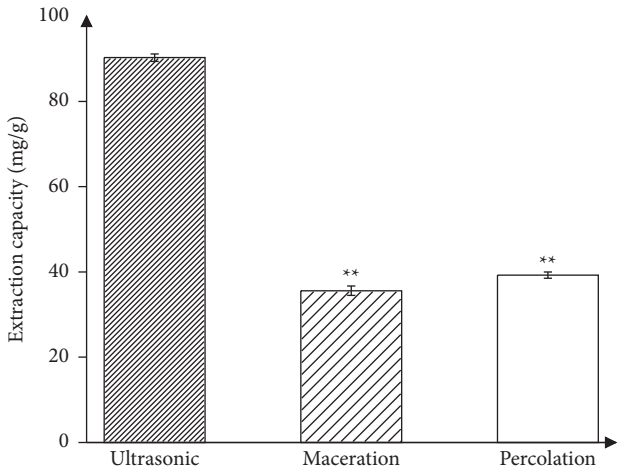


Figure 3: Extraction of flavonoid from *S. vaninii* by different methods.

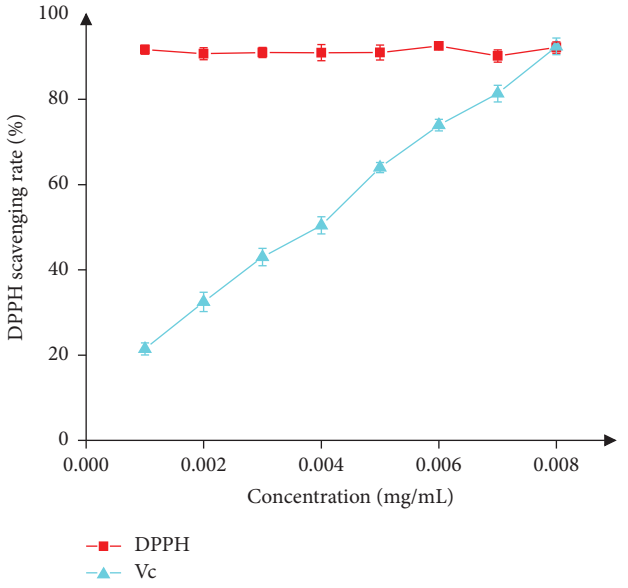


Figure 4: DPPH radical scavenging activity of flavonoid from *S. vaninii* fruit bodies.

### 3.3. Antioxidant Activity of Flavone

*3.3.1. DPPH Radical Scavenging Activity of Flavonoid from S. vaninii Fruit Bodies.* An increase of flavonoids in concentration has nothing to do with an increase in DPPH radical scavenging rates in Figure 4. The DPPH scavenging rate remains at around $92.17 \pm 1.33\%$. Different from $V_C$ (the maximum scavenging rate is $92.44 \pm 1.98\%$), there was no concentration-dependent effect of flavonoid from *S. vaninii* on the scavenging rate of DPPH. Structural features of flavonoids required for efficient radical scavenging DPPH include an ortho-dihydroxy structure in the B ring (for electron delocalization). The number and position of phenolic hydroxyl groups in flavonoids are the key factors affecting their antioxidant activity [32]. *S. vaninii* flavonoid with 4′ OH and ortho di-OH groups in the B ring. The existence of this structure endows *S. vaninii* flavonoid with DPPH free radical scavenging power and can result in differences in antioxidant activity due to different structures. An ortho-dihydroxy group in the B-ring allowed flavonoid phenoxyl radicals to participate in electron delocalization, leading to dislocation of an electron in the B ring that exhibits antioxidant activity [52]. The clearance capacity of the compound is positively correlated with the B-ring –OH number within a certain range.

*3.3.2. $ABTS^+$ Radical Scavenging Ability of Flavonoid from S. vaninii Fruit Bodies.* *S. vaninii* flavonoid samples were

tested for ABTS $^{+}$ radical scavenging rates. With the increase of flavonoid contents in preparing samples, the $ABTS^{+}$ radical scavenging rate increased in Figure 5. There was a concentration-dependent effect of flavonoid from *S. vaninii* on the scavenging rate of $ABTS^{+}$ radical. The trend is similar to that reported by Sillero et al. [53]. The maximal scavenging rate of $ABTS^{+}$ radical of *S. vaninii* flavonoid and positive control $V_C$ to a was $92.49 \pm 1.55\%$ and $94.69 \pm 1.62\%$, with $IC_{50}$ values of 0.0221 mg/mL and 0.0267 mg/mL, respectively. The lower $IC_{50}$ value of the samples, the higher the antioxidant activity. The antioxidant properties of flavonoids from *S. vaninii* were higher than that of $V_C$. The compound containing catechol structure in the B ring reacts with the free radical to form a relatively stable conjugated semiquinone-type free radical [54, 55], which can form a hydrogen bond with the adjacent hydroxyl group, thereby reducing the energy of the molecular system and the $ABTS^{+}$ are scavenged. Moreover, that semiquinone radical can form o-benzoquinone through resonance, so that the unpaired electron density has more distribution on the *o* atom at the ortho position, the internal energy is small, and the radical is more stable.

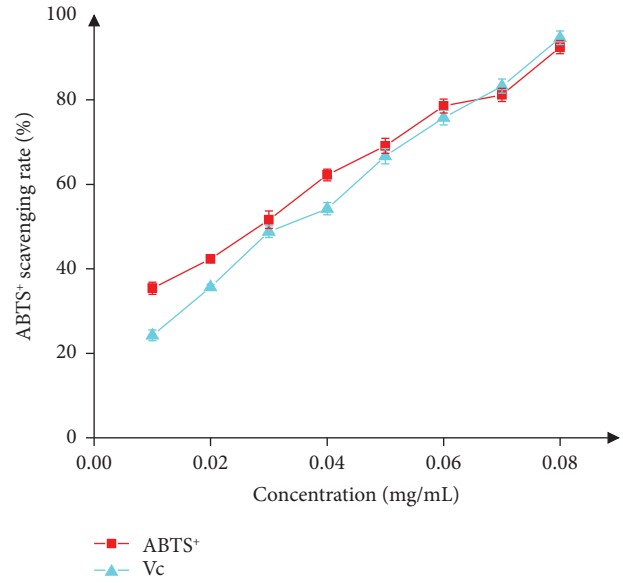


Figure 5: $ABTS^{+}$ radical scavenging activity of flavonoid from *S. vaninii* fruit bodies.

*3.3.3. ·OH Radical Scavenging Ability Flavonoid from S. vaninii Fruit Bodies. S. vaninii* flavonoid samples were tested for ·OH radical scavenging rates. With the increase of flavonoid contents in preparing samples, the ·OH radical scavenging rate increased in Figure 6. There was a concentration-dependent effect of flavonoid from *S. vaninii* on the scavenging rate of ·OH radical. The trend is similar to that reported by Wen et al. [56]. The maximal scavenging rate of ·OH radical of *S. vaninii* flavonoid and positive control Vc to a was $94.85 \pm 1.39\%$ and $91.02 \pm 1.71\%$, with $IC_{50}$ values of 0.0033 mg/mL and 0.0056 mg/mL, respectively. The antioxidant properties of flavonoids from *S. vaninii* were higher than that of Vc. Flavonoid has a 2,3-double bond in conjugation with a 4-oxo functional group in the C ring (providing electron delocalization from the B ring). The ortho-dihydroxy structure on the B ring is also a key group of the flavonoid compounds for resisting ·OH. But its activity is slightly weaker than the hydroxyl group at the $C_3$ position of the C ring [57]. 5-OH and 7-OH, if present in the molecule, also play a role in the free radical scavenging process of the compound [58].

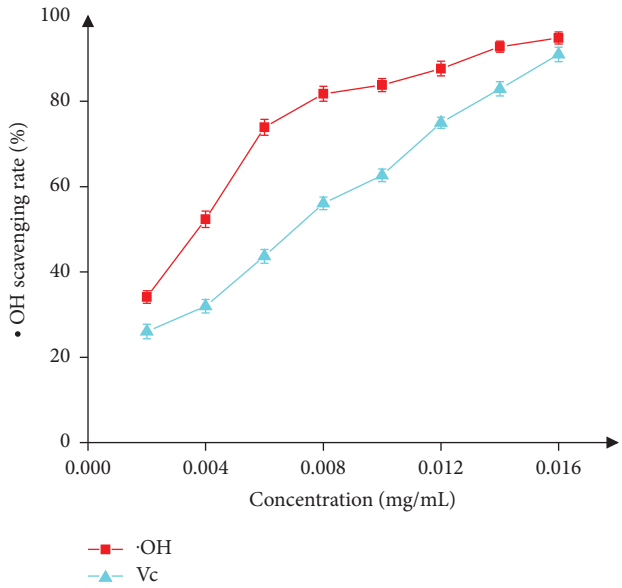


Figure 6: ·OH radical scavenging activity of flavonoid from *S. vaninii* fruit bodies.

*3.3.4. $O_2^{-}$· Radical Scavenging Ability of Flavonoid from S. vaninii Fruit Bodies. S. vaninii* flavonoid samples were tested for $O_2^{-}$· radical scavenging rates. With the increase of flavonoid contents in preparing samples, the $O_2^{-}$· radical scavenging rate increased in Figure 7. There was a concentration-dependent effect of flavonoid from *S. vaninii* on the scavenging rate of $O_2^{-}$· radical. The trend is similar to that reported by Liu et al. [59]. The maximal scavenging rate of $O_2^{-}$· radical of *S. vaninii* flavonoid and positive control Vc to a was $86.01 \pm 1.21\%$ and $89.75 \pm 1.49\%$, with $IC_{50}$ values of 0.0360 mg/mL and 0.0307 mg/mL, respectively. $O_2^{-}$· causes cell damage by first transforming into hydroxyl radicals and then exerting oxidation. $C_3$ hydroxyl group on B ring of flavonoid compound and C ring $C_2$, $C_3$ double bond structure although have certain activity [60], can scavenge $O_2^{-}$·. Interaction between flavonoids and free radicals is related to their charge distribution [61]. The more positive charge that hydroxyl hydrogen atoms of flavonoid compound had, the more easily they would be attacked by the atoms with a negative charge of $O_2^{-}$·, and the stronger the antioxidant activity of the compound would be.

*3.3.5. Reducibility of Flavonoid from S. vaninii Fruit Bodies. S. vaninii* flavonoid samples were tested for reducibility. With the increase of flavonoid contents in preparing

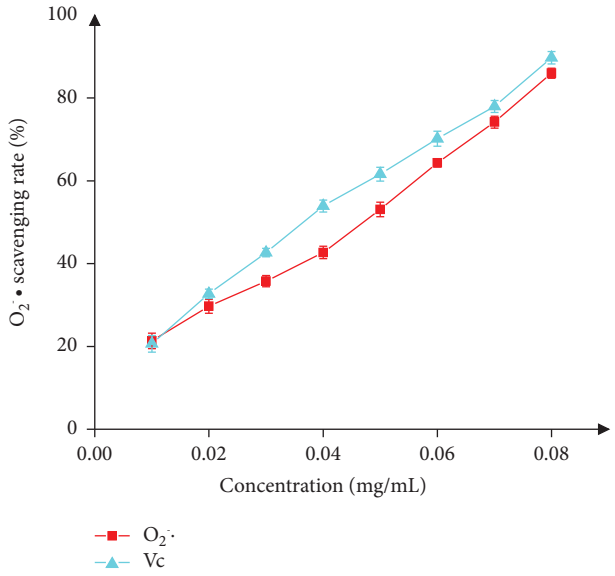


Figure 7: $O_2^{-}$· radical scavenging activity of flavonoid from *S. vaninii* fruit bodies.

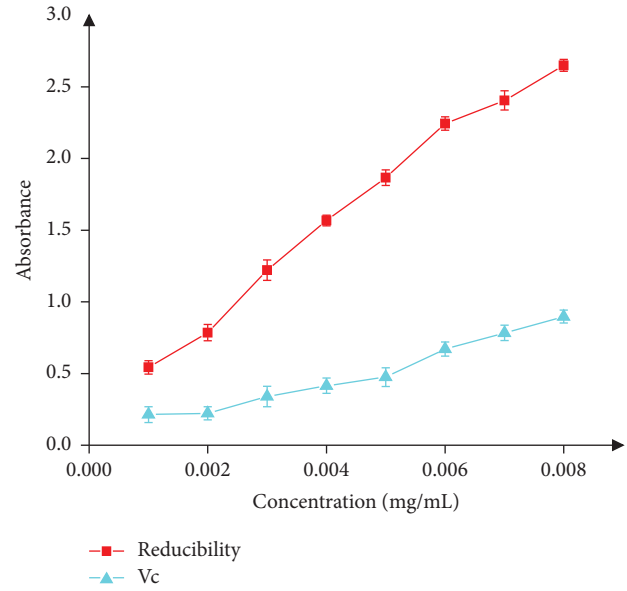


Figure 8: Reducibility of flavonoid from *S. vaninii* fruit bodies.

samples, the reducibility increased in Figure 8. There was a concentration-dependent effect of flavonoid from *S. vaninii* on the reducibility. The trend is similar to that reported by Mohammed et al. [62]. The maximal absorbance of *S. vaninii* flavonoid and positive control Vc to a was $2.65 \pm 0.04$ and $0.90 \pm 0.05$. The interaction between flavonoid compounds and metal ions is related to their charge distribution. Free radicals were more likely to attack the positions where the positive charge distribution of the compound was more extensive. More positive charges the hydroxyl hydrogen atoms in the flavonoid compound had, the more likely they were attacked by the atoms with negative charges on the free radical [63]. Thus, the antioxidant activity of the compound was stronger. The phenolic hydroxyl O atom of the flavonoid compound can provide a lone pair to the *p* orbit of the metal ion and form an M–O bond [64]. When the number of charges distributed on the oxygen atom is larger, the stable M–O formation with metal ions will be favored. Therefore, the phellinus igniarius flavonoid has good reducibility.

*3.4. $NO_2^{-}$ Scavenging Ability of Flavonoid from S. vaninii Fruit Bodies.* *S. vaninii* fruit bodies flavonoid samples were tested for $NO_2^{-}$ scavenging rates. With the increase of flavonoid contents in preparing samples, the $NO_2^{-}$ scavenging rate increased in Figure 9. There was a concentration-dependent effect of flavonoid from *S. vaninii* on the scavenging rate of $NO_2^{-}$. The trend is similar to that reported by Liu et al. [65]. The maximal scavenging rate of $NO_2^{-}$ of *S. vaninii* fruit bodies flavonoid and positive control Vc to a was $90.63 \pm 2.20\%$ and $43.88 \pm 1.69\%$, with $IC_{50}$ values of 0.0146 mg/mL and 0.1734 mg/mL, respectively. The scavenging $NO_2^{-}$properties of flavonoids from S. vaninii was higher than that of Vc. Nitrite is easy to produce active $NO^{+}$, and nitrosation reaction occurs with the reaction substrate [66]. The epoxy cation at the 1 position of the catechol

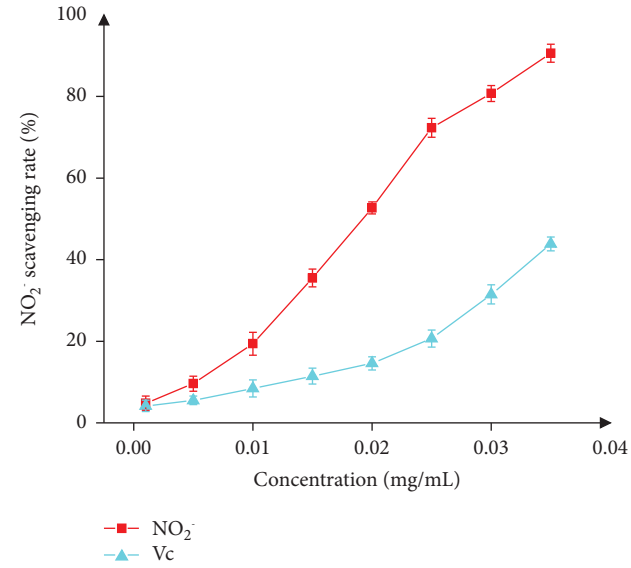


Figure 9: $NO_2^{-}$ scavenging activity of flavonoid from *S. vaninii* fruit bodies.

structure on the B ring may have relatively strong electrophilic ability [67], and the electron cloud on the left benzene ring moves under the action of the epoxy cation, so that the electron cloud density is reduced, the p–$\pi$ conjugated system on the phenolic hydroxyl group is damaged, the activity is enhanced, and the epoxy cation is easily substituted with $NO_2^{-}$ to generate a 1- nitroso substituent, which can effectively remove $NO_2^{-}$.

*3.5. Biological Activity*

*3.5.1. Antibacterial Activity of Flavonoid from S. vaninii Fruit Bodies.* *S. vaninii* fruit bodies flavonoid samples were tested for antibacterial activity. With the increase of flavonoid

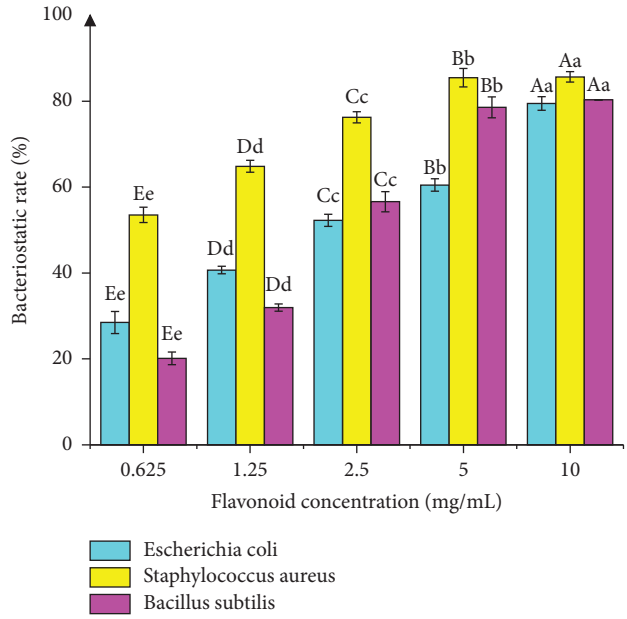


Figure 10: Antibacterial effect of flavonoid from *S. vaninii* fruit bodies.

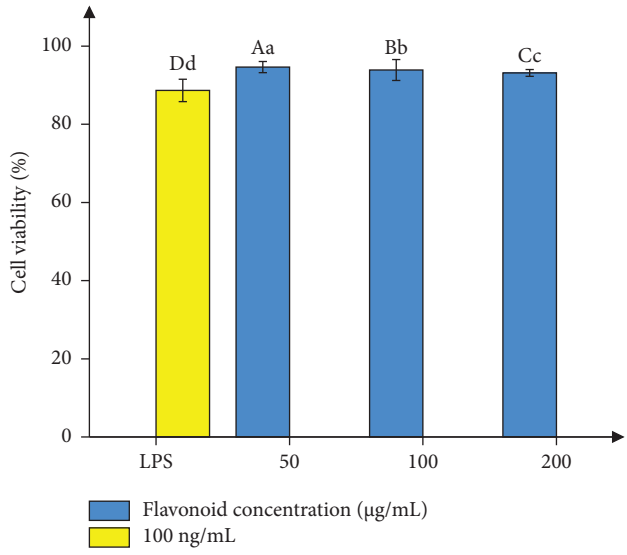


Figure 11: Cell viability of flavonoid from *S. vaninii* fruit bodies.

contents in preparing samples, the bacteriostatic rate increased in Figure 10. There was a negative correlation between flavonoid concentration in *phellinus igniarius* and bacterial growth. The inhibitory rates of flavonoids from *S. vaninii* against *Escherichia coli*, *Staphylococcus aureus,* and *Bacillus subtilis* were $79.48 \pm 1.57\%$, $85.64 \pm 1.22\%$, and $80.29 \pm 0.04\%$, respectively, with $IC_{50}$ values of 2.16 mg/mL, 0.43 mg/mL, and 2.16 mg/mL, respectively. The antibacterial ability of flavonoids may be related to the charge on the $C_3$ atom of hydrophobic molecules. The 3-hydroxy group is an antibacterial active group against plant pathogenic bacteria [68]. The inhibitory concentration of flavonoids from *phellinus igniarius* on *S. aureus* was the lowest; the reason

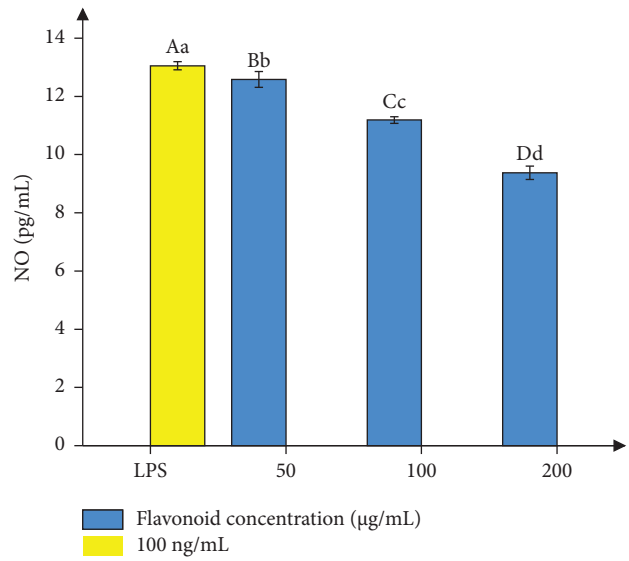


Figure 12: Anti-inflammatory effect of flavonoid from *S. vaninii* fruit bodies.

might be that flavonoids from *S. vaninii* caused dynamic disorder of cell wall peptide synthesis and decomposition, thus damaging the cell wall.

*3.5.2. Anti-Inflammatory Activity of Flavonoid from S. vaninii Fruit Bodies.* MTT method was used to determine the inhibition effect of LPS or *S. vaninii* flavonoid on RAW 264.7 cells viability. As shown in Figure 11, the results indicated that the cell viability was >85% in different treatment groups, which implied that there was no cytotoxicity for LPS, or *S. vaninii* flavonoid at the experiment concentration to RAW 264.7 cells, and it can be adopted for follow-up studies.

Inflammation is considered to be related to the occurrence of various diseases, such as diabetes and cardiovascular diseases [69]. Macrophages produce large amounts of inflammatory factor NO to attack pathogens and maintain the inflammatory environment in the body [70]. Excess inflammation leads to substantial necrosis and dysfunction of organs such as the heart, liver, and kidneys [71]. As shown in Figure 12, the flavonoid NO content of 200 $\mu$g/mL Phellinus igniarius was $9.38 \pm 0.23$ pg/mL. The trend is similar to that reported by Rong et al. [72]. The concentration had a positive correlation with the anti-inflammatory. Flavonoids regulate protein kinases by inhibiting the transcription factor nuclear factor $\kappa$–B(Nf-kB) [73]. This transcription factor participates in the regulation of different chemokines, cytokines, and various cell adhesion molecules by transferring from the cytoplasm to the nucleus and induces expression associated with proinflammatory genes [74].

## 4. Conclusions

Herein, the optimization of flavonoid extraction capacity with *S. vaninii* fruit bodies was studied by implementing a Box–Behnken experimental design and modeling the response surface methodology. Optimal flavonoid extraction

capacity $90.24 \pm 0.89$ mg/mL is found when temperature, solid-to-liquid ratio, ultrasonic time, and ethanol concentration are at 70°C, 1 : 60, 40 min, and 80%, respectively. Compared with ME and PE, ultrasonic wave could significantly increase the extraction rate of flavonoids. *S. vaninii* flavonoid has good anti-free radical activity. The scavenging activities of *S. vaninii* flavonoid on DPPH radical, $ABTS^{+}$ radical, hydroxyl radical, superoxide radical, and nitrite were $92.17 \pm 1.33\%$, $92.49 \pm 1.55\%$, $94.85 \pm 1.39\%$, $86.01 \pm 1.21\%$, and $90.63 \pm 2.2\%$, respectively. Its reducibility is higher than that of Vc. *S. vaninii* flavonoids have good bacteriostasis and anti-inflammation. Ultrasound-assisted extraction proved to be similarly suitable for the extraction of flavonoid from *S. vaninii*. This work showed that the findings of this research provide further potential resources and information on the bioactivities of *Sanghuangporus vaninii*, with a view toward facilitate the development of bioactive therapeutic agents from *Sanghuangporus vaninii*.

## Data Availability

Some or all data, figures, or tables used during the study are available from the corresponding author upon request. Direct requests for these materials may be made to the provider as indicated in the Acknowledgments.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## Acknowledgments

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