Optimization of the Extraction Process of Bioactive Compounds from the Fruiting Bodies of Yunzhi Mushroom *(Coriolopsis aspera)* in Vietnam by Response Surface Methodology

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Ultrasound-assisted extraction (UAE) is a process that accelerates the diffusion of substances in cells to liquids, thus decreasing extraction time. However, there is little research on the effects of sonication on the yunzhi mushroom, *Coriolopsis aspera*. This study investigated the effects of 4 solvents (acetone, methanol, water and ethanol) on the total polyphenol content (TPC), total flavonoid content (TFC), total triterpenoid content (TTC) and antioxidant capacity in fruiting bodies of *C. aspera*. Ethanol was used for extraction of the fruiting bodies. The results of the BBD experiment for optimization of this process were: TPC = 7.8832 ± 0.1844 mg GAE/gDW, TFC = 1.3521 ± 0.015 mg QE/gDW, TTC = 2.09 ± 0.01394 mg OAE/gDW, and RSA = 4.5832± 0.0455 (µg acid ascorbic/ml), at an extraction temperature of 40 °C, extraction time of 8.04 hours, and ethanol concentration of 79.6 %. At a solid to liquid ratio of 1:53, the experimental results did not differ much from the predicted results, indicating that these conditions were suitable for the extraction of polyphenols, flavonoids and triterpenoids from *C. aspera*.

Keywords: *Coriolopsis aspera*; RSM; total polyphenol content (TPC); total flavonoid content (TFC); DPPH radical scavenging activity

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The *Coriolopsis* spp. belongs to the *Polyporaceae* family, and has been widely used around the world. In Asian countries, *Coriolopsis* spp is traditionally used as a medicinal herb to treat many diseases as it has several active compounds. There are many reports on the qualitative screening of bioactive components such as triterpenoids, polyphenols and flavonoids that are abundant in the fruiting body of *Coriolopsis* [2]. Among them, the triterpene compounds are secondary metabolites, which are one of the most important biologically active components that confer cytotoxicity, hemolysis, antifungal, antibacterial, antiparasitic, and antiviral properties [3]. The polyphenol compounds in *Coriolopsis* spp. are antioxidants, possessing redox properties that act as reducing agents, hydrogen donors, free radical scavengers, and monoatomic oxygen reducing agents [4, 5]. Research in Malaysia has shown that polyphenols in yunzhi mushrooms are associated with a reduced risk of cardiovascular diseases, stroke and some types of cancer [6]. In addition, the yunzhi mushroom also contains flavonoids with excellent antioxidant capacity [7]. The antioxidant properties of these flavonoids support anticancer, antitumor and cardioprotective activity [8, 9]. Bioactive compounds can be obtained from various living organisms, such as fungi, plants, algae, fruits and bacteria, but these require different extraction techniques [10].

In previous reports, the optimal extraction processes for bioactive compounds in yunzhi mushrooms were complicated for various reasons, e.g., the extracted compounds had different properties, the location of compounds in different plant tissues, compounds were combined with sugars/proteins or formed certain polymerized derivatives with different solubilities. Thus, there were difficulties in choosing suitable solvents and extraction conditions. Moreover, these compounds may be easily oxidized, thus high temperatures and an alkaline environment could also cause a deterioration in the quality of the extracted compounds. Therefore, the extraction process and subsequent stages of experimental preparation for the quantification of these compounds must be carefully controlled [11, 12]. For these reasons, this study focused

Figure 1. The natural yunzhi mushroom *Coriolopsis aspera* from Pu Mat, Vietnam.

on obtaining the optimized extraction conditions, including extraction time, extraction temperature, liquid to material ratio, and solvent concentration, for determining TPC, TFC, TTC and RSA in yunzhi mushrooms.

MATERIALS AND METHODS

1. Materials

Natural yunzhi mushrooms were collected from Pu Mat National Park, located at 18° 46' North latitude and 104° 24' East longitude in Nghe An province, Vietnam. The sample was then dried at 45 °C to less than 7% moisture for preservation. After drying, the mushroom sample was ground, stored in a vacuum PE bag and preserved at 4 °C.

2. Chemicals and Reagents

Folin–Ciocalteu reagent, gallic acid, oleanolic acid, ascorbic acid, quercetin and 2, 2-diphenyl-picrylhydrazyl (DPPH), were purchased from Sigma Aldrich (Sigma Chemicals Co., St. Louis, MO, USA). All other chemicals used in this study were obtained commercially and were of analytical grade.

3. Preparation of Samples

The dry powdered fruiting bodies of *C. aspera* (2 g) were extracted with a solvent (acetone 80%, methanol 80%, water, ethanol 80%) using ultrasound (ultrasonic processor model GE 750, USA). The mixture was filtered through Whatman filter paper No. 4 to obtain a solid extract. The solids were washed twice. The eluate was mixed with the extract and concentrated under vacuum at 40 °C with a rotary evaporator. The extract was then diluted to 10 ml.

4. Determination of Total Polyphenol Content (TPC)

Total polyphenol content was determined by the UV-VIS method using the Folin-Ciocalteu reagent based on a previously published method [13], with additional modifications. The diluted extract (1 ml) was added to the Folin-Ciocalteu reagent (0.5 ml), shaken and left to stand for 3 min. Then, Na_2CO_3 (2.5 ml) was added to the solution to neutralize the reaction, followed by distilled water to a final volume of 10 ml. The absorbance of the solution was measured at 765 nm (Agilent 8453 UV-Visible Spectrophotometer). The standard calibration curve was constructed with gallic acid standards (0, 10, 20, 30, 40, 50 ppm). The total content of polyphenols was expressed in mg of gallic acid equivalent/g dry weight (mg GAE/g DW) [14]. All experiments were performed in triplicate.

$$\text{Absorbance} = 0.0108\ Cx + 0.01768\ (R^2 = 0.99)$$

$$\text{TPC}\ \left(\frac{mg\ gallic\ acid}{g\ sample\ dry}\right) = \frac{C_x}{10^3} \text{x} \frac{1}{a_g} \text{x} \frac{V_{cv}}{100-W} \text{x K}$$

Where:

TPC: total polyphenol content (mg gallic acid/ g sample dry)

Cx: gallic acid concentration (ppm)

V_{cv}: calibrated volume of the sample (ml)

K: dilution factor

a_g: weight of the wet sample (gram)

w: moisture content of the sample (%)

10^3: conversion coefficient

5. Determination of Total Flavonoid Content (TFC)

The sample extract (1 ml) was incubated with $NaNO_2$ (0.3 ml) for 5 min, and then reacted with $AlCl_3$ (0.3 ml) for another 5 min. Finally, NaOH (2 ml, 1 M) and distilled water (6.4 ml) were added to the mixture. The absorbance at 510 nm was determined with a spectrophotometer. The standard calibration curve was constructed with quercetin standards (0, 10, 20,

30, 40, 50 ppm) and TFC was expressed in mg quercetin equivalent/g dry weight (mg QE/g DW) [15]. All experiments were performed in triplicate.

Absorbance = 0.00046 C_x + 0.00137 (R^2 = 0.99)

$$\text{TFC } \left(\frac{mg\ quercetin}{g\ sample\ dry}\right) = \frac{C_x}{10^3} \text{x} \frac{1}{a_g} \text{x} \frac{V_{cv}}{100-W} \text{xK}$$

Where:

TFC: total quercetin content (mg quercetin /g sample dry)

C_x: quercetin concentration (ppm)

V_{cv}: calibrated volume of the sample (ml)

K: dilution factor

a_g: weight of the wet sample (gram)

w: moisture content of the sample (%)

10^3: conversion coefficient

6. Determination of Total Triterpenoid Content (TTC)

The sample extract (0.2 ml) was added to acetic acid (0.2 ml, 5%) and perchloric acid (1.2 ml, 70 - 72%). The mixture was incubated in a thermostatic bath for 15 min. The solution was then cooled and diluted with ethyl acetate (3.4 ml). Absorbance at 550 nm was determined with a spectrophotometer. The standard calibration curve was constructed with gallic acid standards (0, 2, 4, 8, 8, 10 ppm). The TTC was expressed in mg of oleanolic acid equivalents (mg OAE /g DW). All experiments were performed in triplicate.

Absorbance = 0.01677 C_x + 0.00081 (R^2 = 0.99)

$$TTC\ \left(\frac{mg\ \text{oleanolic acid}}{g\ sample\ dry}\right) = \frac{C_x}{10^3} \text{x} \frac{1}{a_g} \text{x} \frac{V_{cv}}{100-W}$$

Where:

TTC: total oleanolic content (mg oleanolic acid/ g sample dry)

C_x: oleanolic acid concentration (ppm)

V_{cv}: calibrated volume of the sample (ml)

K: dilution factor

a_g: weight of the wet sample (gram)

w: moisture content of the sample (%)

10^3: conversion coefficient

7. DPPH Radical Scavenging Activity Assay

0.1 mM DPPH solution was prepared by dissolving 3.94 mg DPPH in 100 ml ethanol, 99.5%. The sample extract (0.1 mL) was mixed with DPPH solution (4 ml, 0.1 mM) and ethanol (0.9 ml), and shaken vigorously for 1 min. Then, it was incubated in the dark at room temperature for 30 min [16]. In the control sample, the yunzhi extract was replaced with distilled water. The absorbances of the sample (Abs_{sample}) and the control ($Abs_{control}$) were measured at 517 nm. The standard calibration curve was constructed with Vitamin C standards (0, 1, 3, 6, and 9 ppm) and the radical scavenging activity (mg Vitamin C equivalent/g DW) of sample was described by:

Absorbance = - 0.00783C_x + 0.41348 (R^2 = 0.99)

$$\text{RSA (mg Vitamin C/g sample dry)} = C_x \times \frac{V_{đm}}{10^3} \times \frac{100}{a \times (100-W)} \times K$$

Where:

RSA: Radical-scavenging activity (mg Vitamin C /g sample dry)

C_x: Vitamin C concentration of the sample (ppm)

V_{cv}: calibrated volume of the sample (ml)

K: dilution factor

a_g: weight of the wet sample (gram)

w: moisture content of the sample (%)

10^3: conversion coefficient

8. Optimization of Extraction

Optimization of the extraction process was performed according to the following steps:

Step 1: We investigated the influence of solvents on the extraction of bioactive compounds. We performed an independent factor survey on solvents including acetone (80%), methanol (80%), ethanol (80%) and water. The tracked objective functions were total polyphenol content (TPC), total flavonoid content (TFC), total triterpenoid content (TTC) and antioxidant capacity (RSA). The processing mode of the extracted samples was the same, e.g., the ratio of solvent to raw materials was 30:1, sonication for 30 minutes at 375 W (ultra-sonic processor model GE 750, USA) with 2 g of yunzhi mushroom powder.

Step 2: We carried out single-factor experiments for finding the range of independent variables. Then, four independent variables (extraction temperature, X1, solvent- material ratio, X2, extraction time, X3, and ethanol concentration, X4) and four dependent variables (total polyphenol content, Y1, total flavonoid content, Y2, total triterpenoid content, Y3, and radical scavenging activity, Y4) were chosen for optimization.

Step 3: The Box-Behnken design (BBD) mathematical model was selected to perform optimization by Response Surface Methodology (RSM). JMP 10.0.0 and Design-Expert 6.07 software were used to analyse the data.

Table 1. The survey results for solvents.

Metabolite	Solvent			
	Acetone (80%)	Methanol (80%)	Water	Ethanol (80%)
TPC (mg GAE/g DW)	$6.7^{b} \pm 0.3$	$7.23^{c} \pm 0.14$	$4.45^{a} \pm 0.21$	$7.34^{c} \pm 0.23$
TFC (mg QE/g DW)	$1.34^{b} \pm 0.02$	$1.42^{c} \pm 0.04$	$0.93^{a} \pm 0.02$	$1.48^{d} \pm 0.01$
TTC (mg OAE/g DW)	$1.75^{b} \pm 0.01$	$2.10^{c} \pm 0.03$	$0.78^{a} \pm 0.02$	$2.13^{c} \pm 0.04$
RSA (µg acid ascorbic/ml)	$4.01^{b} \pm 0.07$	$5.62^{c} \pm 0.12$	$2.12^{a} \pm 0.05$	$5.83^{d} \pm 0.11$

Notes:

1. Results are expressed as mean ± SD (n = 3); different letters (a, b, c, d) in the same row indicate that there is a statistically significant difference with $p \leq 0.05$.
2. The ratio of raw materials to solvent was 1:30. Samples were subjected to 30 minutes of sonication at 375 W.

9. Statistical Analysis

Design–Expert 6.0.7 and JMP 10.0.0 software were used to design optimized experiments following Response Surface Methodology. Statgraphics Centurion XV software was used to analyse variance (ANOVA) and standard deviation, while graphs were drawn using Microsoft Excel.

RESULTS AND DISCUSSION

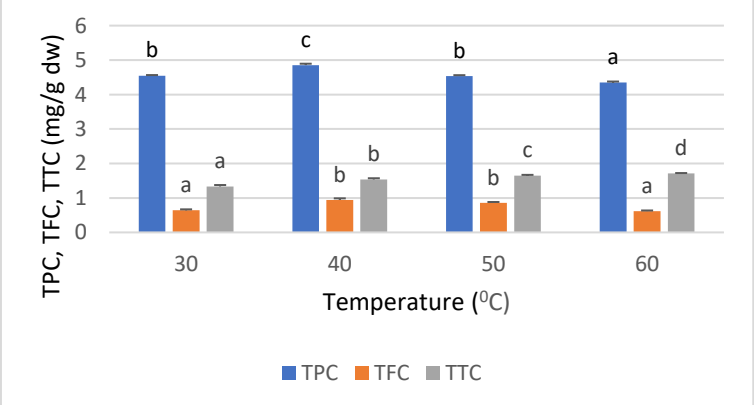
1. The Effect of Solvents on the Extraction of Bioactive Compounds

The results (Table 1) show that methanol and ethanol resulted in the highest TPC yields, while the highest TFC, TTC and antioxidant capacity results were obtained with ethanol. This difference could due to the different polarization ability of substances in materials and liquids. Similar results were observed for TPC in the extract of *Trametes* mushrooms, indicating that ethanol was the most effective solvent for extracting polyphenol [17]. Ethanol is also green and less toxic than methanol [18]. Therefore, we chose ethanol for extracting bioactive compounds in this study.

2. Single-Factor Experiments

2.1. Influence of Temperature on TPC, TFC and TTC

TPC and TFC values were high at the extraction temperature of 40 °C and then showed signs of decreasing at 60 °C due to the temperature-sensitive properties of polyphenols and flavonoids [19], while TTC showed signs of increasing (Figure 2). We chose the temperature of 40 °C for investigation to limit the loss of natural active ingredients and the evaporation of the extraction solvent. Similarly, Ammar Altemimi used sonication for extracting polyphenol and evaluating antioxidants and found that an extraction temperature of 40 °C obtained the highest values for TPC, TFC and free-radical scavenging activity [20].



The results are expressed as mean ± SD (n = 3), different letters (a, b, c, d) show that there is a statistically significant difference with $p \leq 0.05$

Figure 2. Influence of temperature on TPC, TFC and TTC.

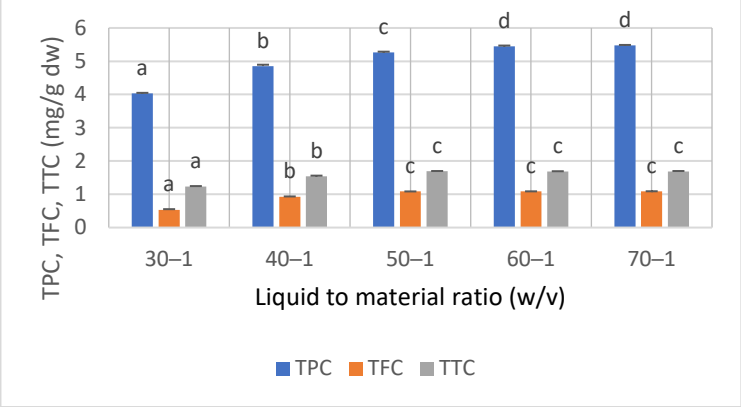


Figure 3: Influence of solvent to raw material ratio on TPC, TFC and TTC

2.2. Influence of Solvent Ratio to Raw Material on TPC, TFC and TTC

The results showed that TPC was highest when the solvent to raw material ratio was 60:1. With a ratio of 50:1, TFC and TTC were highest (Figure 3.). This result may explain the ratio of solvent to raw material that affected the extraction of polyphenols, flavonoids and triterpenes. When the amount of solvent is large, the higher concentration of natural compounds in the yunzhi mushrooms will diffuse into the solvent. ZiLuan Fan investigated the effects of liquid-solid ratios from 10:1 to 60:1 and found that a ratio of 50:1 was suitable for extracting polyphenol from *Lonicera japonica* [21]. With ratios of solvent to raw material from 50:1 to 70:1, TFC and TTC values did not change due to saturation of the extracted compounds [22]. Therefore, we choose the ratio of 50:1 for the following experiments.

2.3. Influence of Extraction Time on TPC, TFC and TTC

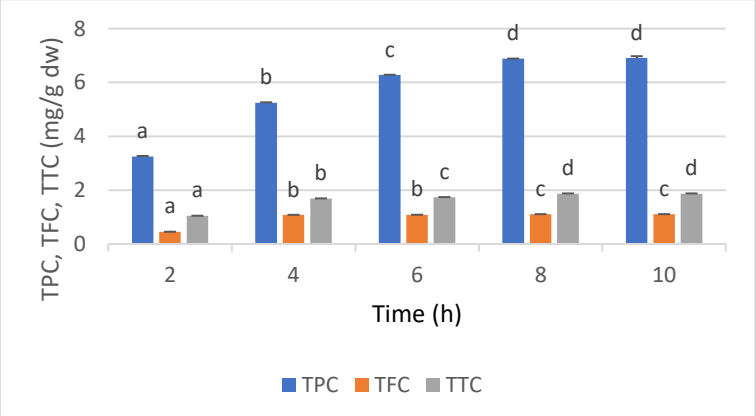
For extraction times of 8 h or more, the TPC value was stable, while TFC was highest for an extraction time of 8-10 h. This data shows that extraction time greatly affects the values of TPC, TFC and TTC. In a study by Sukor, the maximum amount of polyphenol was obtained with 8 hours of extraction time [23]. This supports the results of our study. Thus, we chose an extraction time of 8 hours for the following experiments to determine the influence of ethanol concentration on extraction.

2.4. Influence of Solvent Concentration on TPC, TFC and TTC

The content of TPC, TFC and TTC were highest at a solvent concentration of 80% and did not change significantly at 90%, as the sample contained compounds with a high polarization ability. Similarly, Muhammad Naeem Safdar found that using 80% ethanol gave the highest TPC and antioxidant activity values [24].

3. Optimization of Extraction from *Coriolopsis aspera* TPC, TFC, TTC, RSA

For the single-factor experiment, we selected the lower and upper limits of the factors affecting the TPC, TFC and TTC results for the optimization.



The results are expressed as mean ± SD (n = 3), different letters (a,b,c,d) show that there is a statistically significant difference with $p \leq 0.05$

Figure 4. Influence of time on TPC, TFC and TTC.

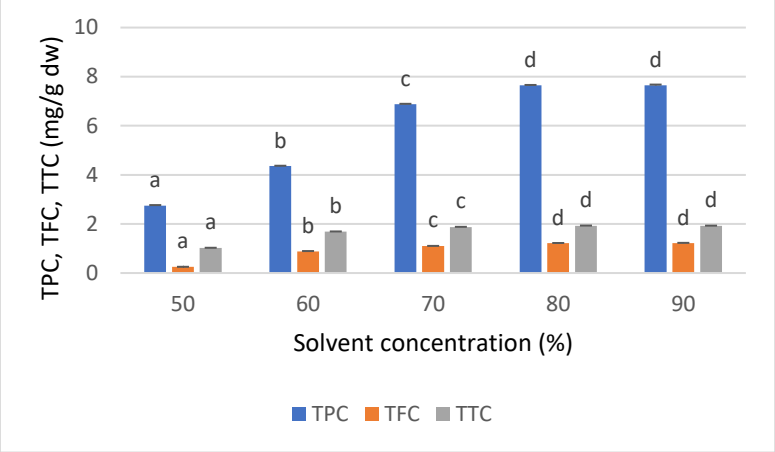


Figure 5: Influence of solvent concentration on TPC, TFC and TTC

Table 2. Experimental matrix with Box-Behnken design.

Treatment	Encode	Factor				Objective function			
		X_1	X_2	X_3	X_4	Y_1	Y_2	Y_3	Y_4
1	0−0+	40	40	8	90	6.642	0.838	1.827	3.127
2	0+0−	40	60	8	70	6.716	0.977	1.627	3.218
3	+00−	50	50	8	70	5.972	0.914	1.969	3.108
4	−0+0	30	50	10	80	5.396	0.779	1.308	3.012
5	0000	40	50	8	80	7.076	1.295	2.078	3.989
6	00+−	40	50	10	70	7.031	1.021	1.788	3.986
7	0−0−	40	40	8	70	7.806	0.956	1.796	4.258
8	−00+	30	50	8	90	5.297	0.739	1.865	3.012
9	−+00	30	60	8	80	5.387	0.786	2.006	3.108
10	00−−	40	50	6	70	6.078	0.857	1.787	3.254
11	+−00	50	40	8	80	6.518	0.925	1.865	3.581
12	++00	50	60	8	80	7.109	1.019	1.707	3.478
13	0000	40	50	8	80	8.127	1.388	2.114	4.865
14	00−+	40	50	6	90	6.546	0.927	1.649	3.021
15	0++0	40	60	10	80	8.109	1.196	2.047	4.634
16	−0−0	30	50	6	80	5.171	0.706	1.606	3.002
17	+0+0	50	50	10	80	7.729	1.106	1.727	4.167
18	0000	40	50	8	80	8.169	1.304	1.988	4.892
19	00++	40	50	10	90	7.967	1.048	1.205	4.023
20	−−00	30	40	8	80	5.272	0.788	1.327	3.076
21	0−+0	40	40	10	80	6.267	0.821	1.144	3.871
22	−00−	30	50	8	70	4.983	0.776	1.327	2.986
23	0+−0	40	60	6	80	6.896	0.784	1.606	3.875
24	0−−0	40	40	6	80	6.346	0.711	1.687	3.321

25	+00+	50	50	8	90	7.764	1.125	1.084	4.321
26	0+0+	40	60	8	90	8.138	1.119	1.897	4.923
27	+0−0	50	50	6	80	6.212	1.027	1.406	3.832

Note: X_1: temperature (°C), X_2: ratio of solvent:material
X_3: time (hours), X_4: ethanol concentration (%)
Y_1: total polyphenol content (mg GAE/g dw)
Y_2: total flavonoid content (mg QE/g dw)
Y_3: total triterpenoid content (mg OAE/g dw)
Y_4: antioxidant capacity (µgVit C/g dw)

Optimized parameters according to the response surface method were: extraction temperature of 30-50 °C, solvent to raw material ratio of 40:1 to 60:1 (v/w), time of 6-10 hours, solvent concentration of 70-90 %.

Based on the results of the independent single-factor experiments, we used RSM for optimizing *C. aspera*. Table 2 presents the experimental matrix using the Box-Behnken design with 27 treatments to optimize four independent variables and 4 dependent variables by response surface methodology (RSM). The independent variables were: temperature, X_1, ratio of solvent-material, X_2, time X_3, and ethanol concentration, X_4. The dependent variables were: total polyphenol content, Y_1, total flavonoid content, Y_2, total triterpenoids content, Y_3, and radical scavenging activity, Y_4. JMP 10.0.0 software was used to analyze the data, the results of which are listed in Table 2.

3.1. Statistical Analysis and Model Fitting

$$Y_1=7.72+0.82X_1+0.29X_2+0.44X_3+0.31X_4-1.31X_1^2-0.26X_2^2-0.45X_3^2-0.30X_4^2+0.12X_1X_2+0.32X_1X_3+0.37\ X_1X_4+0.32X_2X_3+0.65X_2X_4+0.12X_3X_4 \quad (1)$$

In equation (1), Y_1 is the total polyphenol content while X_1: temperature, X_2: ratio of solvent-material, X_3: time, X_4: solvent concentration. Statistical analysis gave $R^2 = 0.9158$, showing a high degree of accuracy in the reliability of the experimental values and a high degree of correlation between the observed and predicted values, while the signal to noise ratio measurement (Adeq precision= 10.248) > 4 indicated an adequate and desired signal [25]. This model can be used to navigate the design space. The F value of the model was significant with $p < 0.001$ and the incompatibility error was minor and not statistically significant with $p > 0.05$, which is desirable. These results prove that the selected model was appropriate. The coefficients X_1, X_1^2 had a dramatic influence ($p<0.001$). The coefficient X_3 had a significant influence ($p<0.01$). The coefficients X_2, X_4, X_2X_4, X_3 had a minor influence ($p<0.05$). The remaining coefficients X_1X_2, X_1X_3, X_2X_3, X_1X_4, X_3X_4 had no influence ($p>0.05$).

$$Y_2=1.33+0.13X_1+0.070X_2+0.080X_3+0.025X_4-0.25X_1^2-0.22X_2^2-0.21X_3^2-0.17X_4^2+0.024\ X_1X_2+1.500E\text{-}003X_1X_3+0.062X_1X_4+0.075X_2X_3+0.065X_2X_4-0.011X_3X_4 \quad (2)$$

In equation (2), Y_2 is the total flavonoid content and X_1: temperature, X_2: ratio of solvent-material, X_3: time, X_4: solvent concentration. Statistical analysis gave $R^2 = 0.9321$, showing a high degree of accuracy in the reliability of the experimental values and a high degree of correlation between the observed and predicted values, while the signal to noise ratio measurement (Adeq precision = 13.115) > 4 indicated an adequate and desired signal. The F value of the model was significant with $p < 0.001$ while the incompatibility error was minor and not statistically significant with $p > 0.05$, which is desirable. These results prove that the selected model was appropriate. The coefficients X_1, X_1^2, X_2^2, X_3^2, X_4^2 had a dramatic influence ($p<0,001$). The coefficients X_2, X_3 had a significant influence ($p<0.01$). The remaining coefficients X_4, X_1X_2, X_1X_3, X_2X_3, X_1X_4, X_2X_4, X_3X_4 had no influence ($p>0.05$).

$$Y_3=2.07+0.027X_1+0.10X_2-0.043X_3-0.064X_4-0.27X_1^2-0.11X_2^2-0.30X_3^2-0.20X_4^2-0.21X_1X_2+0.15X_1\ X_3-0.36\ X_1X_4+0.25\ X_2\ X_3+0.060\ X_2X_4-0.11\ X_3X_4 \quad (3)$$

In equation (3), Y_3 is the total triterpene content and X_1: temperature, X_2: solvent-material ratio X_3: time, X_4: solvent concentration. Statistical analysis gave $R^2 = 0.8561$, showing a high degree of accuracy in the reliability of the experimental values and a high degree of correlation between the observed and predicted values, while the signal to noise ratio measurement (Adeq precision = 7.787) > 4 indicated an adequate and desired signal. The F value of the model was significant with $p <0.01$ while the incompatibility error was minor and not statistically significant with $p > 0.05$, which is desirable. These results prove that the selection model was appropriate. The coefficients X_1^2, X_3^2 had a significant influence ($p < 0.01$). The coefficients X_1X_2, X_2X_3, X_4^2 had an influence ($p < 0,05$). The remaining coefficients X_1, X_2, X_3, X_4, X_1X_3, X_1X_4, X_2X_4, X_3X_4, X_2^2 had no influence ($p>0.05$).

$$Y_4=4.52+0.36\ X_1+0.17X_2+0.28X_3+0.13X_4-0.77X_1^2-0.29X_2^2-0.35X_3^2-0.45X_4^2-0.034X_1X_2+0.081X_1\ X_3+0.30X_1X_4+0.052X_2X_3+0.71X_2X_4+0.068\ X_3X_4 \quad (4)$$

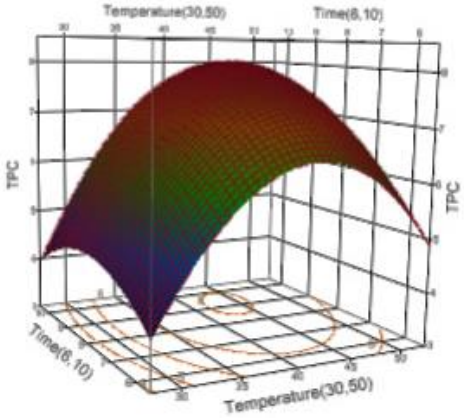
In equation (4) Y_4 is the radical scavenging activity and X_1: temperature, X_2: solvent-material ratio, X_3: time, X_4: solvent concentration. Statistical analysis gave $R^2 = 0.8418$, showing a high degree of accuracy

in the reliability of the experimental values and a high degree of correlation between the observed and predicted values, while the signal to noise ratio measurement (Adeq precision = 7.139) > indicated an adequate and desired signal. The F value of the model was significant with $p < 0.01$ while the incompatibility error was minor and not statistically significant with $p > 0.05$ which is desirable. These results prove that the selection model was appropriate. The coefficient X_1^2 had a dramatic influence ($p < 0.001$). The coefficients X_1, X_2X_4 had a significant influence ($p<0.01$). The coefficients X_3, X_3^2, X_4^2 had a minor influence ($p < 0.05$). The remaining coefficients X_2, X_4, X_1X_2, X_1X_3, X_2X_3, X_1X_4, X_3X_4, X_2^2 had no influence ($p > 0.05$).

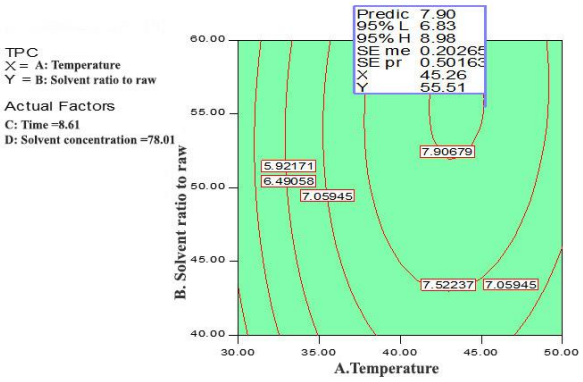
The contour line (A') shows the optimal predicted TPC was 7.9068 mgGAE/g DW and the optimal parameters were: temperature of 45.26 °C, ratio of solvent-material 55.51:1, extraction time of 8.61 hours, and ethanol concentration of 78.01 %.

The contour line (B') shows the optimal predicted TFC was 1.29 mgQE/g DW and the optimal parameters were: temperature of 43.89 °C, ratio of solvent-material 57.23:1 (w/v), extraction time of 8.01 hours, and ethanol concentration of 85.42 %.

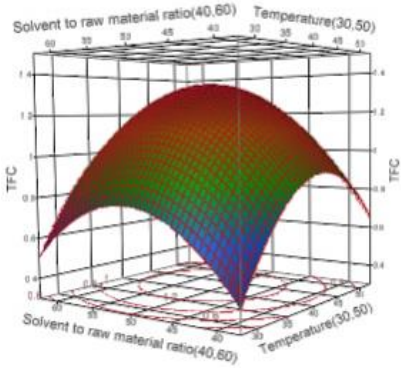
The contour line (C') shows the optimal predicted TTC was 1.96 mgOAE/g DW and the optimal parameters were: temperature of 48.10 °C, ratio of solvent-material 46.26:1 (w/v), extraction time of 6.97 hours, and ethanol concentration of 74.94 %.



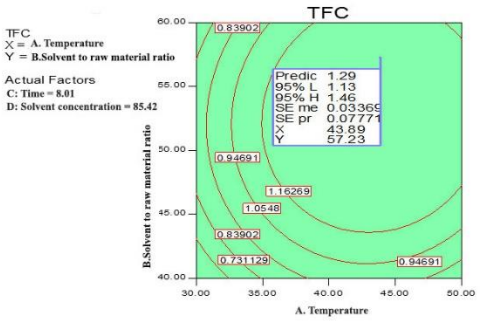
(A)



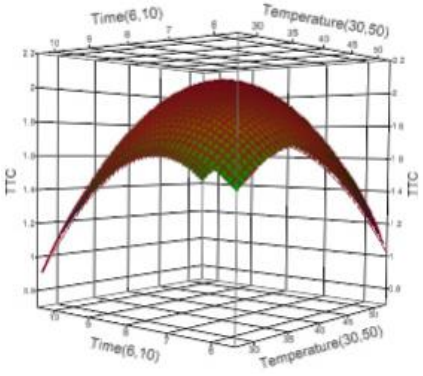
(A')



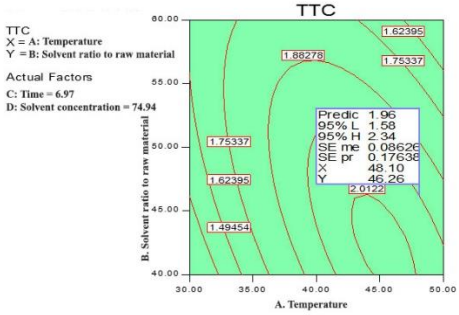
(B)



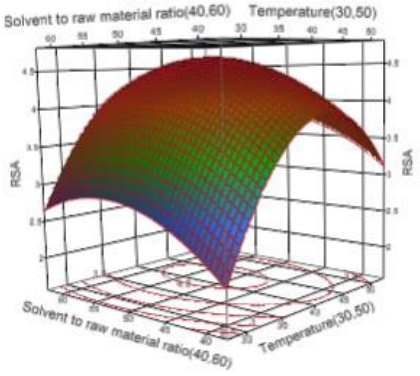
(B')



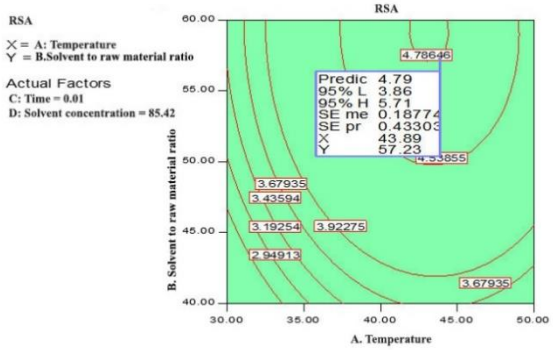
(C)



(C')



(D)



(D′)

Figure 6. (A) 3D graph for TPC; (A') contour line for TPC; (B) 3D graph for TFC; (B') contour line for TFC; (C) 3D graph for TTC; (C') contour line for TTC; (D) 3D graph for RSA; (D') contour line for RSA.

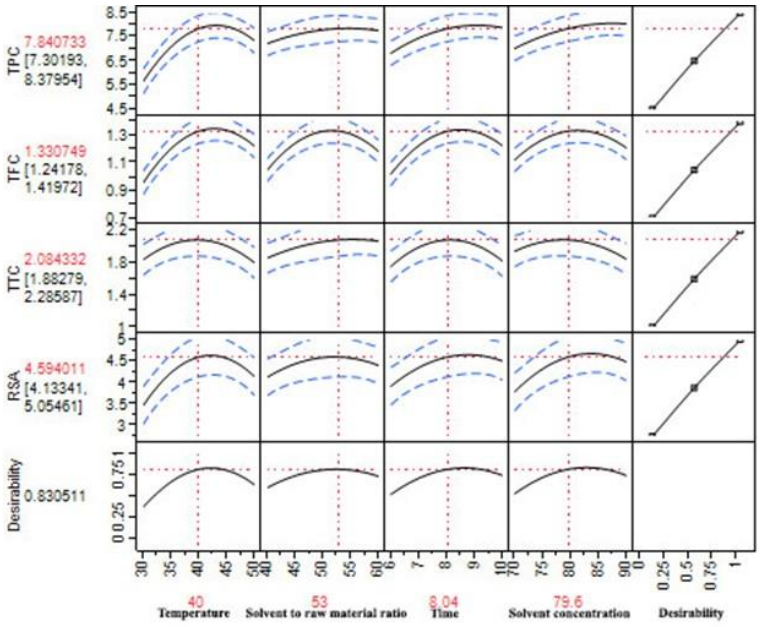


Figure 7: Predicted results.

The contour line (D') shows the optimal predicted RSA content was 4.79 µgVitC/g DW and the optimal parameters were: temperature of 48.10 °C, ratio of solvent-material 46.26:1 (w/v), extraction time of 6.97 hours, and ethanol concentration of 74.94 %.

Figure 7 shows that the 4 factors, extraction temperature of 40 °C, the ratio of ethanol solvent to raw materials of 53:1, extraction time of 8.04 hours, and ethanol concentration of 79.6%, gave the corresponding objective function such that TPC =

Table 3: Empirical tests

Objective function	Predicted value	Experimental value	$P_{\text{-value}}$
TPC	7.8407	7.8832 ± 0.1844	0.71020
TFC	1.3307	1.3521 ± 0.015	0.36218
TTC	2.0843	2.0900 ± 0.01394	0.51800
RSA	4.5940	4.5832 ± 0.0455	0.70404

Using a t-test study with 95% confidence

7.8407 mg GAE/g DW, TFC = 1.3307 mgQE/g DW, TTC = 2.0843 mgOAE/g DW, and RSA = 4.5940 µgVitC /g DW. The predicted values for TPC for R^2 = 0.9158, TFC for R^2 = 0.9321, TTC for R^2 = 0.8561 , RSA for R^2 = 0.8418 were also more than 0.8 [26] ($P < 0.05$), indicating that these parameters were suitable for the determination of TPC, TFC, TTC and RSA in *C. aspera*.

3.2. Verification of Predictive Model

The statistical results of the empirical tests are listed in Table 3, and these show that the difference between the experimental values and the predicted values were not statistically significant. Thus, the predicted values were consistent with the optimal values.

CONCLUSION

We have identified that ethanol was an appropriate solvent to extract the maximum amount of polyphenols, flavonoids and triterpenes from the fruiting bodies of *Coriolopsis aspera*, and to achieve high antioxidant capacity (RSA). The optimal conditions such as temperature, ratio of solvent-material, extraction time and ethanol concentration were identified by applying Response Surface Methodology (RSM). The results of the BBD design experiment indicated that an extraction temperature of 40 °C, extraction time of 8.04 hours, ethanol concentration of 79.6%, and solid to liquid ratio of 1:53 (w/v) were the optimal conditions for extracting bioactive substances from yunzhi mushrooms.

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