OPTIMIZING THE EXTRACTION OF BIOACTIVE COMPOUNDS FROM *GYNOSTEMMA PENTAPHYLLUM* USING RESPONSE SURFACE METHODOLOGY (RSM)

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Abstract

Gynostemma pentaphyllum, a medicinal herb of the *Cucurbitaceae* family with historical significance in Asian traditional medicine, was investigated for the optimization of bioactive compound extraction. This study employed Response Surface Methodology (RSM) utilizing a Central Composite Circumscribed (CCC) design, comprising 17 experimental runs with triplicate replication at the central point, to assess the effects of ethanol concentration (50-80%), extraction temperature (60-80°C), and extraction time (90-150 min) on the extraction efficiency of bioactive compounds. The optimal parameters were determined to be an ethanol concentration of 80%, an extraction temperature of 80°C, and an extraction time of 115 min. Under these conditions, the obtained contents of polyphenol, saponin, saponin, chlorophyll, antioxidant activity, and the obtained extraction yeild reached 0.51 ± 0.01 mg GAE/g, 8.26 ± 0.001 mg OA/g, 0.11 ± 0.0001 mg/ml, 1.54 ± 0.08 mg Vitamin C/g, and 21.53%, respectively. Model validation through three replicate experiments at the optimized conditions demonstrated no statistically significant difference ($p > 0.05$) between experimental and predicted values, confirming the model's reliability.

Keywords: extraction, *Gynostemma pentaphyllum*, optimization, polyphenol, saponin, chlorophyll, antioxidant activity

1. Introduction

Gynostemma pentaphyllum (Thunb.) Makino, a climbing vine from the Cucurbitaceae family, has been widely utilized in traditional Asian medicine due to its therapeutic properties. *Gynostemma pentaphyllum* is also named Jiaogulan in Chinese. Jiaogulan is rich in a variety of bioactive compounds, including saponins (gypenosides), polyphenols, flavonoids, and polysaccharides. Jiaogulan has antioxidant, anti-inflammatory, hypoglycemic, and immunomodulatory activities (Wang et al., 2020). These attributes highlight its potential for development of functional foods, nutraceuticals, and pharmaceuticals. There have been many studies to maximize yield and bioactivities of the extraction of bioactive compounds from Jiaogulan. Traditional extraction methods, such as Soxhlet extraction, often require long extraction times and large amount of solvent, which may lead to compound degradation. Therefore, modern extraction techniques have gained increasing attention.

Several researchers have successfully employed Response Surface Methodology (RSM) for the optimization of *G. pentaphyllum* extractions. Kamkayan and Assatarakul (2021) utilized ultrasound-assisted extraction (UAE) to study the impact of ethanol concentration, extraction time, and ultrasonic power on the antioxidant properties of *G. pentaphyllum* leaves. Their results

indicated that higher ethanol concentrations and moderate extraction times improved total phenolic content and antioxidant activity, demonstrating the combination of solvent and extraction duration to maximize yields. In another study, Meng et al. (2014) applied an enzymatic-ultrasonic method combined with RSM to optimize the extraction of total flavonoids from *G. pentaphyllum*. They reported that enzymatic pretreatment followed by ultrasonic extraction significantly enhanced flavonoid recovery and antioxidant activity compared to traditional methods. These findings emphasize the synergistic effects of combining different extraction technologies. Furthermore, Wang et al. (2020) compared different extraction methods found that different extraction conditions greatly influenced the antioxidant activities of the extracts. Their study reinforced the necessity of careful optimization of extraction parameters such as temperature, solvent concentration, and time to preserve bioactivity. The aim of this work was to optimize the extraction of bioactive compounds from *G. pentaphyllum*.

2. Materials and Methods

2.1 Materials

Giao Co Lam was obtained from a pharmacy in Ho Chi Minh City, and was air-dried to a moisture content below 12% and ground to a particle size smaller than 2mm. The powder after grinding was packed in sample bags and stored at 4°C in a cool cabinet.

2.2 Experimental design

The experiment was designed based on a combination of ethanol solvent concentration (50-80%), extraction temperature (60-80°C), and extraction time (90-150 minutes). Ground *Gynostemma pentaphyllum* with a particle size below 2mm was weighed. Ethanol solvent was added to the extraction flask at a ratio of 1:20 w/v. Experiments were conducted with the ethanol concentrations (X1), temperatures (X2), and times (X3) arranged according to Table 2.1.

Table 2.1: Optimization treatments

Runs	Variables		
	X_1 (%)	X_2 (minutes)	X_3 (^{o}C)
1	50	90	60
2	80	90	60
3	50	150	60
4	80	150	60
5	50	90	80
6	80	90	80
7	50	150	80
8	80	150	80
9	39.77	120	70
10	90.23	120	70
11	65	69.54	70
12	65	170.46	70
13	65	120	53.18

14	65	120	86.82
15	65	120	70
16	65	120	70
17	65	120	70

2.3 Analytical methods

2.3.1 Extraction yield (EY)

Extraction yield was calculated as the percentage of the mass of extract to the mass of raw material dried to constant mass at 105°C.

2.3.2 Total saponin content (TSC)

Saponin content was determined according to the method of Lim et al. (2020). Samples were reacted with acetic acid: sulfuric acid (1:1) and heated in a water bath at 60°C for 45 minutes. The absorbance of the samples was measured at a wavelength of 550 nm. Oleanolic acid was used to construct the standard curve.

2.3.3 Total phenolic content (TPC)

Polyphenolic content was determined by the Folin-Ciocalteau method based on the procedure of TCVN 9745-1:2013. 0.5 mL of the sample solution was pipetted into a test tube, followed by the addition of 2.5 mL of 10% Folin Ciocalteu reagent. The mixture was shaken well for 5 minutes. Subsequently, 2 mL of 7.5% Na_2CO_3 solution was added. The mixture was shaken well, kept in the dark for 60 minutes at room temperature, and then the absorbance (OD was determined at a wavelength of λ=765 nm. The OD values were recorded and a calibration curve was plotted to determine the polyphenol content in the extract samples. The extract samples were processed similarly to the standard polyphenol samples.

2.3.4 Total chlorophyll content (TCC)

Chlorophyll content in the samples was determined by reading the optical density (OD) using a spectrophotometer. Optical density for chlorophyll in the solvent was measured at wavelengths of 645 nm and 663 nm as studied by Porra (2002). The blank sample contained only the solvent at the corresponding concentration. The total chlorophyll (Chl-ab), chlorophyll a (Chl-a), and chlorophyll b (Chl-b) contents were calculated using the following equations:

$$\text{Chl-a} = 12.71 \times A663 - 2.59 \times A645$$
$$\text{Chl-b} = 22.88 \times A645 - 4.67 \times A663$$
$$\text{Chl-ab} = 20.29 \times A645 + 8.04 \times A663$$

2.3.5 Antioxidant capacity (AC)

The relative DPPH radical scavenging capacity of the extract was determined by a colorimetric method using the 2,2'-diphenyl-picrylhydrazyl (DPPH) reagent (Rumpf et al., 2023). A standard

vitamin C solution was used as the reference standard. To 2 mL of the sample, 2 mL of 100 ppm DPPH reagent solution was added, mixed well, and incubated in the dark for 30 minutes. The optical density was measured at a wavelength of 517 nm. The results were calculated in units of mg vitamin C (mg/g, dry matter).

2.4 Statistical analysis

Results were statistically analyzed, calculated, and plotted using Microsoft Excel. Statgraphics Centurion XVI software was used for ANOVA analysis, through Tukey HSD. Optimization was performed using Moddel 5.0 software.

3. Results and discussion

Response surface methodology with a Central Composite Circumscribed (CCC) design, consisting of 17 experimental runs with 3 replicates at the center point, was used to investigate the influence of ethanol concentration (X_1, 50% - 80%), time (X_2, 90 minutes - 150 minutes), and extraction temperature (X_3, 60°C - 80°C) on the extraction efficiency of bioactive compounds in *Gynostemma pentaphyllum*, such as polyphenol content, antioxidant activity, saponin content, chlorophyll content, and extraction yield. Regression analyses were performed, and polynomial equations were obtained. Ignoring non-significant factors, the second-order polynomial model for each studied factor is described as follows:

$$\text{Polyphenol} = 6.50707 + 0.0093523\times X_1 - 0.0283436\times X_2 + 0.521561\times X_3 - 0.248984\times (X_1)^2 - 0.34692\times (X_2)^2 + 0.0365987\times (X_3)^2 + 0.0381311\times (X_1X_2) + 0.00245605\times (X_1X_3) - 0.0606932\times (X_2X_3) \quad (1)$$

$$\text{Saponin} = 0.106814 + 0.00353323\times X_1 - 0.000572874\times X_2 + 0.00495565\times X_3 - 0.0223997\times (X_1)^2 - 0.0237329\times (X_2)^2 - 0.0240112\times (X_3)^2 + 0.000159526\times (X_1X_2) - 0.00237321\times (X_1X_3) + 0.00052481\times (X_2X_3) \quad (2)$$

$$\text{Chlorophyll} = 14.8276 + 5.77295\times X_1 - 0.399329\times X_2 + 0.705593\times X_3 - 0.67674\times (X_1)^2 - 1.41094\times (X_2)^2 - 0.932835\times (X_3)^2 - 0.686346\times (X_1X_2) - 0.00966958\times (X_1X_3) + 0.14648\times (X_2X_3) \quad (3)$$

$$\text{Antioxidant activity} = 5.61069 - 0.243969\times X_1 + 0.106026\times X_2 + 0.169132\times X_3 - 0.718512\times (X_1)^2 - 0.359693\times (X_2)^2 - 0.588824\times (X_3)^2 + 0.0488746\times (X_1X_2) - 0.173444\times (X_1X_3) + 0.121528\times (X_2X_3) \quad (4)$$

$$\text{Extraction yield} = 21.8913 + 5.36333\times X_1 - 0.321665\times X_2 + 0.800544\times X_3 - 2.83965\times (X_1)^2 - 3.31598\times (X_2)^2 - 3.33331\times (X_3)^2 - 0.602885\times (X_1X_2) - 0.328667\times (X_1X_3) + 0.300627\times (X_2X_3) \quad (5)$$

The p-value in the ANOVA analysis for each component in the model was less than 0.05, indicating that the polynomial models are statistically significant. Ethanol concentration, extraction time, and temperature had the greatest impact on the investigated bioactive compounds, especially TPC. The R^2 values of the objective functions were close to 1 (for polyphenol, extraction

yield, saponin, chlorophyll, and antioxidant activity, the R^2 values were 0.994, 0.995, 0.993, 0.997, and 0.995, respectively). All adjusted R^2 values were greater than 0.80, indicating good compatibility and fit between the experimental data and the obtained second-order polynomial equations. In addition, the suitability of the model was evaluated through the Lack-of-fit test. The p-value of the Lack-of-fit was greater than 0.05, indicating that the selected model is appropriate for describing the observed data. With the obtained optimal protocols, the optimization algorithm was performed on Modde 5.0 software, yielding the following results: the optimal conditions for efficient extraction of bioactive compounds from *Gynostemma pentaphyllum* were an ethanol concentration of 80%, a time of 114.67 minutes, and a temperature of 79.17°C; under these optimal parameters, the polyphenol content reached 0.51 ± 0.0005 mg GAE/g dry sample; antioxidant activity reached 1.54 ± 0.0002 mg Vitamin C/g dry sample; saponin content reached 8.26 ± 0.0001 mg OA/g; chlorophyll content reached 0.11 ± 0.0002 mg/ml and the extraction yield reached 21.53%. To validate the usability of the obtained optimal parameters, three validation experiments were conducted. Based on practical conditions, the optimal parameters were adjusted as follows: ethanol concentration 80%, time 115 minutes, and temperature 80°C. The results obtained are shown in Table 3.1

Table 3.1: Optimal parameters

Parameters	Reality content	Predicted content
TPC (mg GAE/g)	0.51 ± 0.01	0.51 ± 0.0005
AC (mg Vitamin C/g)	1.54 ± 0.08	1.54 ± 0.0002
TSC (mg OA/g)	8.25 ± 0.001	8.26 ± 0.0001
TCC (mg/g)	0.11 ± 0.0001	0.11 ± 0.0002
EY (%)	21.48	21.53

Table 3.1 shows that these maximum objective function results (actual) do not differ significantly from the predicted values ($p>0.05$). This result demonstrates that the obtained optimization results are completely consistent with reality and have high reliability.

Table 3.2: Results of optimization treatments affecting the extraction process

Runs	X_1 (%)	X_2 (minutes)	X_3 (°C)	TPC (mg GAE/g)	EY (%)	TSC (mg OA /g)	TCC (mg/g)	AC (mg VitaminC/g)
1	50	90	60	0.36	4.13	1.68	0.02	0.79
2	80	90	60	0.35	18.00	3.51	0.09	1.32
3	50	150	60	0.36	4.31	1.44	0.02	1.33
4	80	150	60	0.37	15.41	3.04	0.08	1.48
5	50	90	80	0.49	5.13	3.58	0.02	1.58
6	80	90	80	0.49	17.73	3.87	0.10	1.24
7	50	150	80	0.46	6.46	3.78	0.03	2.02
8	80	150	80	0.48	16.42	3.82	0.09	1.31
9	39.77	120	70	0.41	2.21	3.14	0.01	1.22
10	90.23	120	70	0.41	21.28	4.72	0.11	1.06
11	65	69.54	70	0.38	10.92	3.77	0.05	1.51

12	65	170.46	70	0.37	10.00	3.53	0.05	2.02
13	65	120	53.18	0.39	9.55	2.55	0.05	1.17
14	65	120	86.82	0.59	12.77	4.81	0.07	1.68
15	65	120	70	0.49	21.92	14.74	0.07	2.39
16	65	120	70	0.49	22.10	14.30	0.07	2.39
17	65	120	70	0.48	22.00	15.45	0.07	2.41

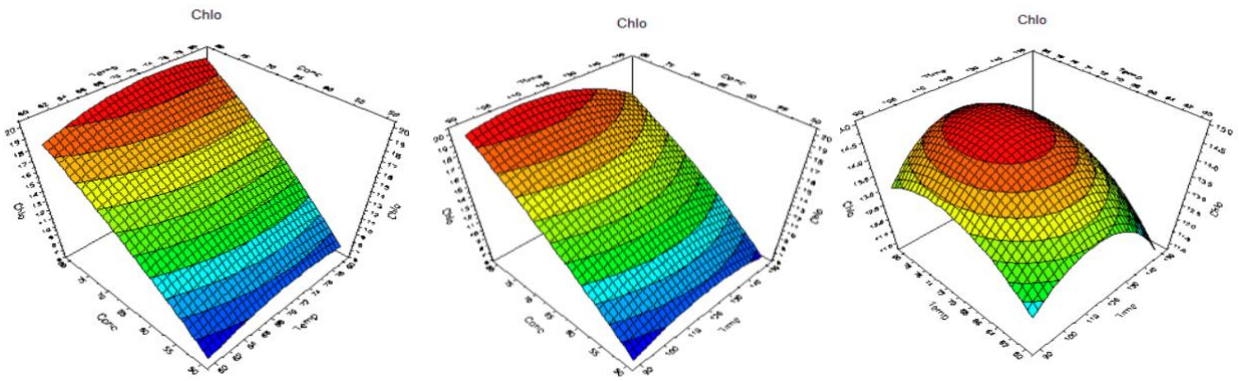

Figure 3.4: Response surface model illustrating the effect of ethanol concentration, time, and temperature on chlorophyll content

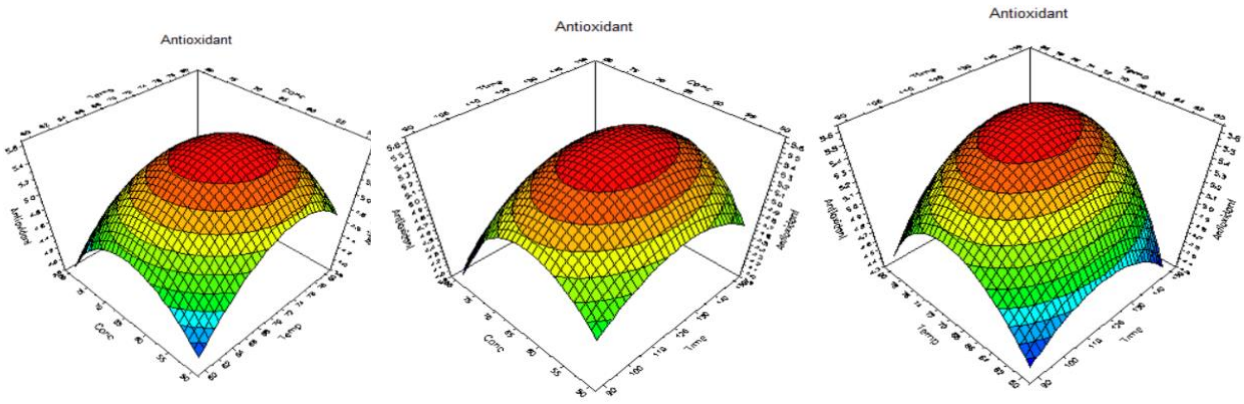

Figure 3.5: Response surface model illustrating the effect of ethanol concentration, time, and temperature on antioxidant activity

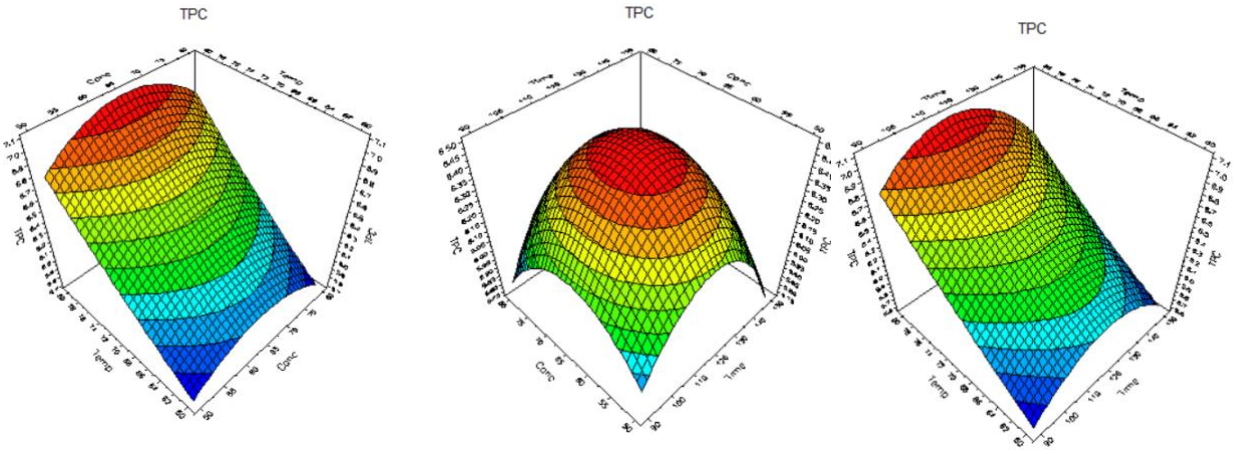

Figure 3.6: Response surface model illustrating the effect of ethanol concentration, time, and temperature on TPC

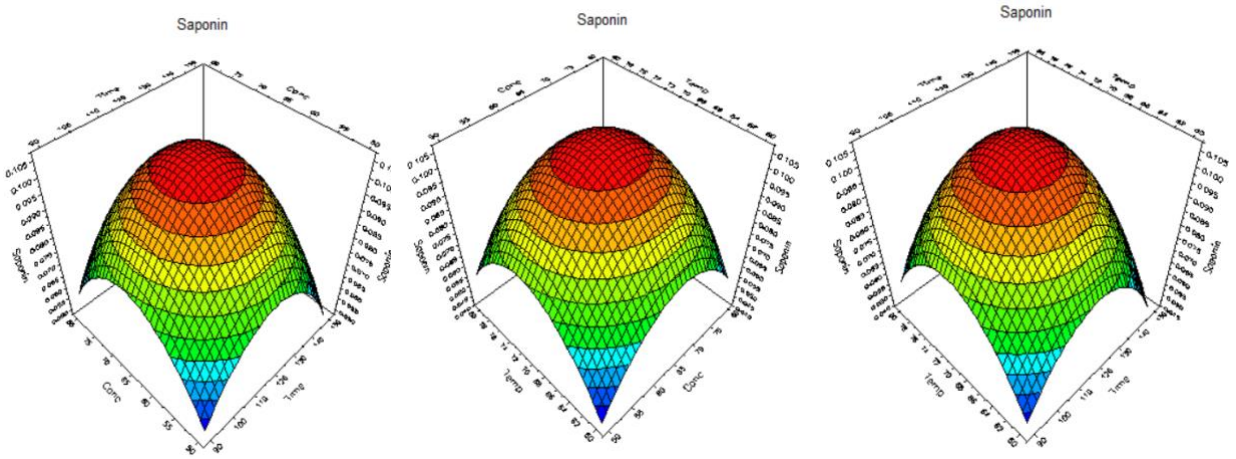

Figure 3.7: Response surface model illustrating the effect of ethanol concentration, time, and temperature on Saponin content

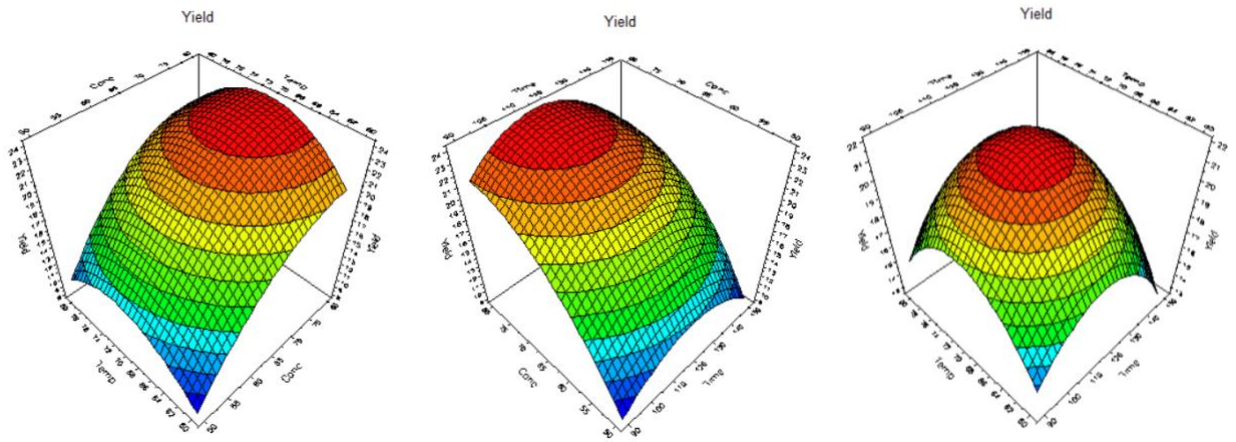

Figure 3.8: Response surface model illustrating the effect of ethanol concentration, time, and temperature on extraction yield

The above research results show that the extraction process of bioactive substances from Gynostemma pentaphyllum can be effectively optimized by using the RSM method. The factors of ethanol concentration, extraction time and temperature all have significant effects on the extraction efficiency and the optimal conditions obtained (ethanol concentration 80%, time 115 minutes, and temperature 80°C) were determined to achieve the highest extraction efficiency. This result not only provides the basis for further research but can also be applied in industrial production to maximize the biological activities of Gynostemma pentaphyllum.

4. Conclusion

This study successfully optimized the extraction process of bioactive compounds from *Gynostemma pentaphyllum* using Response Surface Methodology (RSM), determining that factors such as ethanol concentration, time, and temperature significantly influence the extraction efficiency and the content of bioactive compounds including polyphenol, saponin, chlorophyll, and antioxidant activity. The optimal conditions achieved were an ethanol concentration of 80%, an extraction time of 115 minutes, and a temperature of 80°C, yielding the following values: polyphenol (0.51 ± 0.01 mg GAE/g dry sample), saponin (8.26 ± 0.001 mg OA/g dry sample), chlorophyll (0.11 ± 0.0001 mg/ml), antioxidant activity (1.54 ± 0.08 mg Vitamin C/g dry sample), and extraction yield (21.53%). The optimized model was validated with experimental results showing no significant difference compared to the predicted values ($p > 0.05$), confirming the reliability and suitability of the study. This result provides a scientific basis for applying the extraction process in industrial production to effectively exploit the bioactive compounds of *Gynostemma pentaphyllum* for the food, pharmaceutical, and medical fields, contributing to enhancing the utilization value of the medicinal plant and developing natural health-supporting products.

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