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Original Research Article

Process optimization and insecticidal activity of alkaloids from the root bark of *Catalpa ovata* G. Don by response surface methodology

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Abstract

Purpose: *To optimize the extraction of total alkaloids from the root bark of Catalpa ovata using response surface methodology, and to determine the insecticidal activity of the total alkaloids extracted.*
Methods: *A combination of Box-Behnken design and response surface methodology (RSM) was used to optimize the acid water extraction of total alkaloids from the root bark of Catalpa ovata, with extraction rate of total alkaloids as index and the single factor experiment as basis, and the extraction time, material: liquid ratio and pH as 3 factors. The insecticidal activity of total alkaloids was determined against the three instar armyworm Mythimna separata (Lepidoptera: Noctuidae) and diamondback moth Plutella xylostella (Lepidoptera: Plutellidae).*
Results: *The optimum extraction conditions for total alkaloids were: material: liquid ratio of 1:10; extraction time of 3 h, pH of 1.0, and simmering. Under these conditions, total alkaloid extraction was 8.62 %, which was very close to the experimental value. The results were accurate and reliable, with reference value. The insecticidal activity of the total alkaloids indicate that when the concentration of the total alkaloids was 10 mg/L, the fatality rate of Plutella xyllostella and oriental armyworm was over 89 %, but the insecticidal activity of the total alkaloids was lower than that of avermectin which was employed as a reference.*
Conclusion: *The alkaloids from the root bark of Catalpa ovata are potential botanical insecticides.*

Keywords: *Catalpa ovata, Root bark, Total alkaloids, Response surface methodology, Insecticidal activity*

INTRODUCTION

Catalpa ovata G. Don belongs to family of Bignoniaceae. The phloem and root bark of *Catalpa ovata* are used for medicinal purposes, and its tender leaves are edible [1]. It has been shown that *Catalpa ovata* has insecticidal and diuretic properties [2]. The plant is a source of good wood for making furniture, and it is a shade tree because of its fast-growth [3]. There are lots of wild *Catalpa ovata* in the southern part of Hunan Province, China, where local residents use its root bark for medicinal purposes, such as treating dysentery and killing insects.

The main chemical components of *Catalpa ovata* are iridoids [4–6], naphthoquinones [7,8], phenolic compounds [9], alkaloids [10,11], and a new class of stibene glucosides [12]. Alkaloids have several physiological properties such as anti-cancer, analgesic, anti-virus, anti-inflammation and insecticidal properties [13]. Preliminary studies have found that the main chemical compositions of the root bark of *Catalpa ovata* are alkaloids [10,11]. Alkaloids are secondary metabolites formed as a result of co-habitation of plants and insects [14]. They have high bioactivity, unique modes of action, and are of multiple types [15]. They can be used as a lead compounds for the isolation of active substances with high activity, thereby providing a reference for development of new pesticides. Currently, there are limited reports on the alkaloids of the root bark of *Catalpa ovata*.

Response surface methodology (RSM), an effective optimization process, can be used to determine the interaction and impact of various factors on response value during a process, and presents exact relationship between response value and orthogonal design [17]. In this study, the extraction process of alkaloids from the root bark of *Catalpa ovata* was optimized via RSM and the insecticidal activity of the total alkaloids was studied with the aim of theoretically supporting the development of the genus as a bio-pesticide.

EXPERIMENTAL

Materials

The root bark of *Catalpa ovata* was provided by Hunan Qiyang Yongyuan Biological Technology Co. Ltd. (Yongzhou City, China, Product Code: 161109). The sample was washed, dried and selected through a screen mesh with 0.25 mm aperture.

Reagents

Hydrochloric acid was from Tianjin Damao Chemical Reagent Factory, China; concentrated ammonia was purchased from Tianjin Oubei Kai Chemical Co., Ltd., China; dichloromethane was obtained from Guangzhou Cutai Trade Co., Ltd., China, while ethyl acetate ((Klamar) was product of Shanghai Fortuneibo-tech Co. Ltd, China. Bismuth potassium iodide (Jining Huakai Resin Co., Ltd.) and acetone (Shanghai Yuqin Chemical Co., Ltd.) were of analytical grade.

Instruments

The instruments used were PHS-802 Acidity Meter (Guiyang Xuetong Instruments Co., Ltd.), AY220 electronic analytical balance (Japan Shimadzu), DHG-9070AS electric constant Temperature oven on forced convection (Ningbo Jiangnan Instruments Co., Ltd.), and CN61M/F120 plants mill (Beijing Zhongxi Yuanda Science and Technology Co., Ltd.).

Studies on optimization of extraction

The powder of the root bark (30 g) was weighed. Solutions of various pH, extraction time and solid: liquid ratio were considered as single factors and their effects on the extraction of alkaloids of the root bark of *catalpa ovata* were studied on the basis of single factor experiments. Solution pH, extraction time and solid: liquid ratio were taken as three factors and the extraction rate of alkaloids as index. An RSM with three factors and three levels was carried out, involving 17 experimental points and 5 central points. Software design-Expert 8.0 was used to regressively analyze the experimental data and calculate the mathematical model, so as to confirm the optimizing process conditions for extraction of alkaloids from *Catalpa* ovate root bark.

Extraction of the total alkaloids

According to the optimized process conditions of RSM, 30 g of root bark powder *Catalpa ovata* was extracted with distilled water using known solid-liquid ratio, and the pH of the extracting medium was adjusted with 0.5 % HCl. Then extraction was continued for some time under simmering, and the mixture was filtered to remove residues. The fat-soluble portion was extracted using acetic ether, and the pH was adjusted to 9 - 10 with concentrated aqueous ammonia. The solution was re-extracted with dichloromethane until no alkaloid was detectible using bismuth potassium iodide test. The extracts were pooled, dried using anhydrous sodium sulfate and concentrated through decompression to get the total alkaloids.

Detection of total alkaloids

The total alkaloids were qualitatively identified with iodine-potassium iodide, Dragendorff's reagent and Bertrand's reagent as described earlier [18].

Determination of insecticidal activity

Three different concentrations of the total alkaloids of the root bark of *Catalpa ovata* (600mg/L, 100mg/L and 10 mg/L) were prepared in acetone for use in the determination of insecticidal activity against *Plutella xyllostella* (Linnaeus) and oriental armyworm, using Airbrush [19]. Cabbage leaves (for feeding *Plutella xyllostella*) were punched into 2 cm diameter disks using a punching bear, and corn leaves (for feeding oriental armyworm) were cut into 2cm sections with a disinfected pair of scissors. The front and back of each leaf disc were sprayed with Airbrush at a pressure of 10 psi (about 0.7 kg/cm^2) and spray volume of 0.5 mL. The leaf disks were shade-dried, and 15 third instar test insects were placed on each of them. The leaf discs with test insects were placed in an environment with a relative humidity of 60 to 70 % at 25 ℃. Three days later, the number of surviving insects was counted to calculate the mortality rate. Avermectin was used as positive control.

Statistical analysis

All data are presented mean ± standard deviation (SD). Comparisons between different groups were done with analysis of variance (ANOVA). $P < 0.05$ was taken as indicative of statistically significant difference.

RESULTS

Total alkaloids of *Catalpa ovata* root bark

The results of qualitative analysis with iodine-potassium iodide, Dragendorff's reagent and Bertrand's reagent showed amorphous precipitate, brownish-red precipitate and crystalline precipitate respectively which indicated presence of alkaloids.

Effect of solid-liquid ratio on the extraction of alkaloids from *Catalpa ovata*

With extraction time of 3 h and pH of 2, the effect of different solid-liquid ratios (1:6, 1:8, 1:10, 1:12 and 1:14) on the extraction rate of alkaloids from the root bark of *Catalpa ovata* are shown in Figure 1. As the solid-liquid ratio increased, the extraction rate of the total alkaloids increased initially and then decreased. The possible reason was that the concentration differences between the inside and the outside of cells gave rise to the changes in the extraction rate of the total alkaloids in the root bark of *Catalpa ovate*. The larger the volume of solvent, the bigger the concentration difference between the crude material and the solvent, thereby increasing the mass transfer rate between them and broadening the extraction rate of the total alkaloids. At a certain solvent concentration, the alkaloids were completely extracted, that the extraction rate subsequently decreased. The results show that the optimum solid-liquid ratio was 1:10.

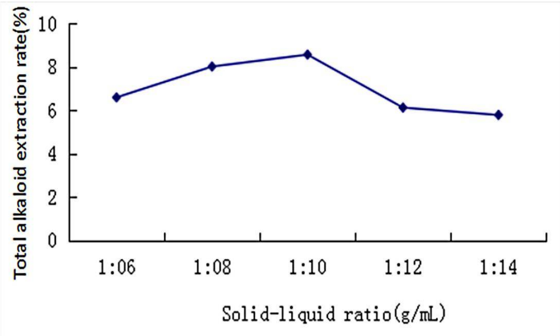

Figure 1: Effect of solid-liquid ratio on amount of extracted alkaloids in the root bark of *Catalpa ovata*

Effect of extraction time on the extraction rate of the total alkaloids

The extraction rates of the total alkaloids at 1, 2, 3, 4 and 5 h with solid-liquid ratio of 1:10 and pH 2 are presented in Figure 2. With increase in extraction time, the extraction rate of the total alkaloids increased gradually, reached a peak at 3 h, and then decreased gradually.

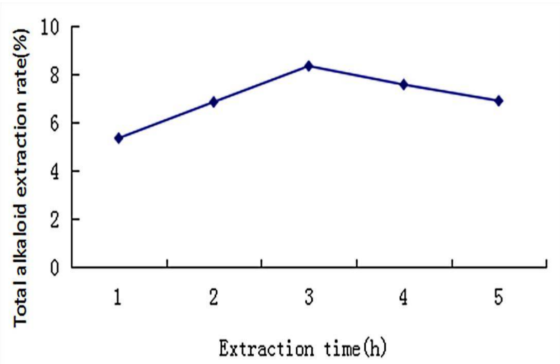

Figure 2: Effect of extraction time on amount of alkaloids extracted from the root bark of *Catalpa ovata*

Effect of pH on the extraction of the total alkaloids

The effect of different pH (1.0, 1.5, 2.0, 2.5 and 3.0) on the extraction of the total alkaloids from the root bark of *Catalpa ovata* at solid-liquid ratio of 1:10 and extraction time of 3 h is shown in Figure 3. With increasing pH, the extraction of the total alkaloids in the root bark of *catalpa ovata* increased, but when pH exceeded 1.5, it decreased rapidly. The most favorable pH was 1.5.

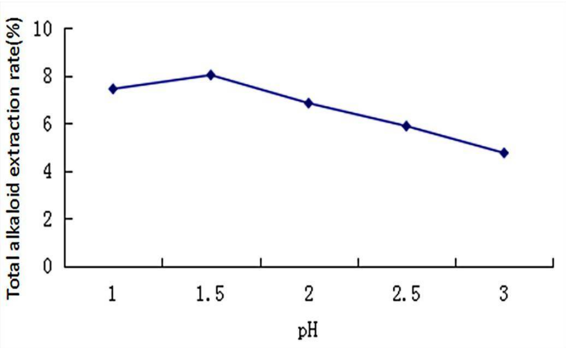

Figure 3: Effect of pH on the extraction of alkaloids from the root bark of *Catalpa ovata*

Response surface methodology data

On the basis of single factor experiment with extraction time, extraction temperature and solid-liquid ratio as examining factors, an RSM test with three factors and three levels was performed to optimize the extraction process of the total alkaloids. Box-Behnken was used for center group and design, with a total of 17 experimental points. Among the points, 12 were analysis points and 5 central points used to evaluate deviation. The factors and levels of RSM test are shown in Table 1. The conceptual design and results are shown in Table 2.

Table 1: Factors and levels of Box-Behnken design

Level	X_1 Solid-liquid ratio (g/mL)	X_2 Extraction time (h)	X_3 pH
-1	1:8	2	1.0
0	1:10	3	1.5
1	1:12	4	2.0

Table 2: Results of Box-Behnken

No.	Factors			Extraction of total alkaloids (%)
	X_1	X_2	X_3	
1	0	0	0	8.16
2	-1	0	-1	7.83
3	0	0	0	8.28
4	1	0	-1	7.56
5	1	0	1	7.29
6	1	1	0	5.54
7	-1	1	0	5.89
8	0	0	0	8.24
9	0	0	0	8.33
10	0	1	-1	6.79
11	1	-1	0	5.21
12	-1	-1	0	5.62
13	0	1	1	6.36
14	0	-1	-1	6.48
15	-1	0	1	7.48
16	0	0	0	8.06
17	0	-1	1	6.15

The data in Table 2 were analyzed by software Design-Expert 8.0 and regressively fitted with quadratic polynomial to get a quadratic multiple regression equation relating the extraction rate of the total alkaloids and independent variables: $Y=8.21-0.18*X_1+0.19*X_2-0.097*X_3+0.015*X_1*X_2+0.070*X_1*X_3+0.025*X_2*X_3-0.83*X_1^2-1.82*X_2^2+0.10*X_3^2$. The correlation coefficient between the extraction rate of the total alkaloids in the response value and the regression equation of the predicted value was equal to 0.9925, which indicated a very good fit. The outcomes of ANOVA on this model are presented in Table 3.

From Table 3, the extraction of alkaloids was affected in the order B > A > C, i.e. extraction time > solid-liquid ratio > pH. Amongst these, A, B, A^2 and B^2 were most significant ($p < 0.01$). The response surface map and contour map of interaction between the factors are depicted in Figure 4, Figure 5 and Figure 6. The best process conditions were: solid-liquid ratio of 1:9.7 (g/mL), extraction time of 3.05 h, and pH 1.0. Under these conditions, the total alkaloid extraction was 8.44 %. The optimal extraction process conditions were adjusted to: extraction time of 3.0 h, solid-liquid ratio of 1:10 (g/mL), pH 1.0, and distilled water as extractant at simmering state. Three portions of root bark powder of *Catalpa ovata* (each 30 g) were used for confirmatory experiment. The findings of the confirmatory experiment revealed that the mean extraction yield of the total alkaloids was 8.38 %, which approximated to the predicted value, demonstrating that these process conditions were reliable and precise.

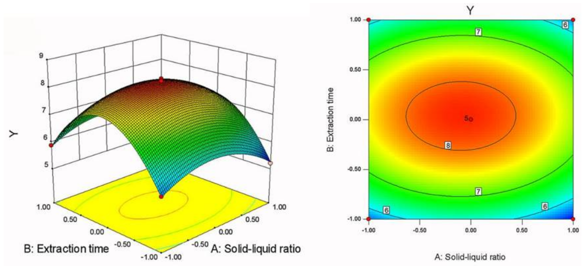

Figure 4: Interaction effect of solid-liquid ratio and extraction time on extraction yield of the root bark of *Catalpa ovata*

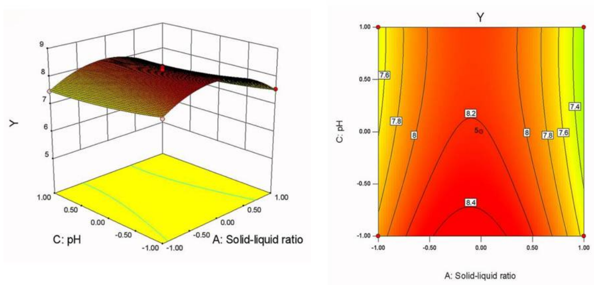

Figure 5: Interaction effect of pH and solid-liquid ratio on extraction yield of the root bark of *Catalpa ovata*

Table 3: ANOVA data from regression analysis

Source	Sum of Squares	df	Mean Square	F-Value	p-Value	Significance
Model	18.22	9	2.02	237.72	< 0.0001	**
X_1	0.25	1	0.25	29.59	0.0010	**
X_2	0.29	1	0.29	33.91	0.0006	**
X_3	0.076	1	0.076	8.93	0.0203	*
X_1X_2	9.000×10^{-4}	1	9.000×10^{-4}	0.11	0.7546	
X_1X_3	0.020	1	0.020	2.30	0.1731	
X_2X_3	2.500×10^{-3}	1	2.500×10^{-3}	0.29	0.6048	
X_1^2	2.88	1	2.88	338.11	< 0.0001	**
X_2^2	13.98	1	13.98	1641.12	< 0.0001	**
X_3^2	0.045	1	0.045	5.24	0.0558	
Residual	0.060	7	8.517×10^{-3}			
Lack of Fit	0.015	3	4.833×10^{-3}	0.43	0.7439	Not significant
Pure Error	0.045	4	0.011			
Core total	18.28	16				
		$R^2 = 0.9967$; $Adj.R^2 = 0.9925$				
		CV % = 1.32 %				

***Extremely obvious, * obvious; p < 0.01 was extremely obvious, p < 0.05 was obvious*

Table 4: Insecticidal potential of the total alkaloids after 72 h

Agent	Fatality of *Plutella xyllostella* (%)			Fatality of oriental armyworm (%)		
	600	***100***	***10***	***600***	***100***	***10***
Total alkaloids of *Catalpa ovata*	100	100	94.61	100	98.64	89.72
Avermectin	100	100	100	100	100	100

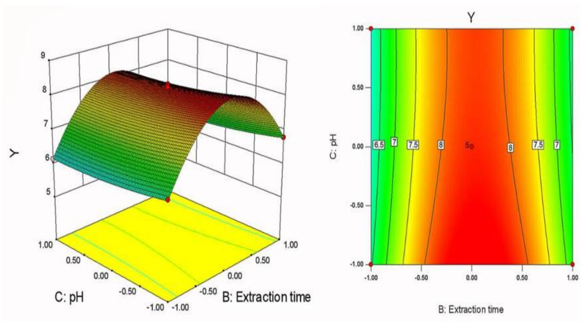

Figure 6: Effect of interaction of pH and extraction time on extraction yield of alkaloids from the root bark of *Catalpa ovata*

Insecticidal activity

The results of insecticidal tests on *Plutella xyllostella* and oriental armyworm are shown in Table 4. A small dose of the total alkaloids was able to kill *Plutella xyllostella* and oriental armyworm, thereby showing appreciable biocontrol potential of the extract. When the alkaloid concentration was 10 mg/L, it killed more than 89 % of *Plutella xyllostella* and oriental armyworm, but its activity was less than that of the standard drug avermectin.

DISCUSSION

The quadratic multiple regression equation relating the extraction of the total alkaloids in the root bark of *catalpa ovata* and independent variables was $Y=8.21-0.18*X_1+0.19*X_2-0.097*X_3+0.015*X_1*X_2+0.070*X_1*X_3+0.025*X_2*X_3-0.83*X_1^2-1.82*X_2^2+0.10*X_3^2$. The correlation coefficient between the extraction of the total alkaloids in the response value and the regression equation of the predicted value was 0.9925, which implies a good fit. The order of impact of factors on the extraction rate of the total alkaloids was extraction time > solid-liquid ratio > pH.

After optimization by Box-Behnken, the best extraction process conditions were solid-liquid ratio of 1:10 (g/mL), extraction time of 3 h, and pH 1.0. Under these conditions, the extraction yield was 8.539 %. Under the same conditions, the experimental yield was 8.38 %. This shows that the experimental data optimized by RSM were accurate and reliable, and have some reference value. The insecticidal activity of the total alkaloids in the root bark of *catalpa ovata* indicated that when the concentration of the total alkaloids was 10 mg/L, the fatality rate of *Plutella xyllostella* and oriental armyworm was over 89%, but the insecticidal activity of the total alkaloids was lower when compared with avermectin. However, it is possible that a single alkaloid from this extract may have stronger insecticidal activity than avermectin. Besides, an alkaloid with new chemical constitutions may be present in the root bark of *Catalpa ovata*, so the total alkaloids can be considered as a lead compound.

CONCLUSION

The findings of this study show that the alkaloids obtained from the root bark of *Catalpa ovata* are potential botanical insecticides.

DECLARATIONS

Acknowledgement

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Conflicts of interest

No conflict of interest is associated with this work.

Contributions of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Jinhua Shao and Fulin He conceived and designed the experiments; Jinhua Shao and Yufei Zhang performed the experiments; Xiaoming Chen and Zhiyong Zhu analyzed the data; Fulin He contributed reagents/materials/ analysis tools; Jinhua Shao prepare the manuscript which was read and approved by all authors.

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