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Study on extraction technology and antioxidant activity of total alkaloids from *Hemsleya chinensis* based on orthogonal design and BP neural network

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ABSTRACT

In this study, total alkaloids from *Hemsleya chinensis* were extracted and tested for their antioxidant properties. To optimize extraction methods, a single factor experiment was conducted to determine the total alkaloid concentrations of *H. chinensis* using the L_9 (3^4) orthogonal design test method and the BP neural network (BPNN), resulting in the optimum extraction conditions for total alkaloids. The optimal conditions for *H. chinensis* alkaloids extraction with acid water are: HCl concentration is 0.50 %, extraction temperature is 85 °C, material-liquid ratio is 1:64.5, and extraction rate of alkaloids is 0.2785 ± 0.0003 mg/mL. The alkaloid from *H. chinensis* exhibited antioxidant activity in a quantity-effect relationship with activity. These findings showed that the procedure to be reasonable, the alkaloid extraction efficiency to be high, and the method could be used to extract the alkaloids of *H. chinensis*, improving the development of natural and healthy medicinal resources for the pharmaceutical and food industries.

1. Introduction

Alkaloids are secondary metabolites found in plants, which are the main organic compounds [1]. Recent studies showed that the alkaloids have high active values associated with anti-cancer, anti-diabetic and anti-inflammatory [2]. Increasing attention has been paid to antioxidants due to their essential role in maintaining health and preventing diseases. Thus, some research have been investigated the effects of natural antioxidants in medical plants [3–5]. The antioxidants activities are typically measured using ABTS, DPPH and FRAP assays [6–8].

A genus of Cucurbitaceae family, *Hemsleya* contains more than thirty species in tropical and subtropical areas of China [9]. Traditional Chinese medicine has traditionally used the tubers of these plants. This genus has been analyzed and evaluated for its phytochemical composition, which included diterpenes, alkaloids, and cucurbitane-type triterpene [10–13]. Additionally, this genus

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contains alkaloid constituents [14]. However, few research have been studied regarding the alkaloid.

For the purpose of further separating and characterizing the chemical components, extraction medicinal plant constituents is the first important step in the analysis of medicinal plant. BP neural network (BPNN), short for error back propagation neural network, is a machine learning method commonly used for image recognition, function approximation, and pattern recognition [15]. Genetic algorithm (GA) simulates the biological evolution process in nature and is a simple, efficient, and easy to operate optimization algorithm in computer mathematics [16]. Recently some GA-BPNN model applied precise extraction/purification [17–19]. Thus, herbal products are likely to be of high quality if extraction techniques are optimized.

Here, based on the results of a single factor experiment, different combinations of temperature, material-liquid ratio and HCl concentration (%) were investigated by orthogonal design, and the optimal extraction process was optimized by orthogonal analysis and BP neural network-genetic algorithm. Application of this optimal extraction process obtain alkaloids from *Hemsleya chinensis*, and evaluate the antioxidant activity of the total extract using several biochemical assays.

2. Materials and methods

2.1. Chemicals and reagents

Hemsleya chinensis was obtained from Yunnan Kezhao Biotechnology Development Co., Ltd. (Kunming, Yunnan Province, China). Ethanol (95 %), hydrochloric acid (≥36 %), sodium hydroxide, ferrous sulfate, salicylic acid, hydrogen peroxide, potassium ferricyanide, trichloroacetic acid, ferric chloride, pyrogallol and ascorbic acid were purchased from Tianjin Fengchuan Chemical Reagent Science and Technology Co., Ltd (Tianjin, China). 2,2-diphenyl-1-picrylhydrazil (DPPH) and 2,2′-azino-bis(3-ethylbenzthiozoline-6) sulfonic acid (ABTS) were purchased from Sigma Chemical (Louis, MO, USA). Trichloroacetic acid, $CHCl_3$ and ascorbic acid with analytical grade were obtained from Tianjin Fengchuan Chemicals and Reagents Co., Ltd (Tianjin, China).

2.2. Determination of total alkaloids content

An analysis of the total alkaloids content was carried out [20]. After the solution was mixed, it was allowed to stand. A layer of chloroform ($CHCl_3$) was collected and its absorbance was measured at 418 nm. A standard curve was drawn based on the data $C = 0.4998A + 0.0081$ ($R^2 = 0.9996$) (A: absorbance, C: berberine hydrochloride concentration, mg/mL, n = 3), calculation of the total alkaloids could be done.

2.3. Preparation of Hemsleya chinensis samples

A 40-mesh sieve was used to grind the samples to particles after they had been cleaned, washed with distilled water, cut into small pieces, and dried overnight in an air dryer at 40 °C. The powder (5 g) was extracted with 250 mL of 0.5% HCl for 50 min at 70 °C.

The filtrate from extraction solution was collected by air pump filtration. The filtrate was adjusted with 5 % NaOH to pH 11–12, transferred into the 250 mL separating funnel and extracted with 100 mL $CHCl_3$ three times. Total alkaloid was obtained by combining the $CHCl_3$ layers and concentrating the filtrate.

2.4. Single factor test and Ortho experimental design

An experimental design using a single-factor experimental design was used to determine the effect of HCl concentration, material-liquid ratio, and extraction temperature on the yield of alkaloids. A triplicate of each experiment was performed. Further optimization of the experimental parameters was achieved through the single-factor experimental design. The optimization process was performed according to the orthogonal experiment design.

2.5. Optimization of orthogonal test data by genetic algorithm and BP neural network

Genetic algorithm and BP neural network were used to optimize the experiment. According to the actual characteristics of alkaloid extraction and combined with relevant literature, the parameters in the neural network toolkit and Genetic Algorithm toolbox (GA) in Matlab 2020a software were adjusted to meet the experimental needs.

To adjust the number of neurons in the model, a GA toolbox was used, and network optimization was performed, and four-parameter values including fitting error (%), prediction error (%), overall error (%), and model fitting degree were obtained.

2.6. Verification test

Six pieces of medicinal materials, each 5 g, were weighed and 3 batches were prepared for verification test using the optimal extraction process optimized by orthogonal test and BP neural network.

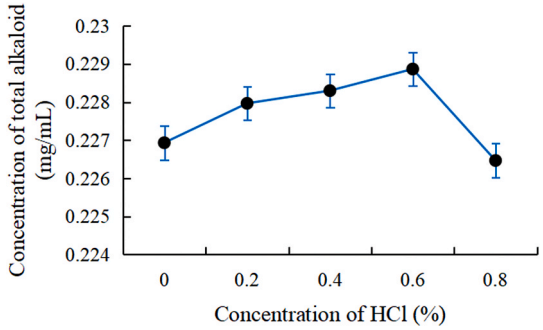

Fig. 1. Effect of HCl concentration on total alkaloids concentration of the *H. chinensis*.

2.7. Antioxidant activity test

2.7.1. Hydroxyl radical-scavenging activity assay

A UV–visible spectrophotometer was used to evaluate the radical-scavenging activity of hydroxyl radicals [21]. This equation was used to calculate radical scavenging activity:

$$\text{Hydroxyl radical scavenging activity } (\%) = (A_0 - A_i)/A_0 \times 100$$

At 510 nm, A_0 represents the absorbance of the blank control, while Ai represents the absorbance of the sample. Positive control was ascorbic acid, and blank control was distilled water.

2.7.2. Superoxide anion radical-scavenging activity assay

The 1.0 mL extracts (1, 3, 5, 7, and 9 mg/mL) were mixed with 1.0 mL of 45 mmol/L pyrogallol and 4.5 mL Tris-HCl (pH 8.2). For the scavenging activity assay at 400 nm, the mixture was incubated at 25 °C for 10 min and then stopped with 1.5 mL HCl (1 mol/L).

$$\text{radical scavenging activity } (\%) = [1\text{-}(\text{As-Ab})/\text{Ac}] \times 100$$

Where As represents the absorbance during the measurement of different alkaloid concentrations, Ab represents the absorbance during the measurement using distilled water rather than pyrogallol as a blank control. The negative control was distilled water instead of the sample for measuring Ac. Controls were performed with ascorbic acid.

2.7.3. DPPH radical-scavenging activity assay

The radical scavenging activity of the extracts was determined by using modified 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays [22]. A series of dilutions were performed using the stock solutions (2 mg/mL) of the extracts. 0.1 mM DPPH solution in methanol (2 mL) was then added to each solution (2 mL each). An incubation period of 30 min was then conducted at 37 °C after the reaction mixture had been vigorously shaken. At 517 nm, the absorbance was measured. The results of a control sample without extract were also calculated and expressed in DPPH radical scavenging activity (%):

$$\text{DPPH radical scavenging activity } (\%) = [(A_D - As)/A_D] \times 100$$

As represents the absorbance of the solution when the extract is added, and A_D represents the absorbance of the DPPH· solution. The positive control was ascorbic acid.

2.7.4. ABTS radical scavenging activity assay

We modified method [7] for the ABTS radical scavenging assay. The mixture of 7 mM ABTS and 2.5 mM potassium persulfate was incubated in an amber bottle at 4 °C in the dark for 12 h to produce ABTS + radical cations. The ABTS + stock solution was diluted with distilled water to get an absorbance of approximately 1.0 at 750 nm. Then, 20 μL of sample extract or standard solution was mixed thoroughly for 10 min with 1.0 mL ABTS + solution. An absorbance measurement was conducted at 750 nm. The following formula was used to calculate ABTS radical scavenging activity (%):

$$\text{ABTS radical scavenging activity } (\%) = [1\text{-}(A_1/A_0)] \times 100$$

A_1 represents the absorbance of the sample, and A_0 represents that of the control. Positive controls were ascorbic acid.

2.8. Statistical analysis

Analysis of the data was carried out using SPSS 19.0. Data were expressed as means ± standard errors. The P value for statistical significance was set at 0.05.

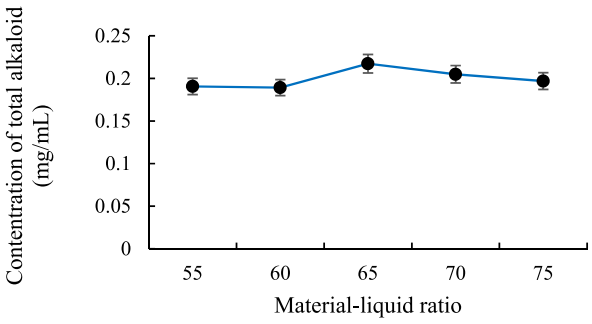

Fig. 2. The effect of the material-liquid ratio on the total alkaloid content of the *H. chinensis*.

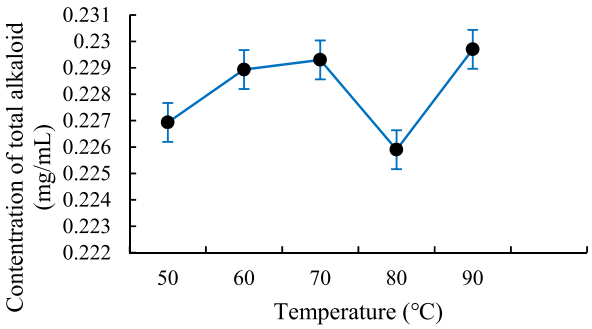

Fig. 3. Effect of extraction temperature on total alkaloid concentration of the *H. chinensis*.

Table 1
The factors and levels of the orthogonal test for determining the extraction rate of total alkaloids.

Levels	A: Temperature (°C)	B: Material-liquid ratio (mg/mL)	C: HCl concentration (%)
1	75	1:55	0.5
2	80	1:60	0.6
3	85	1:65	0.7

3. Results

3.1. Effects of HCl concentration on the total alkaloids content

The influences of HCl concentration on the total alkaloids content are shown in Fig. 1. HCl concentrations from 0.2 to 0.6 % yielded the highest total alkaloids contents, and reduced from 0.6 to 0.8%, respectively. The highest total alkaloids of *H. chinensis* was 0.2285 ± 0.0002 mg/mL at the concentration of 0.6 % HCl.

3.2. Effects of material-liquid ratio on the total alkaloids content

Material-liquid ratio has been investigated for its impact on total alkaloids (Fig. 2). The extraction content was increased from 1:50 to 1:65, although it was reduced from 1:65 to 1:70. The highest total alkaloids of *H. chinensis* was 0.2182 ± 0.0003 mg/mL at the material-liquid ratio of 1:65.

3.3. Effects of temperature on the total alkaloids content

An investigation of the effect of extraction temperature on alkaloids content was conducted (Fig. 3). The highest total alkaloid content of *H. chinensis* is 0.2295 ± 0.0002 mg/mL at the temperature of 90 °C. With an increase in extraction temperature from 50 °C to 90 °C, the total alkaloid content increased.

Table 2
The results of orthogonal optimization experiment in extraction process of total alkaloid.

Number	Factors			
	A: Temperature (°C)	B: Material-liquid ratio (g/mL)	C: HCl concentration (%)	Total alkaloid content (mg/mL)
1	1 (75 °C)	1 (1:55)	1 (0.5 %)	0.2744
2	1	2 (1:60)	2 (0.6 %)	0.2330
3	1	3 (1:65)	3 (0.7 %)	0.2376
4	2 (80 °C)	1	2	0.2326
5	2	2	3	0.2378
6	2	3	1	0.2768
7	3 (85 °C)	1	3	0.2367
8	3	2	1	0.2035
9	3	3	2	0.2761
K1	0.2433	0.2479	0.2527	
K2	0.2491	0.2248	0.2472	
K3	0.2388	0.2600	0.2354	
R	0.0103	0.0352	0.0172	

Table 3
GA-BP neural network optimization threshold and weight error.

Hidden layer neurons	MAE	MSE	RMSE
7	73.2131	5360.1642	73.2131

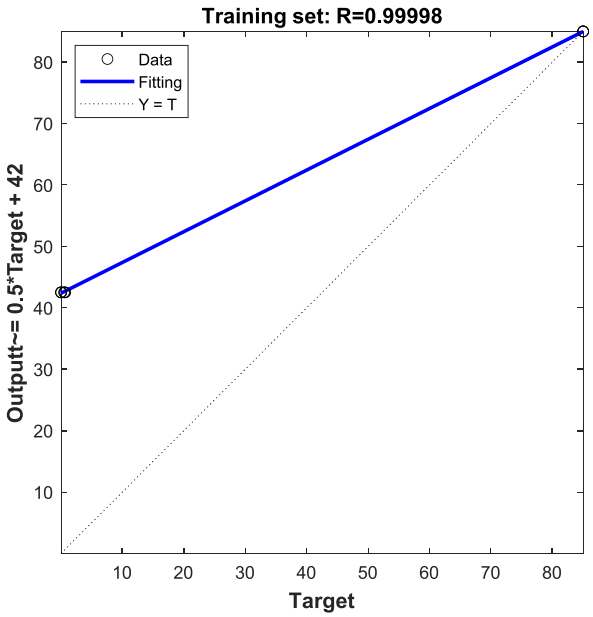

Fig. 4. The fitting degree of threshold and weight of GA-BP neural network.

3.4. Optimization of extraction process

Selecting an appropriate optimization method was the key to improve extraction rate. As a control index for the optimization process, total alkaloid content was expressed using an orthogonal experiment design (Table 1). Therefore, a three-level OAD with an OA 9 (3^4) matrix was chosen. OAD factors and levels for total alkaloid extraction are shown in Table 1.

Extraction effects were significantly affected by extraction temperature, concentration, and material-liquid ratio in the single factor experiment. Therefore, we conducted orthogonal experiments with these three variables. The optimization results are shown in Table 2.

The experimental data from the OAD optimization were interpreted using ANOVA. As shown in Table 2, the influence on the extraction content by the parameters decreased in the following order: B > C > A. The orthogonal results showed a decreasing total alkaloid content from *H. chinensis* with increasing HCl concentration, possibly because there existed interactions between total alkaloid

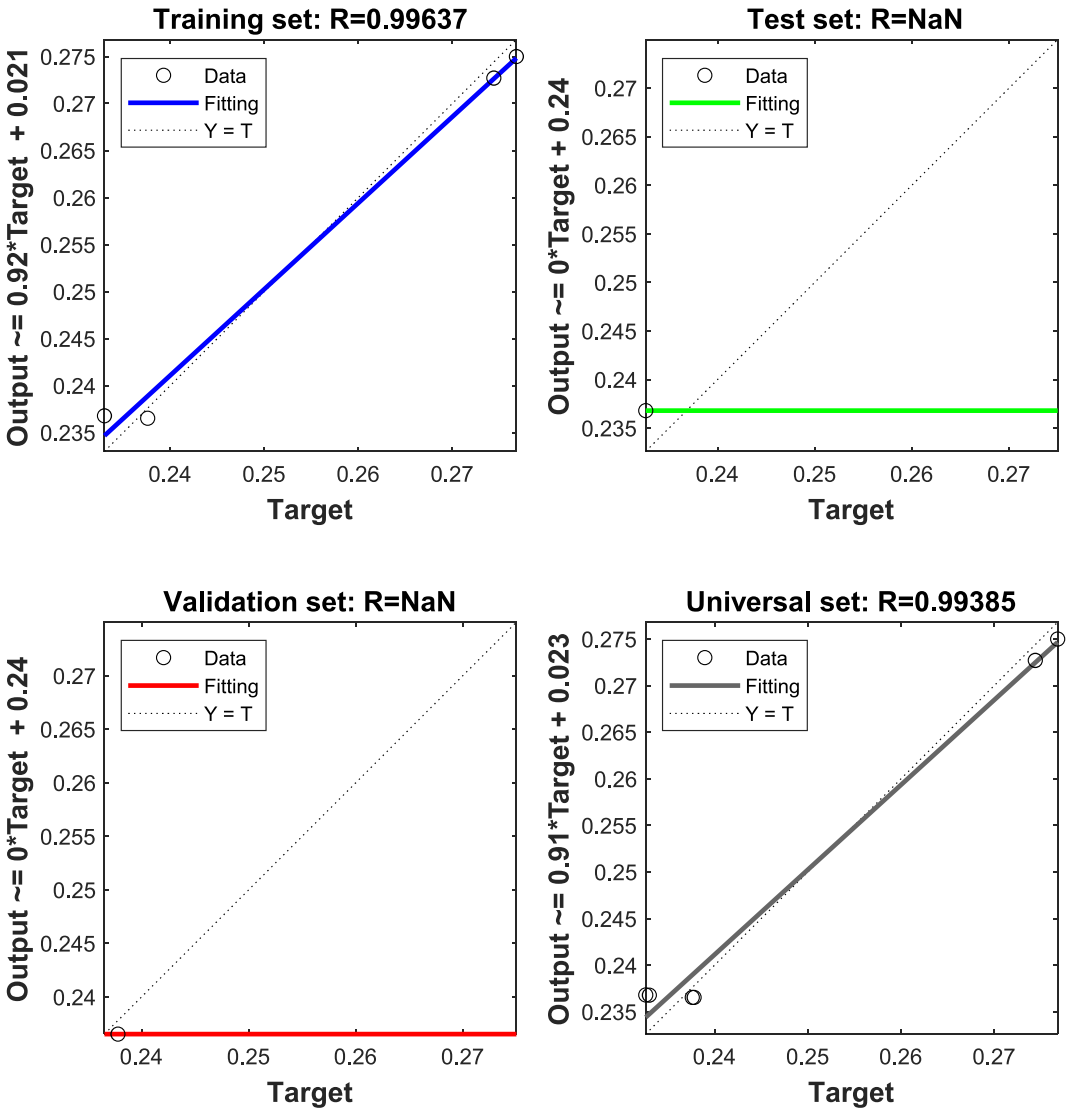

Fig. 5. The fitting degree of GA-BP neural network.

and HCl. The best for total alkaloid extraction conditions is A2B3C1.

3.5. Optimization results of orthogonal test data by genetic algorithm and BP neural network

3.5.1. Building a neural network

A 3-layer BP neural network was constructed by Matlab 2020a software, including 3 layers of input layer (A: temperature; B: solid liquid ratio; C: concentration of hydrochloric acid) and 7 layers of hidden layer (the number of hidden layer nodes will affect the prediction and generalization ability of BP neural network. According to the empirical formula, the number of hidden layer nodes is equal to the output layer *2 + 1, and the output layer is 3*2 + 1), output layer 1 (total alkaloid extraction rate). In neural networks, the output layer nodes use S-type logarithmic function logsig as their transfer function, while the hidden layer nodes use S-type tangent function tansig. Nine groups of experimental samples were divided into 2:1 groups in the orthogonal experiment, of which 6 groups were used as training samples and 3 as validation samples. Training times 1000, training target 0.00001, learning rate 0.1, and other parameters remain at their default values for the neural network.

3.5.2. Optimize the threshold and weight of neural network by genetic algorithm

The GA genetic algorithm is used to optimize the threshold and weight of the BP neural network in order to prevent the BP neural network from falling into local optimum during training and to improve the generalization ability of the model. The optimized threshold and weight are then applied to the existing BP neural network:

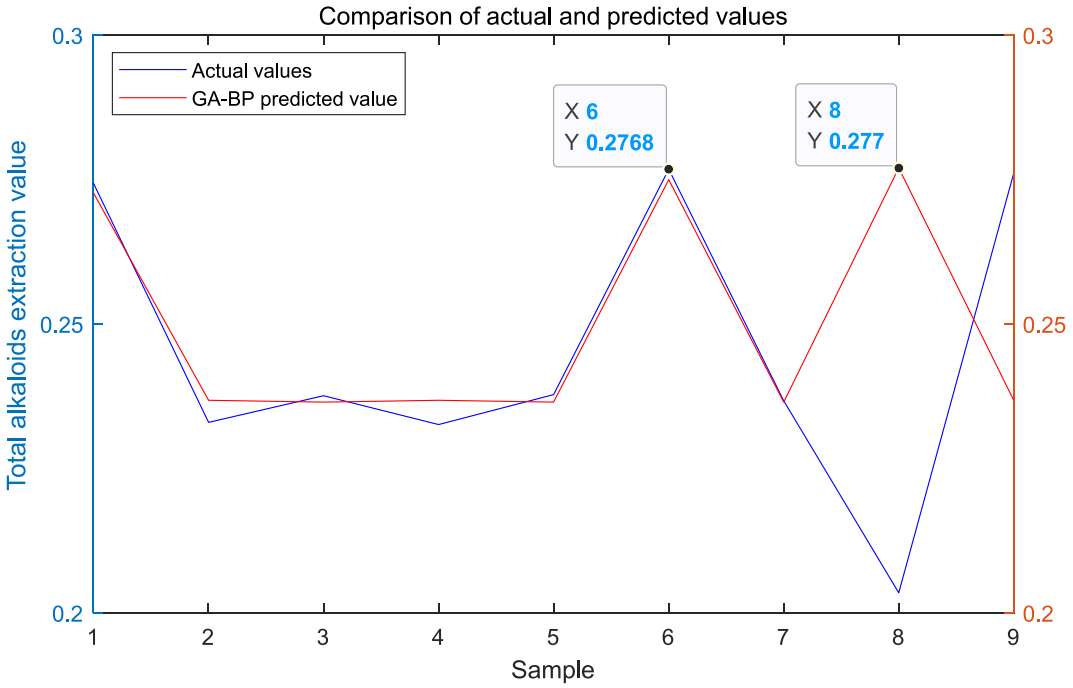

Fig. 6. Comparison of the actual value and the predicted value of GA-BP.

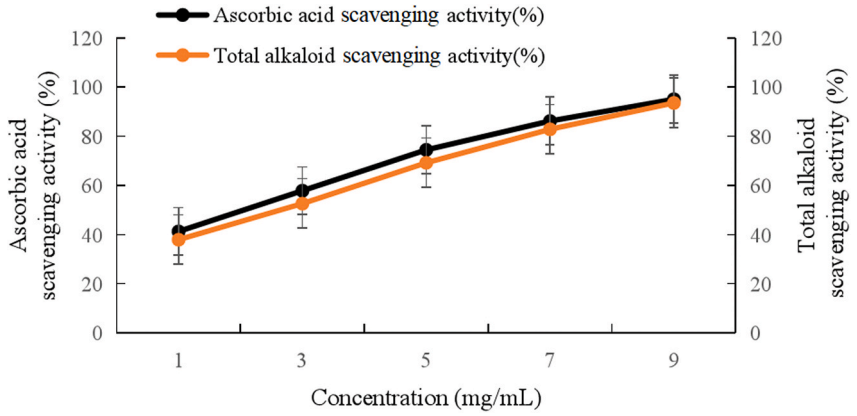

Fig. 7. Effect of total alkaloid on hydroxyl radical-scavenging activity.

Input the number of variables to be optimized N = number of neurons in the input layer * Number of neurons in the hidden layer + number of neurons in the output layer * Number of neurons in the hidden layer + number of neurons in the hidden layer + number of neurons in the output layer = 32.

The genetic algorithm parameters should be set as follows: population size 20, iteration times 100, individual length 10, generation gap 0.95, crossover probability 0.4, mutation probability 0.2, set the initial value of the optimization result, create any discrete random population for optimization, and get the optimized threshold and weight bestX through iteration. Then the optimized threshold and weight are used to train the BP neural network, resulting in a fitting degree of R = 0.99998, indicating good training effects. Moreover, it also calculates the error of optimized threshold and weight of GA-BP neural network (Table 3) and the fitting degree of threshold and weight of GA-BP neural network (Fig. 4).

3.5.3. Using genetic algorithms to find the best model

Using the genetic algorithm toolbox, the GA-BP model above was optimized. The threshold, weight and neural network model were optimized. The upper limit of the conditions was set as follows: temperature was 85 °C, solid-liquid ratio was 0.0182 (1:55) and HCl concentration was 0.7 %, while the lower limit was set as follows: temperature was 75 °C, the solid-liquid ratio was 0.0154 (1:65) and HCl concentration of was 0.5 %. Our neural network was set as follows: training times was 1000, training target was 0.00001, learning

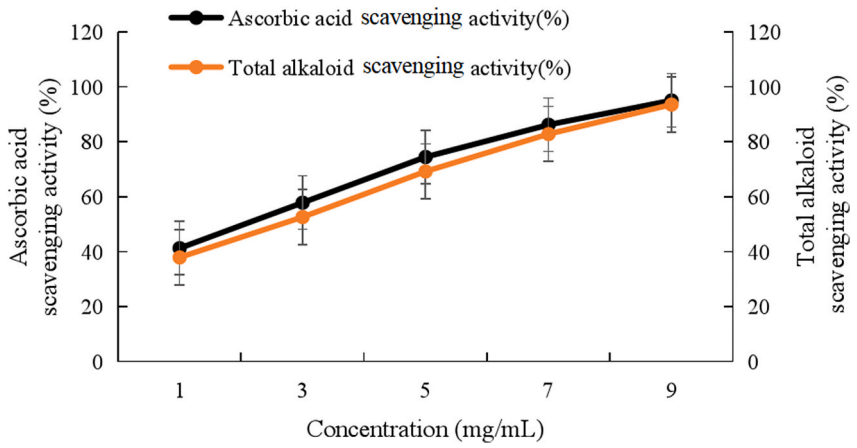

Fig. 8. Effect of total alkaloid on superoxide anion scavenging activity.

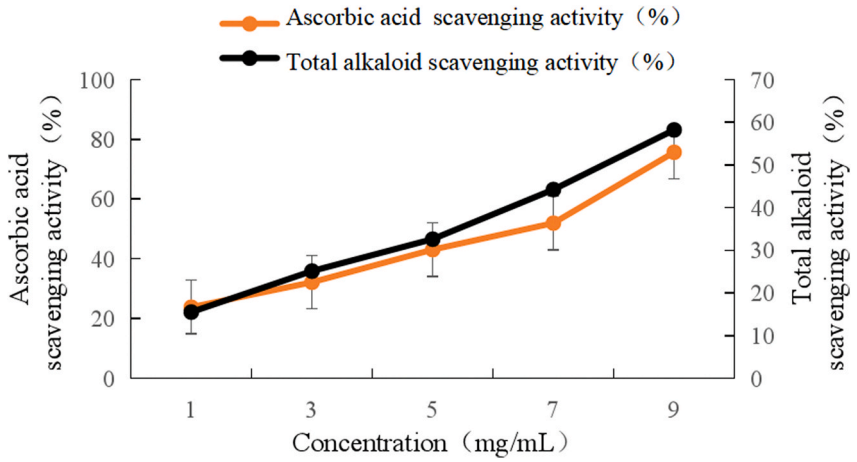

Fig. 9. Effect of total alkaloid on DPPH radical-scavenging activity.

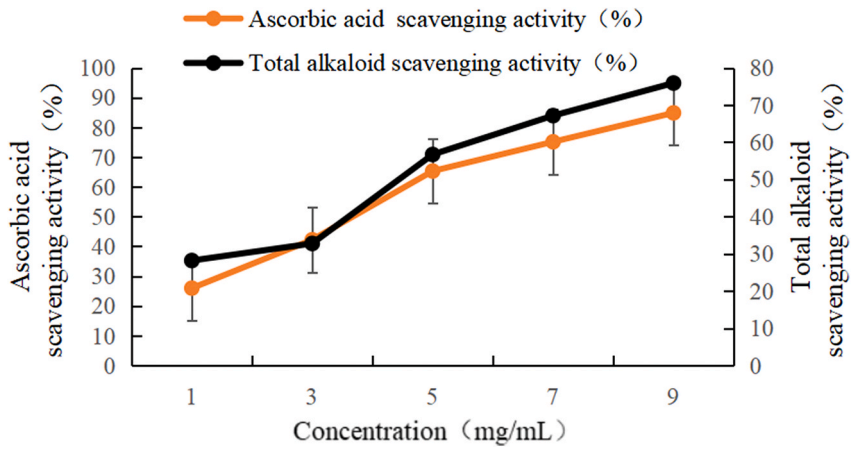

Fig. 10. Effect of total alkaloid on ABTS radical-scavenging activity.

rate was 0.1, and other parameters remained the same. Using the genetic algorithm toolbox for target optimization, we obtained the GA-BP neural network model with R = 0.99385 through continuous training (Fig. 5). The optimal extraction conditions included temperature at 84.9999 °C, liquid-solid ratio of 0.0155 (1:64.5), and HCl concentration at 0.5001 %. The optimal extraction rate of total alkaloids was determined by comparing the predicted value with the real value (Fig. 6).

3.6. Verification test

Six pieces of medicinal materials, each measuring 5 g, were weighed, and 3 batches were prepared to determine the optimal extraction process by using orthogonal tests and BP neural networks. The total alkaloid extraction yield varied by 0.2785 ± 0.0003 mg/mL. Here, we demonstrated that GA-BP neural network optimization can be used for extraction of total alkaloids from *H. chinensis*, and the optimization process is more ideal than orthogonal experiment.

3.7. Antioxidant activities of total alkaloid

The alkaloids in Chinese herbal plants also act as antioxidants and radical scavengers. In this study, alkaloids extracts from *H. chinensis* were primarily evaluated for their antioxidant properties.

A hydroxyl radical-scavenging activity and superoxide anion scavenging activity assays showed that the activity increases while the concentration of total alkaloids increased. The maximum concentration of IC_{50} was found to be 4.17 mg/mL and 2.563 mg/mL, respectively. However, the hydroxyl radical assay showed that hydroxyl radical-scavenging activity was lower than ascorbic acid (Vc) ($p < 0.05$) (Figs. 7 and 8).

A total alkaloid was found to have a higher radical-scavenging effect on DPPH radicals and ABTS radicals, with IC 50 values of 7.78 mg/mL and 4.96 mg/mL, respectively (Figs. 9 and 10). The results indicated that the extracts might have stronger antioxidant properties *in vitro*.

4. Conclusions

In this study, we obtained total alkaloids from *H. chinensis* that were then optimized using an orthogonal design and BPNN-model. The results showed that temperature, material-liquid ratio, and HCl concentration had significant effects on extraction rate. Moreover, the best extraction conditions were 85 °C, 1:64.5 material-liquid ratio, and 0.5% HCl concentration. Furthermore, the total alkaloids from *H. chinensis* in optimal conditions were extracted at a rate of 0.2785 ± 0.0003 mg/mL. DPPH and ABTS assays revealed that total alkaloids from *H. chinensis* were effective at scavenging hydroxyl radicals and superoxide anion radicals. Therefore, extracts may have a high level of antioxidant capacity.

Author contributions

Weiwei Jiang and Yan Zhao conceived and designed the experiments; contributed reagents, materials, analysis tools or data; and wrote the paper; Shaoyu Zheng, Chengxiao Yuan, Qingqing Gao and Chunfan Xiang performed the experiments. Shunwei Tian and Jianmei Li analyzed the data. All authors have read and agreed to the published version of the manuscript.

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Declaration of competing interest

To my knowledge, all of my possible conflicts of interest and those of my coauthors, financial or otherwise, including direct or indirect financial or personal relationships, interests, and affiliations, whether or not directly related to the subject of the paper, are listed in the appropriate sections of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20680.

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