

Extraction Process Optimization of Total Alkaloid from *Actinidia arguta*

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Abstract. To study on optimal conditions for extracting the higher content of total alkaloid from *Actinidia arguta* fruits, the effects of ethanol concentration, temperature, time and solid-liquid ratio on extraction efficiency total alkaloid were explored. The results showed that the optimum extraction conditions included 70% ethanol as extracting solution, temperature on 60C, extraction time by 20 min, ratio of solid- liquid on 1:30 (g/mL). By this method, the total alkaloids of *Actinidia arguta* fruits were extracted to 1.021 mg/g. This experiment of extraction method is simple and easy to operate. It was improved the extraction yield of the total alkaloid extraction from *Actinidia arguta* fruits.

Introduction

The *Actinidia arguta* is an important wild fruit resource. This wild plant distributes in the Northeast China, North China, Shandong, Northwest China and Yangtze valley, and chiefly concentrated in the southern part of the mountainous area in the Northeast [1]. It is rich of many nutrients, such as high content of V_C (200-400mg/100g) [2]. It is an ideal green food and healthy food, the root, stem and fruit are all useful for us because of their medical value and healthy function. The fruits contain lots of SOD, amino acid, sugars and etc. Meanwhile, and have the effect on the hyperlipidemia, hypertension, and stenocardia [3, 4].

In general, alkaloids refer to the natural alkaline nitrogen compounds in plants [5]. Alkaloid has strong physiological role of valuable medicines for people and animals [6]. Alkaloid is the study of a hot spot [7, 8], at present, the researchers have no further research and study in the *Actinidia arguta*. We can only search the reports of fruit SOD, Amino acids, and Enzymes. This article offers the important value on the total exploitation of *Actinidia arguta*.

In this paper a new optimal design of extracting of total alkaloids in *Actinidia arguta* has been studied. The purpose is to improve the peach alkaloid extraction yield. This new extraction process provides a theoretical basis for the resources utilization of the new medicinal plant.

Materials and Methods

Primary materials and instruments. Fruits of the *Actinidia arguta* were purchased from the College of Food, Shenyang Agricultural University, China. UV-type 240 spectrophotometer (SHIMADZU Japan). Aconitine (American Sigma), Other reagents are domestic analytical reagents.

Standard curve. Under the condition of certain pH, alkaloids with hydrogen ion combined into salt. Acid dyes and the salt combined into non-ferrous complex. Aconitine has the nature of the total alkaloid. However, total alkaloids of *Actinidia arguta* determinate by spectrophotography and aconitine as standard. the determination of absorbance values were used the solution from the chloroform layer and joined the Na₂SO₄ for dehydration. The regression equation $Y=0.0682x+0.0077$, $r=0.9997$ ($n=6$).

The sample determination of alkaloids. Using ultrasound, 5g of dried powder of samples was extracted with 100mL of 80% ethanol 3 times at 40C for 30 min. The ethanol extracts were evaporated to dryness by rotary evaporation at 60C under reduced pressure to produce a ethanol

crude extract. The crude extract was dissolved by 20mL 2% HCL and filter. The same volume of chloroform extraction 2 times, pH to 10 by ammonia, and use the same volume of chloroform extraction 3 times again. The extracts were evaporated to dryness by rotary evaporation and constant volume to 25mL. 1mL extracts liquid, 6mL chloroform and 6mL NaOH-KHC₈H₄O₄ for buffer solution fully shock and stewing 1 hour. Finally, the determination of absorbance values were used the solution from the chloroform layer and joined the Na₂SO₄ for dehydration. Alkaloids of *Actinidia arguta* were determined by spectrophotography.

Single factor experiment

The ethanol concentration. Using ultrasound, 5g of dried powder of samples were extracted with 100mL ethanol with different concentrations of 40%, 60%, 70%, 80% and 100% at 40C for 30 min.

The solid-liquid ratio. Using ultrasound, the different solid-liquid ratios of 1:15, 1:20, 1:25, 1:30 and 1:35 were extracted with concentration of 80% at 40C for 30 min.

The extracting time. Using ultrasound, 5g of dried powder of samples were extracted with 100mL ethanol with concentration of 80% at 40C for different times of 20min, 30 min, 40 min, 50min and 60min.

The extracting temperature. Using ultrasound, 5g of dried powder of samples were extracted with 100mL ethanol with concentration of 80% at different extracting temperatures of 30C, 40C, 50C, 60C and 70C for 30 min.

Orthogonal test design. According to the results of single factor experiment, the ethanol concentration(A), the solid-liquid ratio(B), the extracting time(C) and the extracting temperature(D) are the reference factors for design L₉(3⁴) orthogonal experimental (Table 1).

Table.1 The factors and levels of orthogonal test for extraction of alkaloid from *Actinidia arguta*

Level	Concentration(A) %	Solid-liquid(B) g:mL	Time(C) min	Temperature(D) C
1	60	1:20	20	50
2	70	1:25	30	60
3	80	1:30	40	70

Test results

The influence of concentration of ethanol to alkaloid extraction quantity. From Fig. 1A, the best extraction yield is the 70% concentration of ethanol as the solvent and the alkaloid content achieve to 0.96 mg/g. Three concentrations of ethanol (60%, 70% and 80%) were chose for the orthogonal experiment.

The influence of solid-liquid ratio to alkaloid extraction quantity. From Fig. 1B, the best extraction yield is the solid-liquid ratio of 1:25 and the alkaloid content achieve to 1.13 mg/g. Three solid-liquid ratios (1:20, 1:25 and 1:30) were chose for the orthogonal experiment.

The influence of time to alkaloid extraction quantity. From Fig. 1C, the best extraction yield is the time of 30 min and the alkaloid content achieve to 1.05 mg/g. Three times (20min, 30min and 40min) were chose for the orthogonal experiment.

The influence of temperature to alkaloid extraction quantity. From Fig. 1D, the best extraction yield is the temperature of 60C and the alkaloid content achieve to 1.13 mg/g. Three temperatures (50C, 60C and 70C) were chose for the orthogonal experiment.

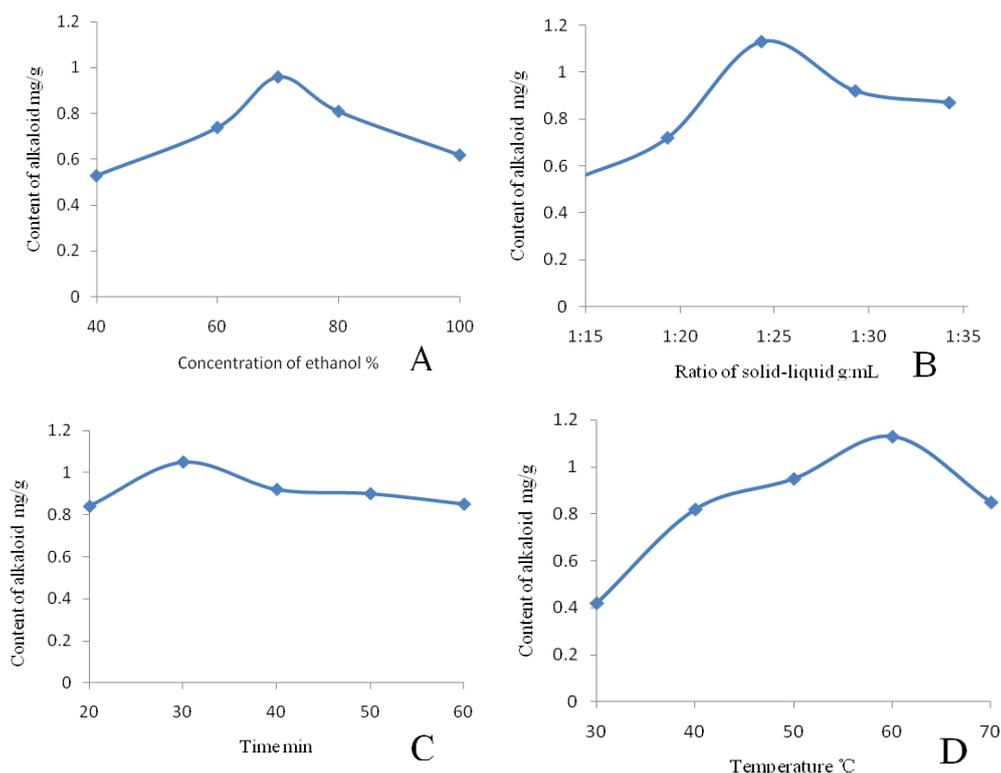


Fig.1 Alkaloid content under different ethanol concentration, solid-liquid ratio, time and temperature

The result of orthogonal experiment. From Table.2, the factors on the influence of extraction yield of alkaloid are in the order to A>C>B>D, namely ethanol>time> temperature >solid-liquid ratio. The best extraction process of total alkaloid from *Actinidia arguta* is A₂B₃C₁D₂, namely 70% ethanol, solid-liquid ratio of 1:20, 20min and 60C. In this condition, the three sets of parallel tests were carried out and the alkaloid content is 1.021mg/g.

Table.2 The result of orthogonal experiment for extraction of alkaloid from *Actinidia arguta*

No.	A	B	C	D	Alkaloid content(mg/g)
1	1	1	1	1	0.462
2	1	2	2	2	0.654
3	1	3	3	3	0.837
4	2	1	2	3	0.925
5	2	2	3	1	1.105
6	2	3	1	2	1.076
7	3	1	3	2	0.856
8	3	2	1	3	0.825
9	3	3	2	1	0.971
K ₁	1.953	2.705	2.363	2.438	
K ₂	3.006	2.484	2.550	2.586	
K ₃	2.757	2.884	2.698	2.587	
R	1.053	0.179	0.335	0.149	

Conclusion

This study has shown that *Actinidia arguta* contains the more alkaloids. Ethanol concentration, solid-liquid ratio, time and temperature all have significant influence on alkaloid extraction, one of the biggest impact is the ethanol concentration. The best extraction process of total alkaloid from *Actinidia arguta* is 70% ethanol, solid-liquid ratio of 1:20, 20min and 60C. Experiments show that this method is feasible and good repeatability[9,10].

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