Establishment and Optimization of Extraction Method of Antimicrobial Peptide from Capsella Bursa Pastoris Seed

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Keywords: Capsella bursa pastoris, Antimicrobial peptide, Extraction efficiency, Resource development

Abstract: In order to improve the utilization rate of Capsella bursa pastoris resources, we extracted the antimicrobial peptides in shepherd's purse seed, and the analyzed the factors affecting the extraction efficiency. The results showed that the degreasing of seeds had no significant effect on the extraction rate of antimicrobial peptides; the extraction rate of peptides by ultrasonic extraction was higher, and NH4OAc was better as the extraction solution. Further experimental results showed that with the increase of the ratio of material to liquid, ultrasonic temperature and ultrasonic time, the concentration of antimicrobial peptide was higher. The best combination was dry seed powder: NH4OAc = 1:25, ultrasonic extraction at 30 ℃ for 30 minutes, and the concentration of peptide was 2.43 mg/g. By using the established method, the protein and peptide concentrations in the seeds from Beijing, Shanghai and Zhengzhou were compared. The results showed that there was little difference between the protein and antimicrobial peptide contents in the seeds from three areas. The results provide a basis for the research and application of antimicrobial peptides and the comprehensive development and utilization of shepherd's purse.

1. Introduction

Shepherd's purse (Capsella bursa pastoris), commonly known as shepherd's purse, local vegetables, chicken cabbage, Jingchangcao, chicken feet, etc., belongs to the genus Capsella bursa, a 1-2 year-old herb. Shepherd's purse is widely cultivated in my country and is the earliest edible wild vegetable in early spring in most areas of northern my country. Shepherd's purse has high nutritional value and delicious taste. It has the effects of name, hemostasis, nourishing liver, nourishing heart and spleen, lowering blood pressure, anti-inflammatory and antibacterial [1]. Shepherd's purse as a wild vegetable and medicinal plant, its use value has been recognized and valued by people. At present, the researches on shepherd's purse are mostly about cultivation, biological characteristics, nutritional value and utilization, but less research on shepherd's purse seeds. In the process of planting shepherd's purse, the amount of shepherd's purse seeds is large, and each silique contains about 20-25 seeds. At present, there is little research on the development and utilization of shepherd's purse seeds.

Antimicrobial peptides, also known as antimicrobial peptides or polypeptide antibiotics, are widely present in all tissues of plants (roots, stems, leaves, flowers, fruits and seeds). They can resist the invasion of pathogens [2] and constitute the immune defense system of organisms. The key part of [3]. Studies have shown that antimicrobial peptides have various biological activities such as antifungal, antibacterial, antiviral, anti-insect, anti-HIV, and anti-tumor [4]. Plants contain a variety of antimicrobial peptides. According to the homology of amino acid sequences, the main family members of plant antimicrobial peptides include thionin, defensin, lipid transporter, ecdysone, cyclic peptides, cell penetrating peptides, etc. [5]. Plant antibacterial peptides have broad-spectrum antibacterial activity and high efficiency, can be used to protect plants and prevent animal diseases, and can also be used as effective substitutes for infection treatment drugs. Therefore, the extraction of antibacterial peptides from plants is an urgent problem to be solved. There have been research reports on the antimicrobial peptides in the shepherd’s purse plants and the roots of

  DOI: 10.25236/civeb.2020.008

shepherd’s purse [6]. This study conducted preliminary extraction of the antimicrobial peptides in the shepherd’s purse seeds, and analyzed the factors affecting the extraction efficiency, and determined the most important Best extraction method, and compare the antimicrobial peptide content in the seeds of shepherd's purse from different producing areas. This study can provide a basis for the development and utilization of shepherd's purse seed resources.

2. Materials and Methods

2.1 Experimental Materials

The shepherd's purse seeds from Beijing, Shanghai, and Zhengzhou were purchased from the market and were identified as healthy shepherd's purse seeds by experts.

2.2 Pretreatment of Materials

Choose the shepherd's purse seeds that are mature, plump, and have no deformities. Wash the seeds with clean water several times. After washing, drain the water from the seeds and set aside. The shepherd's purse with the washed and drained water is put into a 100°C thermostat for drying, and the powder is pulverized into a powder and passed through a 150-mesh sieve. The obtained shepherd's purse powder is transferred to a dry sealed bag for use.

2.3 Shepherd's Purse Seeds Defatted

Take two 50 g shepherd’s purse seed powder in a beaker, add 100 mL petroleum ether and 100 mL acetone respectively; let stand at room temperature for 48 h, after the mixture is separated, transfer to a fume hood to completely volatilize the organic solution, take out and weigh separately Calculate the degreasing rate. Each group has 6 reorganizations.

2.4 Shepherd's Purse Seed Protein Extraction

Two samples of defatted shepherd’s purse seed powder and shepherd’s purse seed powder were 0.2 g of shepherd’s purse dry powder (accurate to 0.001 g), repeated determination of 7 samples, and the formula calculation was carried out to obtain the crude protein content of each sample.

Weigh 0.2 g of dry powder of the sample into the digestion tube, add 0.4 g of copper sulfate, 6 g of potassium sulfate and 20 mL of sulfuric acid to fully nitrify, add 50 mL of water after completion, and measure in an automatic Kjeldahl nitrogen analyzer. The crude protein content in each sample is calculated by the following formula [7]. Analyze the change of seed protein content before and after degreasing, and analyze the influence of different degreasing agents on protein extraction.

$$X = \frac{(V1 - V2) \times c \times 0.0140}{m \times V3/100} \times F \times 100$$

X: The content of protein in the sample, the unit is gram per hundred grams (g/100g);

V1: The volume of the standard titrant that the test solution consumes sulfuric acid or hydrochloric acid; the unit is milliliter (mL);

V2: The volume of the standard titration solution of sulfuric acid or hydrochloric acid consumed by the reagent blank, in milliliter (mL);

c: concentration of sulfuric acid or hydrochloric acid standard titration solution, the unit is moles per liter (mol/L);

3. 0140: 1.0 Ml Sulfuric Acid [C(12h2so4)=1.000 Mol/l] or Hydrochloric Acid [C(Hcl)=1.000 Mol/l] the Mass of Nitrogen Equivalent to the Standard Titration Solution, in Grams (g);

m: The mass of the sample, in grams (g);

V3: The volume of the aspirated liquid, the unit is milliliters (mL);

F: The coefficient of nitrogen conversion to protein. For the conversion coefficient of nitrogen in various foods, see Appendix A;

100: Conversion factor.

3.1 Crude Extraction of Antibacterial Peptides from Shepherd's Purse Seeds

The method reported in the reference [8], take 2.0 g of shepherd's purse seed dry powder, use PBS extraction method, PBS salting-out ultrasonic extraction method, NH4OAC extraction method and NH4OAC salting-out ultrasonic extraction method. The material-to-liquid ratio of the extraction method is 1:20; the leaching is 24 h at 4°C; the material-to-liquid ratio of the salting-out ultrasonic extraction method is 1:20, the ultrasonic time is 30 min, and the extraction temperature is 25°C. The extract was placed in a refrigerated centrifuge at 4°C at 3000 rpm for 15 min to take the supernatant, and the volume of the supernatant was measured. Take 1 mL of the supernatant, add 5 mL of Coomassie Brilliant Blue reagent for color development, use a microplate reader to measure at 595 nm, and bring the result into the curve calculation, and calculate the protein content. There are four treatments in total, with 6 replicates in each treatment.

3.2 The Controlled Variable Method Analyzes the Influence of Different Factors on the Extraction Efficiency of Antimicrobial Peptides

Take 2.0 g shepherd's purse seed dry powder, set up 9 different treatments as shown in Table 1, and repeat each treatment four times to analyze the influence of liquid-to-material ratio, extraction time, and extraction temperature on the amount of protein extraction.

Table 1 Variable Factor Groups for Different Groups

Gr	L/m Ra	Extra time(min)	Extra tem(℃)
1	1:15	30	25
2	1:20	30	25
3	1:25	30	25
4	1:25	30	20
5	1:25	30	25
6	1:25	30	30
7	1:25	10	25
8	1:25	20	25
9	1:25	30	25

3.3 Data Analysis

The experimental data was analyzed by SPSS11.5 software.

4. Results and Analysis

4.1 The Effect of Shepherd's Purse Seed Pretreatment on Protein and Peptide Extraction

Two degreasing agents, acetone and petroleum ether, were used to pre-treat the shepherd’s purse seeds to calculate the degreasing rate. The degreasing rate of petroleum ether is 25%, and the degreasing rate of acetone is 17%, indicating that petroleum ether has a better degreasing effect. The extraction rates of shepherd's purse seed protein before and after defatting were 64.55% and 60.85%, while the extraction rates of crude peptides were 0.54% and 0.66%, respectively. It indicated that degreasing had no significant effect on the extraction of protein and antimicrobial peptides from shepherd's purse seeds.

4.2 Study on the Extraction Method of Shepherd's Purse Seed Crude Peptide

In order to establish a method for extracting crude peptides from shepherd's purse seeds, the effects of different extraction solutions and different extraction methods on the amount of peptides extracted were compared. It can be seen from Figure 1 that the extraction effect of NH4OAC is better than that of the PBS extract, and the extraction efficiency of the salting-out ultrasonic method is higher than that of the extraction method. Therefore, in this experiment, the NH4OAC method was selected to extract the crude peptides of shepherd's purse seeds, and the method was optimized.

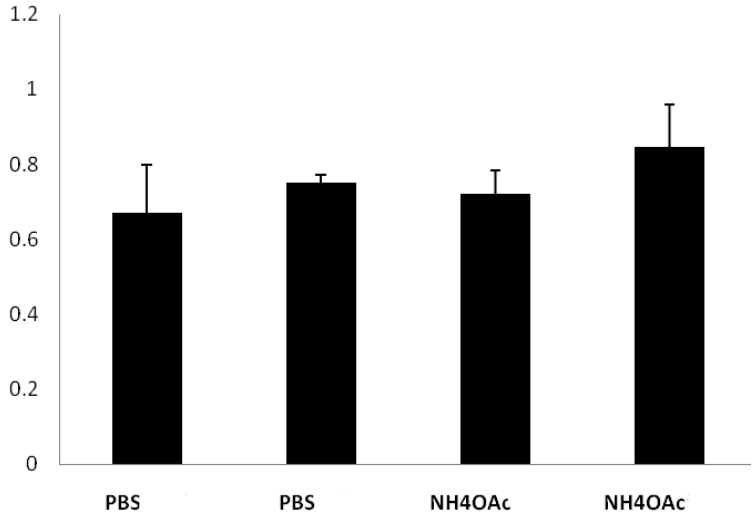

Fig.1 Effects of Different Extraction Methods on the Amount of Polypeptide Extracted from Shepherd's-Purse Seeds

4.3 Optimization of the Extraction Method of Antimicrobial Peptides from Shepherd's Purse Seeds

In order to improve the extraction efficiency of antimicrobial peptides from shepherd's purse seeds, according to the previous experimental results, the experiment set up 9 repeated analysis of the effect of three factors of material-liquid ratio, ultrasonic extraction temperature and ultrasonic extraction time on the extraction efficiency of antimicrobial peptides. It can be seen from the results that as the ratio of the extraction solution increases, the more fully extracted, the higher the concentration of the crude peptide extracted; the extraction concentration of the crude peptide increases with the increase of the extraction temperature and extraction time.

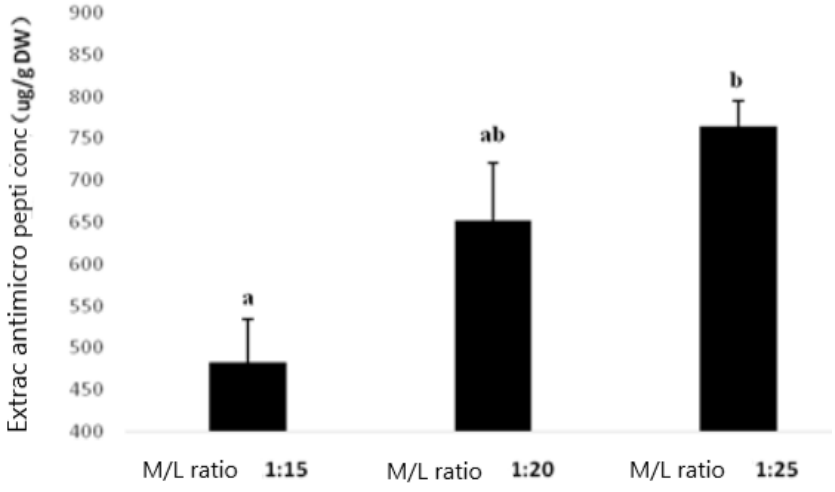

Fig.2 Effects of Different Ratio of Feed to Liquid on the Extraction Concentration of Antimicrobial Peptides (the Different Letters Showed Significant Difference in Treatment At $P<0.05$)

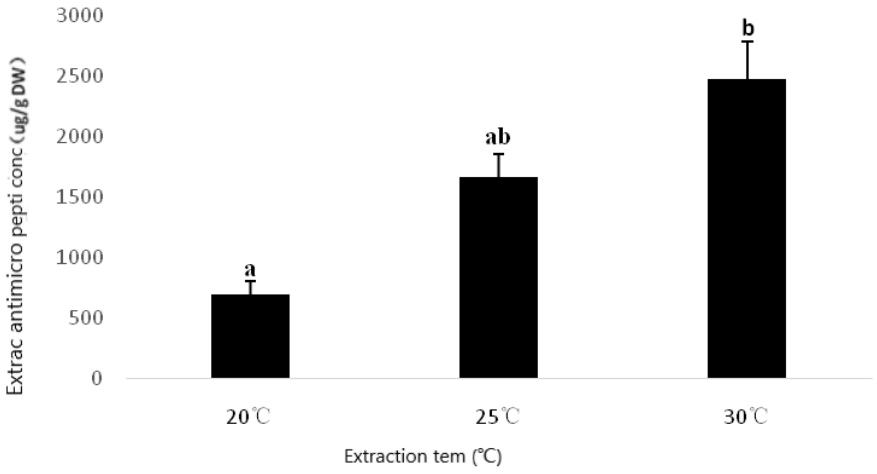

Fig.3 Effects of Different Extraction Temperatures on the Extraction Concentration of Antimicrobial Peptides (the Different Letters Showed Significant Difference in Treatment at P<0.05)

5. Discussion

Shepherd's purse is rich in amino acids and protein. The higher the protein content, the greater the nutritional value; the more types of amino acids and the closer to the human body demand, the better the supplement effect and the better the quality. The determination of the protein content in shepherd's purse is the basis for analyzing the quality of shepherd's purse from different producing areas. At present, the commonly used method for determining protein content is the Coomassie Brilliant Blue method, which uses Coomassie Brilliant Blue G-250 to combine with protein in an acidic solution through van der Waals gravity to form a complex with a maximum absorption peak of 595 nm. The absorbance is related to the protein content. There is a linear relationship between 200-1400 μg/mL, so it can be used for the determination of protein content [10]; however, shepherd's purse crude protein has a complex composition, inconsistent solubility, and different structure. The use of Coomassie brilliant blue method will be interfered by other substances. The content of the measurement result is too small. The Kjeldahl method [7] is the most widely used and frequently used protein determination method. It has simple operation, high accuracy and good repeatability, and it has become a legal standard detection method more at home and abroad [11]. In this experiment, the Kjeldahl method was used to determine the protein content of shepherd's purse in different areas, which provided a scientific basis for the analysis of crude protein content in shepherd's purse from different producing areas.

Park et al. (2000) first isolated and purified two antimicrobial peptides rich in glycine and histidine from the roots of shepherd's purse, and studied their antibacterial activity [12]. Li Yuansheng expressed and purified shepherd's purse in E. coli Antimicrobial peptides in roots [13], and the biological activity of the expressed antimicrobial peptides were identified. The research on shepherd's purse seeds has focused on its germination performance. There are few reports on the research of antibacterial peptides in shepherd's purse seeds. Extracting protein and antibacterial peptides from shepherd's purse seeds is beneficial to the comprehensive and in-depth development of shepherd's purse resources. The primary metabolites are less affected by the environment. The results of this study also show that there is no significant difference in the protein and antibacterial peptide content of shepherd’s purse seeds in different regions. Therefore, the planting range and planting area of shepherd’s purse can be expanded on a large scale to provide abundant Resources. The inhibitory efficiency of shepherd's purse plant extract on tobacco bacterial wilt can be as high as 63.9% [14], and whether the antimicrobial peptides in shepherd's purse seeds have an inhibitory effect on plant pathogens is still unknown. Follow-up research on this part is currently ongoing.

The extraction and purification of antimicrobial peptides from plant seeds is one of the key technologies for the development and utilization of antimicrobial peptides. The oil content in plant seeds is high, and degreasing can improve the quality of the extracted protein. However, whether

degreasing will reduce the yield of shepherd's purse seed protein and antimicrobial peptides is still unclear. The results of this study prove that petroleum ether has a degreasing rate of 25% without reducing the yield of extracted proteins and antimicrobial peptides. Increasing the concentration of antimicrobial peptides in the extract is a problem that scientists have been exploring. This study determined the effect of extraction methods, extraction solvents and other factors on the concentration of antimicrobial peptides. These results can provide a basis for the identification of antimicrobial peptide components and the basis of biological activity research.

Acknowledgment

National College Student Innovation and Entrepreneurship Training Program (201910466014)
Henan Agricultural University Science and Technology Innovation Fund (KJCX2016A17)

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