

## Study on Extraction Technology of Flavonoids from *Pinus Elliottii* Needle

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**Abstract:** Flavonoids were extracted from *Pinus elliottii* needle by ultrasonic extraction. The effects of ethanol concentration, material ratio and extraction time on flavonoids extraction were studied by single factor experiment and orthogonal design. The results showed that the optimum technological conditions for extracting flavonoids from *Pinus elliottii* needles were 90 minutes, 85% ethanol concentration and 1:20 material ratio. The extraction time had the greatest influence on the extraction effect, followed by the material ratio, and the ethanol concentration had the least influence.

### 1. Introduction

The medicinal ingredients of pine needles are higher than those of other parts of pine trees. They contain a lot of bioflavonoids, proanthocyanidins, chlorophyll and many vitamins. The main physiological activities of flavonoids are as follows: effects on cardiovascular system, anti-hepatotoxicity, anti-inflammatory, estrogen-like, anti-bacterial, anti-viral and purgative effects [1,2]. In recent years, for example, there are more studies on *Pinus massoniana* Needles in central and southern regions, and less on *Pinus massoniana* needles. The content of flavonoids in *Pinus massoniana* Needles is higher than that in *Pinus massoniana* needles. Secondly, *Pinus arborescens* is produced in the mountainous areas of Mudanjiang River basin, Changbai Mountains and Eastern Liaohe River in Northeast China. Its branches and trunks are fully utilized by people, but many needles of *Pinus arborescens* are not reasonably utilized. Through the study on the extraction technology of Flavonoids from *Pinus elliottii* needles, a reasonable extraction method was worked out, which will lay a theoretical foundation for the research of pesticide hotspots, namely, the development of Biogenic Pesticides [3,4].

### 2. Materials and Methods

#### 2.1. Materials and Reagents.

*Pinus elliottii* (collected in Jilin Academy of Agricultural Sciences and Technology) was harvested and the stems and leaves were separated and dried in shade for use. Ethanol, activated carbon. Because of the existence of negative pressure, there will be pressure difference between the two sides of the seed metering plate; the driving shaft drives the rotation of the transmission mechanism, and the transmission mechanism drives the rotation of the seed metering disk.

#### 2.2. Instruments and Equipment.

MZ-10 pulverizer (produced by Qingdao Maikelong Powder Technology Equipment Co., Ltd.); ultrasonic extractor; FA1064 electro-optic balance (produced by Shanghai Precision Scientific Instrument Co., Ltd.); rotary evaporator. UV-2045 ultraviolet-visible spectrophotometer (Shimadzu, Shanghai).

## 2.3. Test Method.

### 2.3.1. Single factor test[5-7]

The fine powder of *Pinus elliottii* needle was obtained by grinding it with a grinder and sieving it with 60 meshes. The flavonoids of *Pinus elliottii* needle were extracted with different ethanol concentration, extraction time and material ratio using ethanol as solvent. The active substance of *Pinus psammophila* needle was extracted by ultrasonic extraction method. The filtered solution was filtered by filter paper and evaporated by rotary evaporator to obtain green solution. In order to prevent the influence of pigments on the subsequent ultraviolet determination, activated carbon was selected to adsorb the pigments from the solution, and then the final solution was diluted to 25 ml at a constant volume according to the need. The absorbance was determined at the maximum absorbance wavelength, and the optimum extraction process was deduced according to the absorbance.

1) Ethanol concentration: weigh 4 parts of *Pinus elliottii* needle powder, 2.0g each, and wrap them with filter paper. The ethanol concentration was set to 65%, 75%, 85%, 95%, the material ratio was set to 1:20, the extraction time was set to 90 min, the extraction temperature was set to 40 C, and then the extraction solution was obtained.

2) Extraction time: weigh 4 parts of *Pinus elliottii* needle powder, 2.0g each, and wrap them in filter paper. The extraction time was set to 30 min, 60 min, 90 min and 120 min, the material ratio was set to 1:20, the ethanol concentration was set to 85%, the extraction temperature was set to 40 C, and then the extract was extracted.

3) Material ratio: weigh 4 parts of *Pinus elliottii* needle powder, 2.0g each, and wrap them in filter paper. Material ratio was set to 1:10, 1:15, 1:20, 1:25, extraction time was set to 90 minutes, ethanol concentration was set to 85%, extraction temperature was set to 40 C, and then extraction was carried out to obtain the extract.

### 2.3.2. Orthogonal test[8-10]

On the basis of single factor experiment, three factors, ethanol concentration, extraction time and material ratio, were selected as the main influencing factors. Orthogonal experiments were carried out at three levels to find the best extraction process of Flavonoids from *Pinus elliottii* needle. The factors and level design were shown in Table 1.

Table 1 Factor Level Table

level	Factor		
	A:Time(min)	B:Ethanol concentration(%)	C: Material ratio(g.ml)
1	30	65	1:15
2	60	75	1:20
3	90	85	1:25

## 3. Results and Analysis

### 3.1. Effect of Ethanol Concentration on Extraction

The original solution was diluted 20 times and its absorbance was measured at 510 nm.

As can be seen from Fig. 1, with the increase of ethanol concentration, the absorbance of the coarse body fluid of *Pinus elliottii* increased gradually. When the ethanol concentration was 85%, the absorbance was the largest. Then, with the increase of ethanol concentration, the absorbance gradually decreased. Therefore, when the ethanol concentration was 85%, the extraction of fresh fruits had the greatest impact. Considering comprehensively, the ethanol concentration of 65%, 75% and 85% was chosen as the three levels of ethanol concentration factor in the orthogonal experiment.

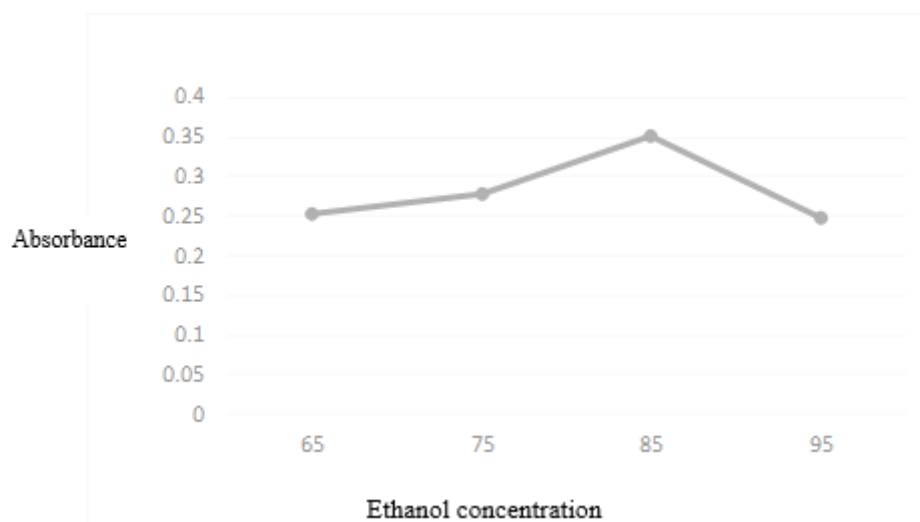


Fig. 1 Effect of ethanol concentration on extraction efficiency

### 3.2. Effect of Extraction Time on Extraction Effect

The original solution was diluted 20 times and its absorbance was determined at 510 nm. Fig. 2 shows that the absorbance increases with the increase of extraction time and decreases after 90 minutes. Considering the extraction efficiency and cost, the optimal extraction time is finally determined to be 90 minutes. Considering the experiment comprehensively, the three levels of extraction time of orthogonal experiment were determined as 30 min, 60 min and 90 min.

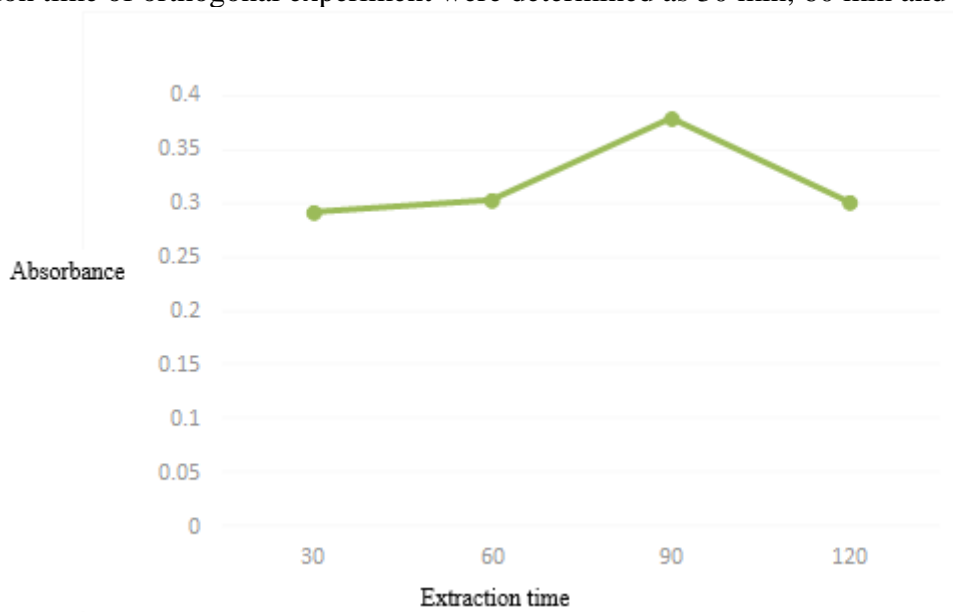


Fig. 2 Effect of extraction time on extraction effect

### 3.3. Effect of Material Ratio on Extraction Efficiency

The original solution was diluted 40 times and its absorbance was determined at 510 nm.

As can be seen from Fig. 3, with the increase of material ratio, the absorbance increases gradually. When the material ratio is 1:20, the absorbance reaches the maximum, so 1:20 is the best extract material ratio of Flavonoids from *Pinus elliottii*. Considering the influence of various factors, the orthogonal experiment finally determined the three levels of extraction ratio as 1:15, 1:20 and 1:25.

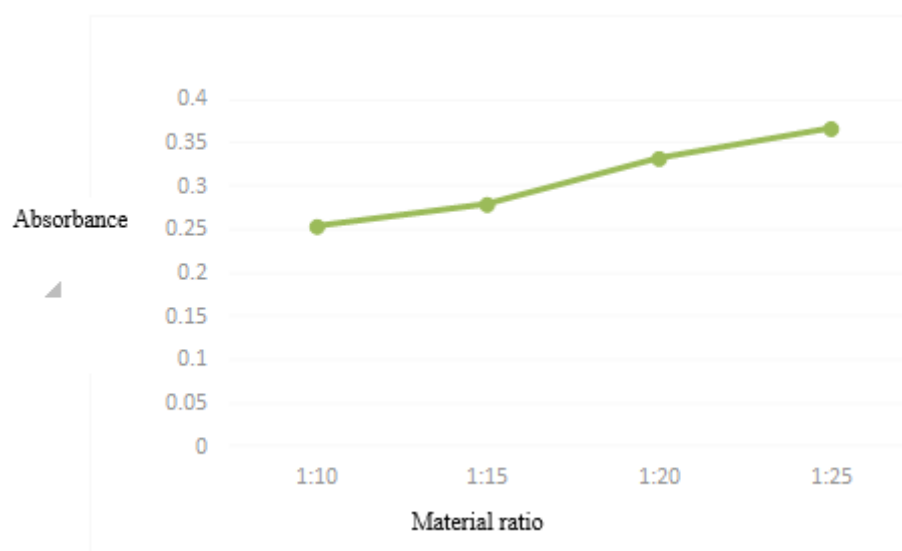


Fig. 3 Effect of Material Ratio on Extraction

### 3.4. Orthogonal Test Results

Because the factors of ethanol concentration, extraction time and material ratio have significant influence on the test results, orthogonal experiments are carried out with time (A), ethanol concentration (B) and material ratio (C), the results are shown in Table 2.

Table 2 Orthogonal test results

Test number	A	B	C	Absorbance	Extraction rate
1	1	1	1	0.538	4.21
2	1	2	2	0.321	2.48
3	1	3	3	0.475	3.71
4	2	1	2	0.489	3.82
5	2	2	3	0.374	2.9
6	2	3	1	0.481	3.76
7	3	1	3	0.435	3.39
8	3	2	1	0.498	3.89
9	3	3	2	0.571	4.48
K <sub>1</sub>	10.4	11.42	10		
K <sub>2</sub>	10.48	9.27	10.78		
K <sub>3</sub>	11.76	11.95	11.86		
R	10.88	10.57	10.87		
Primary and secondary order		A>C>B			
Excellent level	A <sub>3</sub>	B <sub>3</sub>	C <sub>2</sub>		
Excellent combination		A <sub>3</sub> B <sub>3</sub> C <sub>3</sub>			

From the data in Table 2, it can be seen that the extraction effect of Flavonoids from *Pinus elliottii* needles is affected by different factors. Through range analysis, it can be seen that the order of influence of three factors on the extraction effect of crude body fluid is A > C > B, and the extraction time has the greatest impact on the extraction effect, followed by the material ratio, and the ethanol concentration has the smallest impact. According to the extraction effect, the optimum extraction conditions were A<sub>3</sub>B<sub>3</sub>C<sub>3</sub>, that is, extraction time was 90 minutes, ethanol concentration was 85%, and material ratio was 1:20.

#### 4. Conclusion

In this study, ultrasonic extraction method was used to extract flavonoids from *Pinus elliottii* needles, which was affected by many factors, including extraction time, ethanol concentration and material ratio. The extraction time had the greatest impact on the extraction effect, followed by material ratio, ethanol concentration had the smallest impact. The optimum technological combination was extraction time of 90 minutes, ethanol concentration of 85%, material ratio of 1:20. There are still many factors affecting the extraction efficiency of Flavonoids from *Pinus elliottii* needles. Further optimization of extraction conditions is needed to improve the extraction rate in the future, which will provide new ideas for the application of Flavonoids from *Pinus elliottii* needles in the development of biogenic pesticides.

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