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Optimizing the extraction of antibacterial compounds from pineapple leaf fiber

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Abstract: Five different solvents (petroleum ether, chloroform, ethyl acetate, acetone, and distilled water) were used to extract antibacterial compounds from pineapple leaf fiber. Compounds extracted using acetone showed the greatest antibacterial effect against *Escherichia coli*, measured by inhibition zone diameter. Three extraction parameters including temperature, time and solid-liquid ratio were optimized through orthogonal experiment based on single factor investigations for achieving maximum active substance extraction rate and bacteriostatic effect. Results showed that using acetone, the optimum extraction conditions for temperature, time and solid-liquid ratio were 45°C, 8 h, and 1:40 (g/ml), respectively.

Keywords: pineapple leaf fiber, antibacterial component, *Escherichia coli*, orthogonal experiment

1 Introduction

Pineapple (*Ananas comosus*), belonging to Bromeliaceae family, is a kind of perennial monocotyledon herbaceous plant as well as one of the most known tropical fruits [1]. In China, pineapple is mainly planted in 6 provinces including Hainan, Guangdong, Guangxi, Yunnan, Fujian, and Taiwan. In recent years, the average planting area of pineapple has been maintained at approximately 70 000 hm² [2]. The wasted leaves per mu is about 5–10 t,

thus national total wasted amount is accumulated to nearly 10,000,000 t per year, leading to large amount of resource waste and eco-environmental pollution [3]. The fiber content in pineapple leaf is only around 1.5% (dry basis). However, extricated fiber from the leaf has similar uses as hemp, and unique advantages. It is prescribed in “Chinese Materia Medica” that pineapple leaf has effects of promoting digestion and anti-diarrhea and is also used to treat summer diarrhea, dyspepsia and gastral cavity pain. There have been studies indicating that with three major functions including natural antimicrobial function, anti-mite function and dispelling unpleasant smells [4–5], the fiber has a stronger antibacterial property than existing bast-fibres, and this is the significance which lies in the qualitative and quantitative researches on effective chemical components included in pineapple leaf fiber. This experiment focuses on the optimization of extrication technology for antibacterial components in pineapple leaf fiber, providing a theoretical basis and basic data for separation and purification of the active substances from pineapple leaf fiber.

2 Material and Methods

2.1 Material

Pineapple leaves were obtained from a planting base in Xuwen County, Zhanjiang City. Fiber was extracted from the leaves using a self-developed semi-automatic extractor, and then dried and stored. *Escherichia coli* ATCC25922 was used to test suspected antibacterial substances from extracted fiber.

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2.2 Experimental method

2.2.1 Preparation of polar crude extracts

Dry fibers were ground into powder and 10 g were placed in each of five 500 mL round-bottom flasks. 400 mL of a single solvent (one of: petroleum ether, chloroform, ethyl acetate, acetone, or distilled water) was added to each flask, which were placed in a water bath, heated for 2 h, and cooled to 25°C. The mixtures were filtered by qualitative filter paper and rotating evaporation concentrated into extractum which was put in 10 mL EP tubes and stored in 4°C refrigerator until needed for analysis.

2.2.2 Qualitative analysis and antibacterial tests of chemical components under different polarity sections

Bacteriostatic activity was measured by agar diffusion; qualitative analysis on the 5 extract samples was made using special color developing agents.

2.2.3 Determination of optimal parameter of extrication technology.

In single factor experiment, only corresponding acetone solution is added in blank group; by successively changing temperature, reaction time, and solid-liquid ratio, based on *Escherichia coli* as indicator bacteria as well as extraction yield $\times$ bacteriostatic diameter as active tracking indicator, related research and analysis can be performed, moreover the optimal parameters of orthogonal experiment of three factors three levels can be determined.

3 Results and Discussion

3.1 Qualitative analysis and antibacterial test of chemical components under different polarity sections

Escherichia coli was used as an indicator in the antibacterial tests, and the antibacterial activity was measured with the agar diffusion method. Test results are shown in Table 1. Extracts obtained from ethyl acetate, acetone, and distilled water exhibited antibacterial activity, with acetone-extracted samples showing the largest inhibition zone (14.76 mm).

Qualitative analysis was applied to the extracts at 5 polarity intervals (A, B, C, D, E) using a special color

developing agent. Compounds identified from the extracts are shown in Table 2, and include flavonoid and phenols from samples B, C, D, and E, with D showing the greatest color response. According to related literature reports *Cannabis sativa* and *Apocynum venetum* contain flavonoid and phenols [6-7], and both compounds have antibacterial effects, from which we can infer that the antibacterial substances in pineapple leaf fiber were likely two such compounds [8-9].

3.2 Single factor experiment result and analysis

3.2.1 The effect of temperature extraction rates and antibacterial.

As the acetone-extracted samples demonstrated the greatest antibacterial effect, this solvent was used to determine the effect of temperature on the extraction process. Different temperatures were set using a consistent extraction time of 8 h and a solid-liquid ratio of 1:40. The results are shown in Fig. 1a. The extraction rate increased with the temperature, reaching a peak value at 45°C and then decreasing with the continuous increase of temperature, leveling off at 65°C. Such variation was probably due to the overly high temperature which led to increasing evaporation rate of acetone, thus leading to the decrease of extraction efficiency. On the other hand, the antibacterial effect increased with the temperature, reached a peak value at 55°C, and then with the increase of temperature the inhibitory zone diameter (IZD) decreased, which may have been due to overly high temperature leading to the denaturation of antibacterial substance, and thus damping the antibacterial effect. This experiment was based on extraction rate $\times$ bacteriostatic diameter as active tracking indicator, and by comprehensively evaluating the two indexes, 45°C was selected as the optimal temperature for extraction [10-11].

3.2.2 The effect of time on extraction rates and antibacterial

Different extraction times were set using acetone at 45°C and a solid-liquid ratio of 1:40. The results are shown in Fig. 1b. The antibacterial activity reached and maintained a peak at 6 h, while extraction rates peaked at 6 h and decreased after 8 h. Through comprehensive consideration of both indexes, the optimal extrication time was determined as 8 h [12].

Table 1. Antibacterial effect of chemical composition of different polarity.

Extraction agent	petroleum ether (A)	chloroform (B)	ethyl acetate (C)	acetone (D)	distilled water (E)
diameter of inhibition zone (mm)	0	0	10.20	14.76	12.32
	0	0	10.10	13.56	12.10
	0	0	9.10	13.50	11.30

Note: inner diameter of Oxford cup is 7.8 mm, outer diameter of Oxford cup is 8.0 mm

Table 2. Qualitative analysis results of pineapple fiber extracts at 5 polarity intervals (A, B, C, D, E) treated with color developing agents.

Substance	Reagent	Petroleum ether (A)		Chloroform (B)		Ethyl acetate (C)		Acetone (D)		Distilled water (E)	
		Color development phenomenon	Conclusion	Color development phenomenon	Conclusion	Color development phenomenon	Conclusion	Color development phenomenon	Conclusion	Color development phenomenon	Conclusion
volatile oil	qualitative filter paper	none	—	oil spot	+	oil spot	+	none	—	oil spot	++++
anthraquinone	1% sodium hydroxide	none	—	none	—	none	—	red, disappear after acidification	++++	none	—
alkaloid	bismuth potassium iodide	none	—	none	—	none	—	none	—	yellow precipitate	++
phenol	ferric trichloride - 1% potassium ferricyanide	blue spot	++	blue spot	++	blue spot	++++	blue spot	+	blue spot	++
lactone, coumarin	hydroxylamine hydrochloride	prunosus spot	+	prunosus spot	++++	none	—	prunosus spot	++	prunosus spot	++++
saponin	foam test	none	—	none	—	none	—	foam	++	none	—
cardiac glycosides	Kedde reagent	none	—	none	—	prunosus spot	++++	none	—	none	—
flavonoid	hydrochloric acid - magnesium powder	none	—	red spot	++	red spot	++++	red spot	++	red spot	++
organic acid	0.05% bromophenol blue	none	—	none	—	none	—	blue	++	none	—
amino acid	ninhydrin	purple ring spot	+	purple ring spot	+	none	—	purple ring spot	++	none	—
reducing sugar, glycosides with polysaccharide	Fehling reagent	none	—	none	—	none	—	erythrine precipitate	+	none	—
steroid and terpenoid	concentrated sulfuric acid	none	—	brown ring spot	++	brown ring spot	++	brown ring spot	+	brown ring spot	++
cyanophoric glycoside	picric acid sodium test paper	none	—	none	—	none	—	none	—	none	—

Note: “++++”—very significant color development, “++”—significant color development, “+”—insignificant color development, “—”no color development

3.2.3 The influence of solid-liquid ratio extraction rate and antibacterial effect of antibacterial active substances extricated from pineapple leaf fiber

Different solid-liquid ratios were set using acetone at 45°C and 8 h. The results are shown in Fig. 1c. The extraction rate increased with the solid-liquid ratios, reaching a peak value at 1:40 (g/mL). Although the extrication rate continued to increase with the solid-liquid ratio, increases slowed after 1:40. According to Table 3 (b), the active tracking indicator is positively related to solid-

liquid ratio, and the active tracking indicator remains unchanged when solid-liquid ratio reaches 1:40. Through comprehensive consideration of both indexes, the optimal solid-liquid ratio was determined as 1:40 (g/mL) [13].

3.3 Optimization of extrication condition by orthogonal experiment

Based on the theory of second order regression current rotation design, and through comprehensive analysis of single factor experiment, acetone solution was selected as extraction agent. In the experiment, the three factors (temperature, extrication time, and solid-liquid ration) that significantly influenced the extrication effect and antibacterial effect of fiber antibacterial material were selected, the interaction effects were not considered, and $L_9(3^3)$ design was selected. The results are shown in Table 3. Through comparing the maximum difference among the three factor including temperature, extraction time, and solid-liquid ratio, it can be concluded that temperature exhibits the greatest effect followed by time and then solid-liquid ratio [14].

Antibacterial test of extract under optimal process condition. To further verify the reliability of optimal process, 10 g fiber was taken and ground into powder, under the condition that solid-liquid ratio was 1:40, temperature was 45°C and extrication time was 8 h. Through agar diffusion method, the antibacterial activity of the extract was measured, and the IZD was measured as 30.15 mm. Therefore, it indicated that the extrication condition optimized by orthogonal experiment was suitable for the extrication of antibacterial substance from pineapple leaf fiber, providing theoretical basis and basic data for future studies on separation and purification [15].

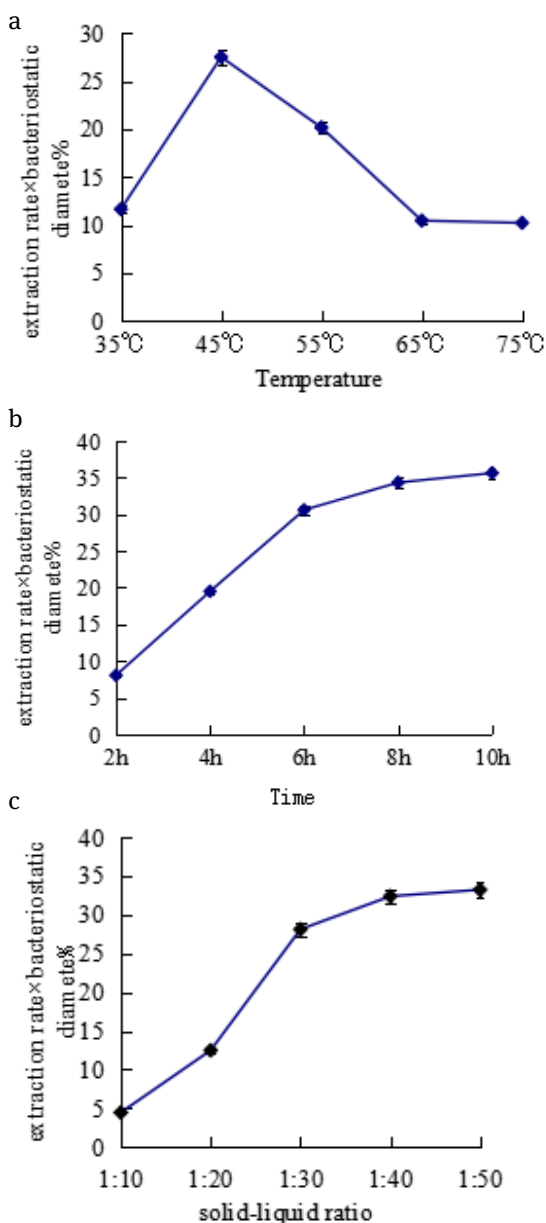


Fig. 1. A) Effect of temperature, B) Effect of extracting time C) Effect of solid-liquid ratio on the extraction rate and antibacterial activity of bacteriostatic material from pineapple leaf fiber.

4 Conclusion

This experiment investigated the influences of different solvents on the extraction of antibacterial compounds from pineapple leaf fiber. Through comprehensively considering the antibacterial activity of each extract and relative qualitative analysis, acetone was finally selected as extraction solvent.

The extrication technology was optimized through single factor test and orthogonal experiment, and the optimal extrication condition was determined as 45°C for extrication temperature, 8 h for extrication time, and solid-liquid ratio for 1:40. In addition, the orthogonal experiment was proved to be reasonable and reliable, and

Table 3. Results of orthogonal test for the extrication effect and antibacterial effect of fiber antibacterial material.

Factor	Temperature (W)	Solid-liquid ratio (L)	Time (T)	IZD (mm)
1	1	1	1	9.44
2	1	2	2	10.65
3	1	3	3	10.9
4	2	1	2	12.29
5	2	2	3	10.5
6	2	3	1	11.5
7	3	1	3	10.3
8	3	2	1	9.3
9	3	3	2	9.0
k1	10.297	10.230	10.320	
k2	11.050	10.310	10.200	
k3	9.700	10.507	10.527	
Maximum difference	1.350	0.277	0.327	
Order of importance	W > T > L			
Optimal level	W2	L3	T3	
Optimal combination	W2T3L3			

can effectively extricate the antibacterial substance from pineapple leaf fiber. The antibacterial test result showed that IZD was as large as 30.15 mm. The order of influence of the three factors is: temperature> time> solid-liquid ratio.

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Conflict of interest: Authors declare nothing to disclose.

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