



Research Article

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**Antibacterial activities of extracts from *Pinus yunnanensis* Franch. var. *pygmaea* pollen and application of the study in teaching**

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**ABSTRACT**

In this article, the antimicrobial effect of extracts from the pinus pollen was studied to provide the theoretical basis for application of the pinus pollen, and the research methods were used in course experiments to enhance students experiment skill. the pinus pollen extracts was extracted by alcohol abstraction method, and antimicrobial activities of extracts against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas stutzeri*, *Asparagillus niger* and *Saccharomyces cerevisiae* were studied by inhibitory zone with filter paper and analysis of minimum inhibitory concentration. As a result, the extracts could inhibit *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae*, and the extracts showed antibacterial effects against six bacterial stains: *E. coli* > *B. subtilis* > *P. stutzeri* > *S. aureus* > *A. niger* > *S. cerevisiae*. Extracts had the strongest antibacterial activity on *E. coli* and the weakest antibacterial action on *S. cerevisiae*, and inhibition rate increased gradually with increase of concentration. The antimicrobial experiments could improve students' experiment skill. On the whole, the pinus pollen could inhibit the growth of microbes, and the antibacterial experiment of the pinus pollen could be used in biochemistry experiment and microorganism experiment.

**Key words:** The pinus pollen, Extracts, Antimicrobial activity, Teaching

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**INTRODUCTION**

*Pinus yunnanensis* var. *pygmaea* belonged to *Pinus*, and the pinus pollen contained rich protein, polysaccharides, lipids, mineral elements, amino acids and vitamin, etc. And the pinus pollen had been traditionally used as health foods and folk medicines for treating illnesses in China for several thousands years. The pinus pollen contained abundant secondary metabolites with more biological activities, including antimicrobial, antioxidation and antitumor properties<sup>[1-6]</sup>.

The pinus pollen extracts had different degrees of antimicrobial activities against one or more microbe, and had antitumor activities. However, few studies has been done on researching the pinus pollen extracts to inhibit food microorganism. In this paper, extracting and antibacterial activities of extracts from *Pinus yunnanensis* var. *pygmaea* were studied in order to study nutritive value and food preservatives effect of the pinus pollen in food preservation. In this study, the alcohol abstraction method was used to gain extracting solution, and inhibitory zone with filter paper and analysis of minimum inhibitory concentration(MIC) were used to investigate antimicrobial effects of extracts from the pinus pollen against *Bacillus subtilis* Cohn, *Staphylococcus aureus* Rosenbach, *Escherichia coli* Castellani et Chalmers, *Pseudomonas stutzeri*, *Asparagillus niger* V. Tiegh and *Saccharomyces cerevisiae* Hansen which were isolated from food. And the antibacterial experiment of the pinus pollen was used in teaching in order to enrich the student experiments and improve students' experimental skills.

## EXPERIMENTAL SECTION

### The pinus pollen samples collection

The pinus pollen samples were collected from Gesala county of Panzihua city in SiChuan province. And the collected samples were dried and preserved.

### The pinus pollen extracts

Ten gram pinus pollen samples were weighed, and the impurities was removed. Further, the pinus pollen samples were broken by mechanical method and soaked in 80% ethanol for 48 hours. Lastly, the pollen samples were smashed and filtered, and filtrate was diluted with distilled water to 50 mL.

### Antimicrobial sensitivity assay

*Bacillus subtilis* Cohn, *Staphylococcus aureus* Rosenbach, *Escherichia coli* Castellani et Chalmers, *Pseudomonas stutzeri*, *Asparagillus niger* V. Tiegh and *Saccharomyces cerevisiae* Hansen were separated and purified from food. The antimicrobial effects of extracts against *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae* were tested by inhibitory zone with filter paper. The diameter of inhibition zones was measured and the average was calculated. And the inhibition rate was assayed by the inhibition zone diameters<sup>[7]</sup>.

The inhibition rate (%) = (the inhibition zone diameters - filter diameter) / the inhibition zone diameters × 100%

Toxicity regression equations and 50% inhibiting concentration (IC<sub>50</sub>) were got in order to determine antibacterial property of the pinus pollen extracts.

### MIC measure

The minimal inhibitory concentration (MIC) was scaled by agar dilution method<sup>[8-10]</sup>.

### Application of antibacterial experiment in teaching

The antibacterial experiment of the pinus pollen was used in students' experiment, and the experiment effect was researched in order to improve the students' ability of experiment.

## RESULTS AND DISCUSSION

### Antibacterial assay of the pinus pollen extracts

As shown in figure 1, the inhibition rate of the pinus pollen extracts against *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae* increased while contents of extracts increased. When extracts concentration increased from 0.2 mg/mL to 125 mg/mL, the inhibition ratio against *E. coli* was the highest, with 78.86% ± 1.98%; it was followed by *S. aureus*, *P. stutzeri*, *B. subtilis* and *A. niger*; and it showed the lowest inhibiting rates against *S. cerevisiae*, with 54.16% ± 2.63%. Therefore, the pinus pollen extracts had antimicrobial activities to *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae*, the extracts had the strongest antimicrobial effect on *E. coli* and the weakest antimicrobial effects on *S. cerevisiae*.

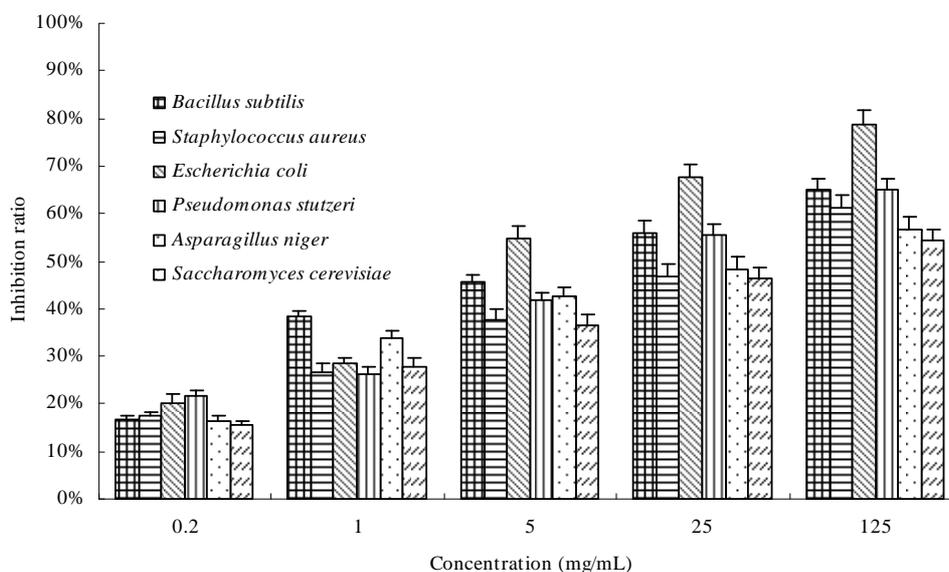


Figure 1 The antibacterial action of extracts in *Pinus yunnanensis* var. *pygmaea* pollen

### Regression analysis of antimicrobial activity from the pinus pollen extracts

The table 1 showed that antimicrobial regression equations and IC<sub>50</sub> were got from the inhibition rates when *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae* were inhibited by the pinus pollen extracts. IC<sub>50</sub> against *E. coli* was the lowest, with 30.89 mg/mL; it was followed by *B. subtilis*, *S. aureus*, *P. stutzeri*, and *A. niger*; and it showed the highest IC<sub>50</sub> against *S. cerevisiae*, with 94.45 mg/mL. There was significant difference between IC<sub>50</sub>. In a word, through testing IC<sub>50</sub>, the value of IC<sub>50</sub> descend orderly: *S. cerevisiae* > *A. niger* > *S. aureus* > *P. stutzeri* > *B. subtilis* > *E. coli*. So the pinus pollen extracts had the strongest antibacterial activity on *E. coli* and the weakest antibacterial action on *S. cerevisiae*.

**Table 1 IC<sub>50</sub> of antibacterial activities in extracts of *Pinus yunnanensis* pollen**

Strains	Regression Equation	R <sup>2</sup>	IC <sub>50</sub> (mg/mL)	T0.01
<i>Bacillus subtilis</i>	y = 0.0025x + 0.3641	0.9556	54.36	C
<i>Staphylococcus aureus</i>	y = 0.0027x + 0.2932	0.908	76.59	B
<i>Escherichia coli</i>	y = 0.0036x + 0.3888	0.9546	30.89	D
<i>Pseudomonas stutzeri</i>	y = 0.0028x + 0.3328	0.9366	59.71	C
<i>Asparagillus niger</i>	y = 0.0021x + 0.3301	0.9438	80.90	B
<i>Saccharomyces cerevisiae</i>	y = 0.0022x + 0.2922	0.9088	94.45	A

### Analysis of minimum inhibitory concentration (MIC)

It could be seen from table 2 that minimum inhibitory concentration (MIC) were got when *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae* were inhibited by extracts within the pinus pollen. MIC against *E. coli* was the lowest, with 98 mg/mL; it was followed by *B. subtilis*, *P. stutzeri*, *S. aureus* and *A. niger*; and it showed the highest MIC against *S. cerevisiae*, with 168 mg/mL. And there were significant difference between MIC. Through testing the value of MIC, the MIC descended orderly: *S. cerevisiae* > *A. niger* > *S. aureus* > *P. stutzeri* > *B. subtilis* > *E. coli*. Therefore, extracts within the pinus pollen had the strongest antibacterial activity on *E. coli* and the weakest antibacterial action on *S. cerevisiae*.

**Table 2 Minimum inhibitory concentration (MIC) of extracts of *Pinus yunnanensis* pollen**

Strains	MIC (mg/mL)	T0.01
<i>Bacillus subtilis</i>	104	C
<i>Staphylococcus aureus</i>	139	B
<i>Escherichia coli</i>	98	D
<i>Pseudomonas stutzeri</i>	112	C
<i>Asparagillus niger</i>	144	B
<i>Saccharomyces cerevisiae</i>	168	A

### Application of antibacterial experiment in teaching

The antibacterial experiment of the pinus pollen was used in biochemistry experiment and microorganism experiment. And alcohol abstraction method was used to attain extracts of the pinus pollen, and it taught students about extraction protocols in biochemistry experiment. And the bacteriostasis experiment was used in microorganism experiment, and it made students learn aseptic manipulation and filtering paper method. Through the experiments, students' experiment skill was increased, so the antibacterial experiment of the pinus pollen could be used in biochemistry experiment and microorganism experiment.

## CONCLUSION

*Pinus yunnanensis* Franch. var. *pygmaea* was distributed widely in China, and the pinus pollen contained rich proteins, amino acids, lipid material, polysaccharides, etc., and these compounds had antitumor, antioxidant and antibacterial effects. However, few study was researched on inhibitory effect.

Plant extracts could inhabit bacteria growing and breeding. In this study, through study on IC<sub>50</sub> and MIC of antibacterial effects on the pinus pollen extracts, the extracts could inhibit *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae*. Inhibition rate increased gradually with increase of concentration, and extracts had the strongest antibacterial activity on *E. coli* and the weakest antibacterial action on *S. cerevisiae*.

The results of the present study suggested the pinus pollen had certain bacteriostatic action against bacteria and fungi, and it had certain treating function and preservative effect. And the antibacterial experiment of the pinus pollen could be used in biochemistry experiment and microorganism experiment in order to improve students' experiment skill.

So the pinus pollen had edible and medicinal value, and it could be made to health care product. And antibacterial experiment of the pinus pollen could be used in course experiments.

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