Comparison Study of Antibacterial Effects of Wound Secretions of Different Strains of Chinese Fir

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Article

Comparison Study of Antibacterial Effects of Wound Secretions of Different Strains of Chinese Fir

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Abstract: To reveal the antibacterial effect of wound secretions of different Chinese fir strains on five kinds of bacteria. Three 3-year-old seedlings of *Taxus chinensis var.koraiensis* and Yangkou and the 3-year-old container seedlings of *Taxus chinensis var.pendula*, Yang061 and Yang020, were used as the research objects. The antibacterial effect of wound secretions was determined by the filter paper diffusion method, and the antibacterial effect was analyzed. Results showed that the wound secretions of different strains of Chinese fir bark had noticeable bacteriostatic effects on *Bacillus subtilis*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. However, the bacteriostatic effects of different strains of Chinese fir secretions differed. The minimum inhibitory concentrations (MICs) of the wound secretions of different strains of Chinese fir against the five test bacteria differed. The mean MIC of *Pseudomonas aeruginosa* was the highest, and the mean MIC of *Escherichia coli* was the lowest. The antibacterial ability of the wound secretions of different Chinese fir strains was mainly determined by the composition of the wound secretions. There was no noticeable relationship with the number of wound secretions.

Keywords: Chinese fir; species; wound exudate; antibacterial effect

1. Introduction

After injury, the wounds in many plants secrete various phenolic secondary compounds and terpenoids of different colors. These secretions have different ecological functions [1,2]. The secretions secreted by plants after injury quickly cover the wound and play a role in sealing and protecting the wound, preventing the invasion of foreign organisms [3]. A large number of studies have shown that shikimic acid, isovitexin, isopimaric acid, and epicatechin can be extracted from plant wound secretions [4–8], have various pharmacological effects, such as anti-oxidation, anti-oxidation, anti-inflammatory, and antibacterial effects. Therefore, research on plant wound secretion is of great practical significance.

Chinese fir (*Cunninghamia lanceolata*) is the main afforestation tree species in the forest areas of southern China. Chinese fir grows rapidly and has high yield and good material quality; moreover, it is the largest afforested tree species in China [9]. In recent years, the number of small animals has increased with improvements in the ecological environment in various forested areas. The bark of Chinese fir forests in some areas is vulnerable to small animal bites and injuries. After bites and injuries, the bark of Chinese fir secretes light yellow or milky white secretions, but the function of these wound secretions of Chinese fir is lacking. Our previous study found that different strains of Chinese fir have formed different environmental response characteristics in long-term adaptation to different environments, and there are significant differences in the number and composition of wound secretions after dry skin injury of different strains of Chinese fir [10]. However, there is a lack of understanding of the biological functions of these different strains of Chinese fir wound secretions, especially whether these wound secretions have antibacterial functions. This significantly limits the configuration and management of artificial afforestation of different strains of Chinese fir.

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Therefore, this study used different strains of Chinese fir plantations created in 2019 as the research object, collected wound secretions through an artificially simulated bark mechanical damage test, and studied the antibacterial effect of wound secretions of different strains of Chinese fir on five different bacteria using the filter paper diffusion method. The differences in the antibacterial effects of wound secretions of different strains of Chinese fir were compared, and the minimum inhibitory concentration (MIC) of wound secretions of different strains of Chinese fir was determined, providing a scientific basis for screening high-disease-resistant Chinese fir strains.

2. Materials and Methods

2.1. Overview of the Test Site

The experimental site is situated in the Yangkou State-owned Forest Farm in Shunchang County, Fujian Province's Banqiao working region (26 ° 50 ′ N, 117 ° 53 ′ E). The location is grade II, the soil type is mountain red, and the elevation is 260 m. With an average annual temperature of 18.5 °C, an average annual sunlight duration of 1740 h, an average annual frost-free period of 230 d, and an average annual precipitation of 1880 mm, it is a mid-subtropical maritime monsoon climate. In 2019, various experimental Chinese fir forest strains were cultivated. Chinese fir clonal container seedlings Yang 061 and Yang 020 were grown in the Forest Farm of Fujian Province, which is controlled by Yangkou State, for afforestation purposes, and Hubei Chuizhisha developed clonal container seedlings that become Chinese firs. Yangkou 3 is a seedling raised at the Forest Farm of Fujian Province, owned by Yangkou State, using seeds from a Chinese fir seed orchard dating back to the third generation. Red-heart Chinese fir seedlings were raised at the Jiangxi Province's Chenshan Forest Farm in Anfu County.

2.2. Experimental Design

Artificial afforestation of different strains of Chinese fir was performed in February 2019 using a randomized block design. Each experimental plot from the foot of the mountain to the top of the mountain strip afforestation, each experimental plot area of more than 500 m^2, with Schima superba as the isolation zone, each experimental plot planting density of 3000 plants · hm-2. A total of three blocks and 15 experimental plots were constructed. The growth of different strains in Chinese fir plantations is shown in Table 1.

Table 1. Growth of 3a Chinese fir plantations of different strains.

Chinese fir species	DBH/cm	Tree height/m
Chuizhisha	3.35±0.11	3.22±0.07
Yangkou 3	4.78±0.06	3.49±0.11
Hongxinsha	4.22±0.01	3.65±0.07
Yang020	3.47±0.62	3.98±0.35
Yang061	4.27±0.53	3.76±0.26

Fifteen typical standard trees were chosen in October 2021 for the artificial mechanical damage wound simulation treatment for each strain of Chinese fir in a three-year-old Chinese fir experimental forest. Wound secretions were collected using a disposable syringe in the early morning when sap flow was slow. The samples were stored at room temperature and returned to the laboratory for further analysis.

Based on the management status, pests, and diseases of Chinese fir plantations, *Bacillus subtilis*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* were selected as the test strains. Test bacteria were purchased from Beijing Lvyuan Bird Biotechnology Co., Ltd.

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The collected wound secretions of Chinese fir were used as bacteriostatic agents to compare the bacteriostatic effects of the wound secretions of different strains of Chinese fir bark.

2.3. Test Treatment Implementation

The antibacterial effects of wound secretions were ascertained using the filter paper diffusion method. A total of 3 g of beef extract, 5 g of sodium chloride, and 10 g of agar powder (20 g) were weighed using an electronic balance and placed in a 1000-ml beaker. Ultrapure water was then added to the beaker and the mixture was heated to slight boiling until the solids completely dissolved. The pH was then adjusted to 7.0, the mixture was sterilized for 30 minutes at 121 °C, and the mixture was refrigerated at 4 °C.

The preparation procedure for the liquid medium without the agar powder was the same as that for the solid medium. Test tubes were subpackaged after processing. The bacterial stock solution was obtained by removing the dry powder test bacteria from the refrigerator at 4 °C, adding them to a test tube filled with sterile water, and shaking the mixture in a 37 °C constant temperature shaker on the sterile ultra-clean bench. An appropriate amount of bacterial stock solution was inoculated onto the plate and activated in a constant temperature incubator at 37 ° C for 24 h. The activated bacterial plate was inoculated with an inoculation rod, and the strain was transferred to a test tube containing sterile water. The bacterial concentration was diluted to 105-8 CFU/mL using a McBurney turbidity tube and stored in a refrigerator at 4 ° C for later use.The sensitivity determination of test bacteriostatic ring is shown in Table 2.

Table 2. Sensitivity determination of test bacteriostatic ring.

	Inhibition zone diameter (mm)				
	0	<10	10-14	15-20	>20
Sensitivity	Insensitivity	Slightly	Moderately	Highly	Extremely

2.4. Determination of Antibacterial Effect

One hundred microliters of the bacterial suspension were placed on a plate and evenly coated with a coating rod. Sterile filter paper infiltrated by wound secretions (6 mm in diameter) was attached to a solid plate, and sterile water infiltration filter paper was used as the blank control. The prepared plates were placed in a constant temperature incubator at 37 ° C for 24 h. The plate was removed, and the diameter of the inhibition zone was measured using a Vernier caliper. Each experiment was performed in triplicates.

2.5. Determination of MIC

The sterile water was used as the diluent, and six concentration gradients of wound secretion were set up, which were 8 mg/ml, 4 mg/ml, 2 mg/ml, 1 mg/ml, 0.5 mg/ml, and 0.25 mg/ml, respectively. A total of 100 uL of bacterial suspension was added to 9 ml of liquid medium containing wound secretions and fully mixed. After incubation in a constant-temperature incubator for 24 h, the plate was inoculated and placed in a constant-temperature incubator for 24 h to observe colony growth on each plate. Each concentration gradient was repeated thrice, with sterile water as the control, and the presence of growing colonies on the plate was used as the MIC criterion.

2.6. Data Processing and Analysis

SPSS software (version 24.0) was used for statistical analysis. Single factor T test was used to compare the effects of wound secretions of five strains of Chinese fir on different strains ($\alpha = 0.05$). Excel 2010 software was used to create the charts.

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3. Results

3.1. Comparison of Bacteriostatic Effects of Wound Secretions from Different Strains of Chinese Fir on Bacillus subtilis

The bacteriostatic test results for the different strains of Chinese fir secretion against *Bacillus subtilis* are shown in Table 3. The wound secretions of the five Chinese fir bark strains showed significant antibacterial effects against *Bacillus subtilis* ($P<0.05$). Among them, the average diameter of the inhibition zone of Yangkou 3, Yang020, Red-heart Chinese fir, Yang061, and Chuizhisha was 2.70 times, 2.48 times, 3.07 times, 2.56 times, and 1.90 times that of the CK, respectively. The antibacterial sensitivity of Taxodium distichum is moderately sensitive, and the other four strains are highly sensitive. The antibacterial effect of the wound secretions of the five strains of Chinese fir was ranked as Red-heart Chinese fir > Yangkou 3 > Yang 061 > Yang 020 > Chuizhisha.

Table 3. Comparison of antibacterial effect of wound exudate of different species of Chinese fir on *Bacillus subtilis*.

Chinese fir species	Inhibition zone diameter (mm)		Sensitivity
	CK	Chinese fir secretions	
Yangkou 3	6±0	16.22±4.39ab	Highly
Yang 020	6±0	14.85±0.77b	Highly
Red-heart Chinese fir	6±0	18.44±2.25a	Highly
Yang 061	6±0	15.37±2.8ab	Highly
Chuizhisha	6±0	11.42±0.33c	Moderately

Different lowercase letters in the Table indicate that the bacteriostatic effect of different strains of Chinese fir secretions is significantly different ($P < 0.05$).

The MIC test results for different strains of Chinese fir wound secretions against *Bacillus subtilis* are shown in Table 4. The MIC of Red-heart Chinese fir was 0.5 mg/ml, the MIC of Yangkou 3 and Yang 061 was 1 mg/ml, the MIC of Yang 020 was 2 mg/ml, and the MIC of Chuizhisha was 4 mg/ml. The MICs of wound secretions of Chuizhisha, Yang 020, and Yang 061 on *Bacillus subtilis* were higher than those of Yangkou 3 and Red-heart Chinese fir. The MIC of wound secretions of Yangkou 3rd generation on *Bacillus subtilis* was higher than that of Red-heart Chinese fir, and the wound secretions of Red-heart Chinese fir had the strongest inhibitory effect on *Bacillus subtilis*.

Table 4. Minimum inhibitory concentration (MIC) of wound exudate from different species of Chinese fir against *Bacillus subtilis*.

Chinese fir species	Secretion concentration (mg·ml^{-1})					
	0.25	0.50	1	2	4	8
Yangkou 3	++	+	-	-	-	-
Yang 020	+++	++	+	-	-	-
Red-heart Chinese fir	+	-	-	-	-	-
Yang 061	++	+	-	-	-	-
Chuizhisha	+++	++	+	+	-	-

'+++'indicates that the colony grows vigorously at this secretion concentration,'++'indicates that the colony grows more at this secretion concentration,'+'indicates that the colony grows a small amount at this secretion concentration, '-'indicates that the colony grows aseptically at this secretion concentration, the same below.

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3.2. Comparison of Antibacterial Effect of Wound Exudate of Different Species of Chinese Fir Against Salmonella paratyphi B

The bacteriostatic test results for the different strains of Chinese fir secretions against *Salmonella paratyphi B* are shown in Table 5. The wound secretions of the five Chinese fir strains showed significant antibacterial effects against *Bacillus subtilis* (*P<0.05*). Among them, the average diameter of the inhibition zone of Yangkou 3, Yang020, Red-heart Chinese fir, Yang061, and Chuizhisha was 2.21 times, 1.72 times, 2.00 times, 2.00 times, and 1.83 times that of the CK, respectively. The bacteriostatic sensitivity of wound secretions of different strains of Chinese fir to *Salmonella paratyphi B* was moderately sensitive. The antibacterial effect of the wound secretions of the five strains of Chinese fir was ranked as Yangkou 3 >Red-heart Chinese fir > Yang 061 > Chuizhisha> Yang 020.

Table 5. Comparison of antibacterial effect of wound exudate of different species of Chinese fir against *Salmonella paratyphi B.*

Chinese fir species	Inhibition zone diameter (mm)		Sensitivity
	CK	Chinese fir secretions	
Yangkou 3	6±0	13.27±1.71a	Moderately
Yang 020	6±0	10.30±0.21c	Moderately
Red-heart Chinese fir	6±0	12.01±0.87b	Moderately
Yang 061	6±0	11.97±0.75b	Moderately
Chuizhisha	6±0	10.97±0.68bc	Moderately

Different lowercase letters in the Table indicate that the bacteriostatic effect of different strains of Chinese fir secretions is significantly different ($P < 0.05$).

The MIC test results for different strains of Chinese fir secretions against *Salmonella paratyphi B* are shown in Table 6. The MICs of Yangkou 3, Red-heart Chinese fir, and Yang 061 were 2 mg/ml, and those of Yang 020 and Chuizhisha were 4 mg/ml. The MICs of the wound secretions of the five strains of Chinese fir Yang020 and Chinese fir on *Salmonella paratyphi B* were greater than those of Yangkou 3, Red-heart Chinese fir, and Yang061. Yangkou 3 had the strongest antibacterial effect on *Salmonella paratyphi B*.

Table 6. MICs of wound exudate from different species of Chinese fir against *Salmonella paratyphi B.*

Chinese fir species	Secretion concentration (mg • ml^{-1})					
	0.25	0.50	1	2	4	8
Yangkou 3	++	+	+	-	-	-
Yang 020	+++	++	+	+	-	-
Red-heart Chinese fir	++	+	+	-	-	-
Yang 061	++	+	+	-	-	-
Chuizhisha	+++	++	+	+	-	-

3.3. Comparison of Antibacterial Effect of Wound Exudate of Different Species of Chinese Fir Against Pseudomonas aeruginosa

The antibacterial test results of the wound secretions of different Chinese fir strains against *Pseudomonas aeruginosa* are shown in Table 7. The wound secretions of the five Chinese fir strains showed significant antibacterial effects against *Pseudomonas aeruginosa* (P<0.05). Among them, the average diameter of the inhibition zone of Yangkou 3, Yang020, Red-heart Chinese fir, Yang061, and Chuizhisha was 1.70, 1.35, 1.93, 1.83, and 1.51 times that of the CK, respectively. It is moderately sensitive, and the average diameter of the inhibition zone was 9.07 mm, which was less sensitive. The antibacterial effect of the wound secretions of the five strains of Chinese fir was ranked as Red-heart Chinese fir > Yang 061 >Yangkou 3 > Chuizhisha> Yang 020.

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Table 7. Comparison of antibacterial effect of wound exudate of different species of Chinese fir against *Pseudomonas aeruginosa.*

Chinese fir species	Inhibition zone diameter (mm)		Sensitivity
	CK	Chinese fir secretions	
Yangkou 3	6±0	10.21±0.46c	Moderately
Yang 020	6±0	8.12±0.48e	Slightly
Red-heart Chinese fir	6±0	11.58±0.29a	Moderately
Yang 061	6±0	10.96±0.76b	Moderately
Chuizhisha	6±0	9.07±0.53d	Slightly

Different lowercase letters in the Table indicate that the bacteriostatic effect of different strains of Chinese fir secretions is significantly different ($P < 0.05$).

The MIC test results for the different strains of Chinese fir secretions against *Pseudomonas aeruginosa* are shown in Table 8. The MIC of Yangkou 3, Hongxinshan, and Yang 061 was 4 mg/ml, whereas that of Yang 020 and Chuizhishan was 8 mg/ml. The MICs of wound secretions of Yang020 and Chuizhisha on *Pseudomonas aeruginosa* were higher than those of Yangkou 3, Red-heart Chinese fir, and Yang061.

Table 8. MICs of wound exudate from different species of Chinese fir against *Pseudomonas aeruginosa.*

Chinese fir species	Secretion concentration (mg • ml^{-1})					
	0.25	0.50	1	2	4	8
Yangkou 3	+++	++	+	+	-	-
Yang 020	+++	+++	++	+	+	-
Red-heart Chinese fir	+++	++	+	+	-	-
Yang 061	+++	++	++	+	-	-
Chuizhisha	+++	+++	++	++	+	-

3.4. Comparison of Antibacterial Effect of Wound Exudate of Different Species of Chinese Fir Against Escherichia coli

The bacteriostatic test results for the different strains of Chinese fir secretions against *Escherichia coli* are presented in Table 9. Wound secretions of the five Chinese fir strains showed antibacterial effects against *Escherichia coli*. Among them, the average diameter of the inhibition zone of Yangkou 3, Yang020, Red-heart Chinese fir, Yang061, and Chuizhisha was 2.68 times, 2.87 times, 2.79 times, 2.92 times, and 2.91 times that of the CK, respectively. The bacteriostatic sensitivity of the five Chinese fir strains toward *E. coli* was also high. The antibacterial effects of the five strains on *E. coli* were ranked as follows: Yang 061 > Chuizhisha > Yang 020 > Red-heart Chinese fir > Yangkou 3.

Table 9. Comparison of antibacterial effect of wound exudate of different species of Chinese fir against *Escherichia coli.*

Chinese fir species	Inhibition zone diameter (mm)		Sensitivity
	CK	Chinese fir secretions	
Yangkou 3	6±0	16.09±0.76a	Highly
Yang 020	6±0	17.22±0.45a	Highly
Red-heart Chinese fir	6±0	16.76±2.00a	Highly
Yang 061	6±0	17.52±0.92a	Highly
Chuizhisha	6±0	17.45±0.76a	Highly

Different lowercase letters in the Table indicate that the bacteriostatic effect of different strains of Chinese fir secretions is significantly different ($P < 0.05$).

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The MIC test results for the different strains of Chinese fir secretions against *Escherichia coli* are shown in Table 10. The MICs of Yang020, Red-heart Chinese fir, Yang061 and Chuizhisha were all 0.5 mg/ml, and the MIC of Yangkou 3 was 1 mg/ml. The MIC of wound secretions of Yangkou 3 on *Escherichia coli* was higher than that of Yang020, Red-heart Chinese fir, Yang061, and Chuizhisha. This showed that Yangkou 3 had the worst bacteriostatic effect on *Escherichia coli*.

Table 10. MICs of wound exudate from different species of Chinese fir against *Escherichia coli.*

Chinese fir species	Secretion concentration (mg • ml^{-1})					
	0.25	0.50	1	2	4	8
Yangkou 3	++	+	-	-	-	-
Yang 020	+	-	-	-	-	-
Red-heart Chinese fir	+	-	-	-	-	-
Yang 061	+	-	-	-	-	-
Chuizhisha	+	-	-	-	-	-

3.5. Comparison of Antibacterial Effect of Inhibition Zone of Wound Secretion of Different Species of Chinese Fir Against Staphylococcus aureus

The bacteriostatic test results for different strains of Chinese fir secretions against *Staphylococcus aureus* are shown in Table 11. Five strains of Chinese fir secretions exhibited antibacterial effects against *Staphylococcus aureus*. Among them, the average diameter of the inhibition zone of Yangkou 3, Yang 020, Red heart Chinese fir, Yang 061, and Chuizhisha is 2.49 times, 2.55 times, 2.41 times, 2.44 times, and 2.53 times that of the CK, respectively. Yangkou3, Red heart Chinese fir and Yang 061 were moderately sensitive to S.aureus, while Yang 020 and Chuizhisha were highly sensitive. The antibacterial effect of the five strains on *Staphylococcus aureus* was ranked as Yang 061 > Chuizhisha > Yang 020 > Red-heart Chinese fir > Yangkou 3.

Table 11. Comparison of antibacterial effect of inhibition zone of wound secretion of different species of Chinese fir against *Staphylococcus aureus.*

Chinese fir species	Inhibition zone diameter (mm)		Sensitivity
	CK	Chinese fir secretions	
Yangkou 3	6±0	14.93±0.84ab	Moderately
Yang 020	6±0	15.29±0.45a	Highly
Red-heart Chinese fir	6±0	14.44±0.32b	Moderately
Yang 061	6±0	14.64±0.66ab	Moderately
Chuizhisha	6±0	15.17±0.30a	Highly

Different lowercase letters in the Table indicate that the bacteriostatic effect of different strains of Chinese fir secretions is significantly different ($P < 0.05$).

The MIC test results for the different strains of Chinese fir secretions against *Staphylococcus aureus* are shown in Table 12. The MIC of Yang 020 was 1 mg/ml, and that of Yangkou 3, Red-heart Chinese fir, Yang 061, and Chuizhisha was 2 mg/ml. The MIC of Yang020 wound secretions against *Staphylococcus aureus* was lower than that against the other four strains of Chinese fir.

Table 12. MICs of wound exudate from different species of Chinese fir against *Staphylococcus aureus.*

Chinese fir species	Secretion concentration (mg • ml^{-1})					
	0.25	0.50	1	2	4	8
Yangkou 3	++	++	+	-	-	-
Yang 020	++	+	-	-	-	-
Red-heart Chinese fir	++	++	+	-	-	-
Yang 061	++	+	+	-	-	-
Chuizhisha	++	+	+	-	-	-

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4. Discussion

The extraction of natural active antibacterial substances from plants has attracted significant attention from scholars [11–13]. In the 1960s, the American Institute of Water Oncology performed antitumor bioassays on plant extracts [14]. Eight kinds of plant extracts, such as honeysuckle, have inhibitory effects on *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, etc. [15–17]. These extracts play an antibacterial role and contain terpenes, alkaloids, flavonoids, phenols, and glycosides [18]. A previous study of the research group showed that the number of wound secretions in Chinese fir was significantly higher than that of other Chinese fir strains, and the composition of wound secretions was significantly different from that of the other three strains [10]. This study showed that the wound secretions of five strains of Chinese fir had bacteriostatic effects on *Bacillus subtilis*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. However, the bacteriostatic effects of the same strain of Chinese fir secretions on different strains differed, and the bacteriostatic effects of different strains on the same strain were also different. The antibacterial effect on *Bacillus subtilis* was as follows: Red-heart Chinese fir > Yangkou 3 > Yang 061 > Yang 020 > Chuizhisha. The antibacterial effect on *Salmonella paratyphi B* was as follows: Yangkou 3 >Red-heart Chinese fir >Yang 061 > Chuizhisha> Yang 020. The antibacterial effect on *Pseudomonas aeruginosa* was as follows: Red-heart Chinese fir >Yang 061 >Yangkou 3 > Chuizhisha> Yang 020. The antibacterial effect on *Escherichia coli* was as follows: Yang 061 >Chuizhisha> Yang 020 > Red-heart Chinese fir >Yangkou 3. The antibacterial effect on *Staphylococcus aureus* was as follows: Yang 061 >Chuizhisha> Yang 020 > Red-heart Chinese fir >Yangkou 3;

Because the secretion is usually a mixture, the antibacterial effect caused by the superposition of antibacterial substances may increase or decrease in multiples [19]. Shi et al. combined the concentrations of different antibacterial extracts, and the antibacterial effect significantly improved, indicating that the compound had a synergistic antibacterial effect [20]. Chen et al. revealed the inhibitory effect of essential oils on different strains using the minimum inhibitory test on 18 types of plant essential oils [21]. This study showed that the MICs of the wound secretions of different strains of Chinese fir for the five test bacteria differed. The MIC was highest for B. subtilis and lowest for P. koraiensis. The MICs of *Salmonella paratyphi B* and *Pseudomonas aeruginosa* were Yang 020 and Huizhisha > Yangkou 3, Red-heart Chinese fir, and Yang 061. The MIC of *Escherichia coli* showed that the MIC of Yangkou 3 was higher than that of the other four strains. The MIC of *Staphylococcus aureus* was Yang 061, Chuizhisha, Yangkou 3, and Red-heart Chinese fir > Yang 020. The antibacterial effect on *Pseudomonas aeruginosa* was the lowest among the five bacteria tested, and the antibacterial effect on *Escherichia coli* was the strongest among the five bacteria. This is related to the fact that *Pseudomonas aeruginosa* is a commonly acquired pathogenic bacteria with strong drug resistance [22,23]. Its changeable adaptation mechanism and active metabolic ability can protect the bacteria from external damage, and the aromatic and sesquiterpene compounds in the secretions are the main substances inhibiting *Escherichia coli* [24], which may be the reason for its strong antibacterial effect on the wound secretions of Chinese fir.

The number of wound secretions of Chuizhisha was significantly higher than that of other strains of C. lanceolata, but the antibacterial effect of Chuizhisha wound secretions on *Bacillus subtilis*, *Salmonella paratyphi B*, and *Pseudomonas aeruginosa* was poor, whereas the Red heart Chinese fir with the least number of wound secretions had the best antibacterial effect on these three bacteria, indicating that the antibacterial effect of wound secretions may not be directly related to their number. The composition of wound secretions of Chuizhisha and Yang 061 are very similar, and their antibacterial effects on different bacteria are very similar, which indicates that the antibacterial ability may be determined by the type of secretion rather than the number of secretions. Chuizhisha and Yang 061 had the best antibacterial effect on *Escherichia coli* and *Staphylococcus aureus*. The wound secretions of these two Chinese fir strains were mainly composed of shikimic acid, isovitexin, and isopimaric acid, most of which are organic acid compounds. Studies have shown that the antibacterial properties of different substances vary. An antibacterial mixture based on organic acids exhibits a strong antibacterial effect against *Escherichia coli* [25]. This study also obtained similar results. The highest proportion of wound secretions in the Yangkou 3, Yang020, and Red heart Chinese fir was

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carnosol, accounting for more than 55 %, mainly terpenoids, which had the best antibacterial effect on *Bacillus subtilis*. This indicates that carnosol is an effective antibacterial substance against *Bacillus subtilis*, which is similar to the results of previous studies [26].

5. Conclusions

The wound secretions of different strains of Chinese fir bark had obvious antibacterial effects on *Bacillus subtilis, Salmonella paratyphi B, Pseudomonas aeruginosa*, *Escherichia coli,* and *Staphylococcus aureus*; however, the antibacterial effects of different strains of Chinese fir secretions were different. The antibacterial effect of wound secretions of different strains of Chinese fir bark on *Bacillus subtilis* was as follows: Red-heart Chinese fir > Yangkou 3 > Yang 061 > Yang 020 > Chuizhisha. The antibacterial effects on *Salmonella paratyphi B* were as follows: Yangkou 3 > Red-heart Chinese fir > Yang 061 > Chuizhisha > Yang 020. The antibacterial effect on *Pseudomonas aeruginosa* was as follows: Red-heart Chinese fir > Yang 061 > Yangkou 3 > Chuizhisha > Yang 020, and the antibacterial effect on *Escherichia coli* was Yang 061 > Chuizhisha > Yang 020 > Red-heart Chinese fir > Yangkou 3. The antibacterial effects on *Staphylococcus aureus* were as follows: Yang 061 > Chuizhisha > Yang 020 > Red-heart Chinese fir > Yangkou 3.

The effects of the wound secretions of different Chinese fir strains on the MIC of five kinds of test bacteria were different. The MICs of the wound secretions of different strains of Chinese fir on the five types of test bacteria differed. The average MIC for *Pseudomonas aeruginosa* was the highest, and the average MIC for *Escherichia coli* was the lowest. The MIC of *Bacillus subtilis* was highest in Chuizhisha and lowest in Red-heart Chinese fir. The MICs of *Salmonella paratyphi B* and *Pseudomonas aeruginosa* were Yang 020, Chuizhisha > Yangkou 3, Red-heart Chinese fir, and Yang 061. The MIC of *Escherichia coli* showed that the MIC of Yangkou 3 was greater than that of the other four strains. The MIC of *Staphylococcus aureus* was Yang 061, Chuizhisha, Yangkou 3, Red-heart Chinese fir > Yang 020. The antibacterial ability of wound secretions from different strains of Chinese fir is closely related to the composition of wound secretions, and the correlation with their quantity is unclear. Organic acid compounds may play a major antibacterial role against *Escherichia coli*, whereas terpenoids may play a major antibacterial role against *Bacillus subtilis*.

In this paper, the bacteriostatic effect of five strains of Chinese fir secretions on bacteria was compared and discussed. However, due to the limited experimental materials (five strains), the bacteriostatic mechanism of Chinese fir secretions on bacteria may not have been fully revealed. Follow-up studies can be performed on the comparisons of firs of different ages and with different provenances and thus further explore the bacteriostatic effect of Chinese fir secretions on bacteria.

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