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IN VITRO ANTIMICROBIAL ACTIVITIES OF *NOSTOC COMMUNE* EXTRACTS

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ABSTRACT

In the article, the antimicrobial effect of *Nostoc commune* extracts was studied to provide the theoretical basis for application of *Nostoc commune*. *N. commune* 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* > *S. aureus* > *S. cerevisiae* > *B. subtilis* > *A. niger* > *P. stutzeri*. Extracts had the strongest antibacterial activity on *E. coli* and the weakest antibacterial action on *P. stutzeri*, and inhibition rate increased gradually with increase of concentration. On the whole, *N. commune* extracts could inhibit the growth of microbes, and inhibition rate increased gradually with increase of extracts concentration.

Keywords: *Nostoc commune* Vaucher, Extracts, Antimicrobial activity, IC50, MIC.

1. INTRODUCTION

Nostoc commune is a cyanobacterium, which is known as blue-green algae and also called "Di muer" in China. And *N. commune* is widely distributed around the world, with wide distribution in every province of China. *N. commune* had been traditionally used as health foods and folk medicines for treating illnesses in China for several thousand years. And it was eaten as a vinegared, known as "ishikurage" in Japan [1-4]. Wild *N. commune* had been decreasing in quantity because of market demand and environmental pollution; however, *N. commune* could be cultured in BG11 medium [5].

N. commune contains abundant secondary metabolites with more biological activities, including antimicrobial, ant oxidation, antimitotic, cytotoxic, and enzyme inhibitory properties. Antibacterial activities of different substances had been studied in *N. commune*. Diterpenoids comnostin A-E had antibacterial activities against *Bacillus cereus*, *Staphylococcus epidermidis* and *Escherichia coli*. Nostofugicidine had antifungal activity against *Aspergillus candidus* [4]. The polysaccharides in *N. commune* had the anti-tumor, anti-virus, antibacterial, anti-inflammatory effects; it could strengthen immunity ability and could be used to promote crops' growth [6-8]. Inhibiton ratio of polysaccharide on *B. subtilis* and *E. coli* was more than that on *A. niger* [9]. A research found polysaccharides could inhibit the growth of *B. subtilis* and *E. coli* [10]. Total flavonoids of *N. commune* were a natural antibacterial agent against some common microorganisms [11]. Lipid compositions from *N. commune* had antibacterial effects against *E. coli*, *S. aureus*, *B. subtilis*, *A. Niger*, *S. cerevisiae* and *P. stutzeri* [12].

Microalgae extracts had different degrees of antimicrobial activities against one or more microbe, and had antitumor activities [13]. However, few studies has been done on researching *N. commune* extracts to inhibit food microorganism. In this paper, extracting and antibacterial activities of extracts from *N. commune* were studied in order to study food preservatives effect of *N. commune* 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 were used to investigate antimicrobial effects of extracts from *N. commune* against *Bacillus subtilis* Cohn, *Staphylococcus aureus* Rosenbach, *Escherichia coli* Castellaniet Chalmers, *Pseudomonas stutzeri, Asparagillus niger* V. Tiegh and *Saccharomyces cerevisiae* Hansen which were isolated from food.

2. MATERIALS AND METHODS

2.1 *Nostoc commune* samples collection

N. commune samples were collected from PengXi County in Sichuan province. And the collected samples were washed and dried.

2.2 *Nostoc commune* extracts

Ten gram *N. commune* samples was weighed, and the sediment was rinsed with distilled water and drained. Further, *N. commune* samples were soaked in 80% ethanol for 48 hours and swelled. Lastly, the swelled samples were smashed and filtered, and filtrate was diluted with distilled water to 50 mL [14].

2.3 Antimicrobial sensitivity assay

Bacillus subtilis Cohn, *Staphylococcus aureus* Rosenbach, *Escherichia coli* Castellaniet 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.

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

Toxicity regression equations and 50% inhibiting concentration (IC_{50}) were got in order to determine antibacterial property of *N. commune* extracts.

2.4 MIC measure

The minimal inhibitory concentration (MIC) was scaled by agar dilution method [15-17].

3. RESULTS AND THE ANALYSIS

3.1 Antibacterial assay of *Nostoc commune* etracts

As shown in figure 1, the inhibition rate of *N. commune* etracts against *B. subtilis*, *S. aureus*, *E.* Castellaniet, *P. stutzeri*, *A. niger* and *S. cerevisiae* increased while contents of etracts increased. When etracts concentration increased from 0.2 mg/mL to 125 mg/mL, the inhibition ratio against *E. coli* was the highest, with 64.87% ± 2.88%; it was followed by *S. cerevisiae*, *S. aureus*, *B. subtilis* and *A. niger*; and it showed the lowest inhibiting rates against *P. stutzeri*, with 48.96% ± 2.36%. Therefore, *N. commune* etracts 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 *P. stutzeri*.

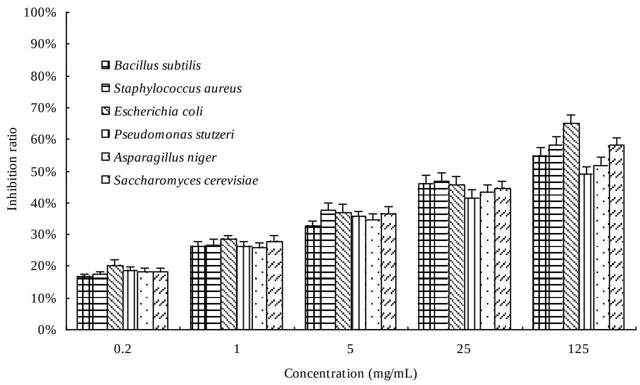

Figure 1. The antibacterial action of extracts in *Nostoc commune*

3.2 Regression analysis of antimicrobial activity from *Nostoc commune* extracts

The table 1 showed that antimicrobial regression equations and IC_{50} were got from the inhibition rates when *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri, A. Niger* and *S. cerevisiae* were inhibited by *N. commune* extracts. IC_{50} against *E. coli* was the lowest, with 68.93 mg/mL; it was followed by *S. aureus*, *S. cerevisiae*, *B. subtilis* and *A. Niger*; and it showed the highest IC_{50} against *P. stutzeri*, with 118.50 mg/mL. There was significant difference between IC_{50}. In a word, through testing IC_{50}, the value of IC_{50} descends orderly: *P. stutzeri* > *A. Niger* > *B. subtilis* > *S. cerevisia* > *S. aureus* > *E. coli*. So *N. commune* extracts had the strongest antibacterial activity on *E. coli* and the weakest antibacterial action on *P. stutzeri*.

Table 1. IC_{50} of antibacterial activities in extracts of *Nostoc commune*

Strains	Regression Equation	R^2	IC_{50} (mg/mL)	T0.01
Bacillus subtilis	y = 0.0024x + 0.2788	0.8799	92.17	C
Staphylococcus aureus	y = 0.0025x + 0.2949	0.885	78.04	E
Escherichia coli	y = 0.0029x + 0.3001	0.9283	68.93	F
Pseudomonas stutzeri	y = 0.0018x + 0.2867	0.9182	118.50	A
Asparagillus niger	y = 0.002x + 0.2834	0.966	108.30	B
Saccharomyces cerevisiae	y = 0.0025x + 0.2942	0.8929	85.32	D

2.3 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 *N. commune*. MIC against *E. coli* was the lowest, with 155 mg/mL; it was followed by *S. aureus*, *S. cerevisiae*, *B. subtilis* and *A. Niger*; and it showed the highest MIC against *P. stutzeri*, with 275 mg/mL. And there were significant difference between MIC through testing the value of MIC, the MIC descended orderly: *P. stutzeri* > *A. Niger* > *B. subtilis* > *S. cerevisia* > *S. aureus* > *E. coli*. Therefore, extracts within *N. commune* had the strongest antibacterial activity on *E. coli* and the weakest antibacterial action on *P. stutzeri*.

Table 2. Minimum inhibitory concentration (MIC) of extracts of *Nostoc commune*

Strains	MIC (mg/mL)	T0.01
Bacillus subtilis	205	C
Staphylococcus aureus	168	E
Escherichia coli	155	F
Pseudomonas stutzeri	275	A
Asparagillus niger	240	B
Saccharomyces cerevisiae	175	D

4. DISCUSSION

N. commune was distributed widely in the world, and it contained rich proteins, amino acids, lipid material, polysaccharides, total flaconoids, vitamins and many kinds of minerals, etc., and these compounds had antitumor, antiviral, antioxidant and antibacterial effects. However, few studies was researched on inhibitory effect.

Plant extracts could inhabit bacteria growing and breeding [16]. The microalgae extracts had different degrees of antimicrobial activity against *S. aureus, B. subtilis*, *E. coli*, *A. Niger* and *P. chrysogenum*, and showed potent activity against the growth of the tumor cells [13]. *N. commune* extracts had obvious inhibition effects on *E. coli*, *S. aureus*, *B. saotilis*, *and Monilia* and *Serratia marcescens* [18].

In this study, through study on IC_{50} and MIC of antibacterial effects on *N. commune* extracts, the extracts could inhibit *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri, A. niger* and *S. cerevisiae*, research result was similar to Deng Wei and Eloff 's reports[13,16]. Inhibition rate increased gradually with increase of concentration, and extracts had the strongest antibacterial activity on *E. coli* and the weakest antibacterial action on *P. stutzeri*, such findings were similar to Qian Senhe's report [18].

The results of the present study suggested *N. commune* extracts had certain bacteriostatic action against bacteria and fungi, and it had certain treating function and preservative effect [18, 19]. So *N. commune* could be used in food preservation and had edible and medicinal value, which contributed to its popularity in China.

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