Antimicrobial Activity and Mechanism of *Schisandra Chinensis* Extract

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Abstract. The methanol extract of *Schisandra chinensis* is evaluated for their antimicrobial activity and mechanism on four common gastrointestinal bacteria. The antimicrobial activity is tested through inhibition zone, MIC and MBC. The antibacterial mechanism is studied based on the growth curve and cell membrane permeability. The results show that the *Schisandra chinensis* extract is against on *E. coli*, and increase *L. bulgaricus numberand*. The antibacterial mechanism about *E. coli* is as follow: the extract decreases the number of cell divisions in logarithmic phases and induces the voids of cell walls to increase, thus the materials in the cell to leak out. The mechanism of increase *L. bulgaricus* is that the extract can make logarithmic growth phase accelerated and keep cells wall intact, so let the *L. bulgaricus* to live long.

1. Introduction

The common gastrointestinal bacteria contain pathogens and probiotics. The pathogens may cause gastrointestinal diseases, such as, Escherichia coli which could cause diarrhea when the numbers are over standard. On the contrary, the probiotics are ease the symptoms of people who often have slightly abnormal gut microbiomes with irritable-bowel syndrome [1], such as, *Bacillus coagulans* (used for treating diarrhea and constipation), *Bacillus subtili* (could block pathogenic bacteria to cause in intestine) and *Lactobacillus bulgaricus* (lacto-bacillus for the intestine, could supplement from yogurt). As new infections and bacterial resistance are constantly emerging, novel drugs or the antibiotics had been used more. However, the drugs are not only impact on pathogens but also kill the probiotics and human cells. So it is very important to find the new drug that has antibacterial activity against bacteria and little affecting on probiotics or increasing probiotics.

Schisandra chinensis (wu wei zi) is an edible and medical plant. The fruit can eat and induce astringency, replenishing and promoting production of body fluid and tonifying the kidney to relieve mental strain [2]. It also could treat for abdominal distension, regurgitation and dyspepsia. The research on *Schisandra chinensis* extract has made great progress recently. Although much study has been published [3], little has been discussed about common gastrointestinal pathogens and probiotics. In this paper, the methanol extract from *Schisandra chinensis* is evaluated for its antimicrobial activity and mechanism against *E. coli* and three kinds of probiotics *B. coagulans, B. licheniformis,* and *L. bulgaricus* respectively.

2. Experimental

2.1 Material

Type cultures of *Escherichia coli* (ATCC 25922; G-; pathogen), *Bacillus licheniformis* (ATCC 11945; G+; probiotic), *Bacillus subtili* (ATCC 6051; G+; probiotic) and *Lactobacillus bulgaricus* (ATCC 10638; G+; probiotic) from Microbiology Institute of Shannxi, were used in the vitro study. Frozen isolates were thawed and their identity reconfirmed using standard methodology. They were inoculated onto Luria-Bertani (LB) and incubated for 3 days at 37.8℃ [4].

At last, the bacteria liquid were harvested and diluted in aquae sterilised at a concentration of 1.5×10^{-8} spores/ml (0.5 Mac Farland Standard Units) for studies[5].

2.2 Collection and Extraction of *Schisandra chinensis* extract

The *Schisandra chinensis* were got from *Taibai* mountain area, Baoji, China, identified by Ph. D. Xiaomei Wang from Baoji University of Arts and Science. The plants were healthy rhizomes and stored after being dried in the shade.

The *Schisandra chinensis* extract were prepared for methanol extraction [6]. The standard protocols were as follow: 500ml methanol (MeOH) and 100g powdery of the material were put into the 1000ml round-bottomed flask to reflux condensation at 55℃ lasted for 2 hours. Then the solution was used for vacuum extraction filtering to get the filtrate. After the MeOH was evaporated, added distilled water and waved under supersonic to get the 100mg/ml turbid liquid to proceed for the tests.

2.3 In vitro antimicrobial activities test

The inhibition zone was formed according to a standard protocol designed on nutrient agar (NA) [5]. Briefly, 200μl liquid of each bacterial species were inoculated on NA plate using a glass rod, then 6 mm diameter paper disks soaked in 20μl of each of the crude extracts at a concentration of 100mg/ml were placed concentrically on the NA plate. The NA plates were incubated for 20h, 37℃. The inhibition zone diameter was the average of three measured replicates.

And then, the crude extract would be determined the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) by double broth dilution method with microdilution checkerboard techniques [7]. The microorganism was seeded into 96 well plates and incubated in an inverted position for 20 hours at 35°C to observe whether it turned red after an increase of 5% MTT. The concentration of the last no-reddened well plate was MIC. After MIC experiment, 200μl taken from each well with the concentration being no more than MIC bacterial suspension was inoculated in blood agar plates for 24h for observation. The lowest drug concentration that yielded no growth was documented as MBC. The crude extracts liquid were conducted three times.

2.4 In vitro activities mechanism test

After the antimicrobial activity test, the bacteria growth curves were drawn by classical method [8]. The control group (without drug, CK group) and drug group (100mg/ml extract liquid) were built for every kind of microorganism. Each group had seven time points of inoculation (0h, 4h, 8h, 12h, 16h, 20h, 24h), then incubated them at 37℃. The ultraviolet spectrophotometer would be adopted to measure the absorbance on 610nm at each time point, and then a curve of absorbance would be drawn accordingly.

And then, the cell membrane permeability of extract would be studied through the conductivity of bacterial liquid, reducing sugar content of bacterial liquid and protein content of bacterial liquid. When measured conductivity [9], each bacterium was divided into two groups: a control group (without drug, CK group) and a dosing group. In each group, there were seven time points (0min, 5min, 15min, 30min, 60min, 90min and 180min) respectively. In the CK group, each tube contained 18ml sterile water and 2ml bacteria liquid (concentration of 1.5×10^{-8} spores/ml). In the drug group 200μl extract liquid (100mg/ml) was added in. Both of the tubes were then cultivated at a constant temperature of 37°C. Later, corresponding samples were picked out to measure the conductivity at each time point.

When measuring the reducing sugar content [10], each bacterium was divided into two groups: a control group and a drug group. In each drug group, there were seven test tubes, which were marked 1h, 2h, 3h, 4h, 5h, 6h, 7h. Each drug group tubes contained 20ml sterile water, 10ml bacteria liquid and 200μl extract liquid. The CK group was without extract. Both groups cultivated at 37°C. Later, corresponding samples were picked out to measure the reducing sugar content at each time point by Fehling's solution [11].

At last the protein content of bacterial liquid would be determined [12]. Every bacterium was divided into two groups: a control group and a dosing group. There were six time points (0h, 4h, 8h, 16h, 32h, 64h) in each group. Each tube of CK group contained 20ml microbial fluid, and the drug group added 200μl extract liquid more to the CK group. Two groups cultivated at 37°C. Later, corresponding samples were picked out 3ml, stay overnight at -20°C, and then took it out and thaw it at room temperature. At last mixed 15ml Tris-HCl (0.01mor/ml) with the 3ml bacterial liquid,

freezing them at -20°C for later use. The protein content of bacterial liquid was measured at each time point through BCA Protein Assay purchased from Boster.

3. Results and Discussion

3.1 In vitro antimicrobial activities test

The antimicrobial activity experimental results of the inhibition zone and the MIC, MBC are shown in Fig. 1, Table 1.

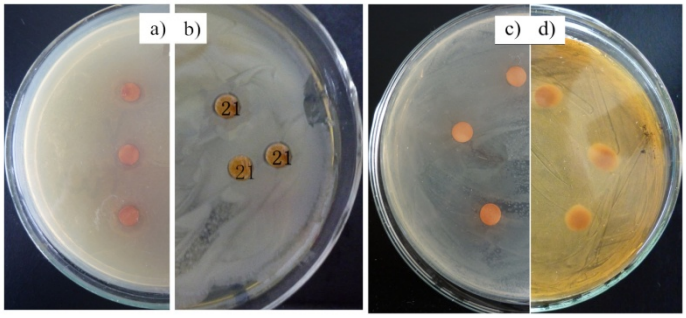

Fig. 1: (a) *E. coli*; (b) *B. lincheniformis*; (c) *B. subtili*; (d) *L. bulgaricus*

The results are that the *Schisandra chinensi* extract not only shown antagonistic action to pathogenic *E. coli*, but also increased the activity of *L. bulgaricus.*

Table 1 The inhibition zones width, MIC and MBC of the extract

Microorganisms	Inhibition zone (mm)	MIC (mg/ml)	MBC (mg/ml)
E. coli	7.1	6.25	12.5
B. lincheniformis	7.2	3.13	6.25
B. subtili	6.2	50	50
L. bulgaricus	increase	Red	Red

3.2 In vitro activities mechanism test

After the antimicrobial activity test, we can find that the *Schisandra chinensis* extract is against on *E. coli* and *B. lincheniformis,* the extract can increase *L. bulgaricus*, but the influence on *B. subtili* was little. The *E. coli* which could cause diarrhea and *L. bulgaricus* which is probiotics were chosen to experiment with mechanism respectively. The growth curves were shown on Figure 2.

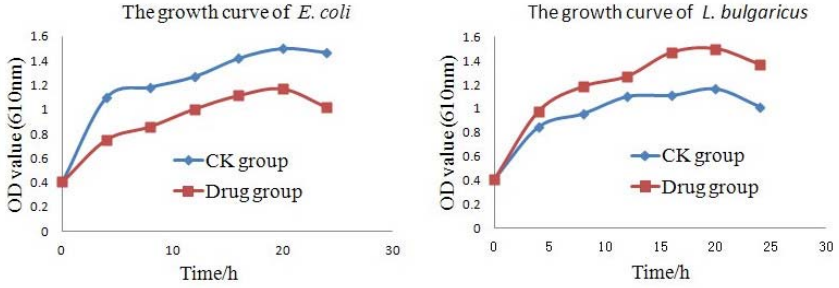

Fig. 2 The growth curves of strains before and after drug added

The extracts inhibited the growth of *E. coli*, whose cell division in logarithmic growth phase were inhibited and logarithmic growth stage did not reach the number in normal conditions. For the probiotics, the extract could make *L. bulgaricus* cell division in logarithmic growth phase accelerated, and the number of *L. bulgaricus* can increase much.

The results of the permeability changes for the bacteria outer membrane is shown on Fig. 4 and Fig. 5. The Fig. 3 was protein standard curve.

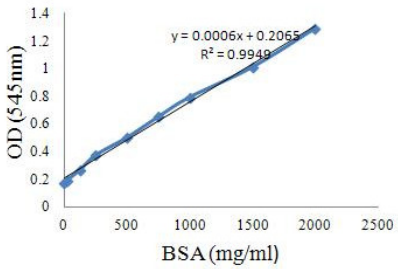

Fig. 3: The curve of standard protein.

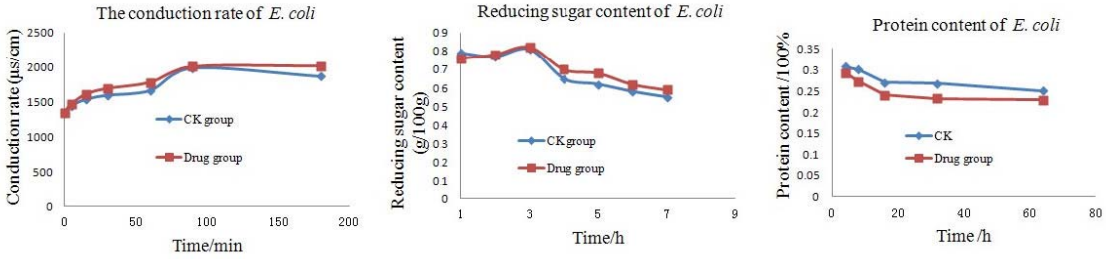

Fig. 4: The permeability changes of *E. coli* membrane before and after drug added.

From Fig. 4, we found that the extract influenced the conductivity of liquid *E. coli*, the reducing sugar content could increase after 3 hours and the protein content significantly seep within 20 hours. Thus, it can be deduced as follows: after drugs were added, the small molecules of *E. coli* seeped from cell of pathogens and leaded the reducing sugar gradually seeped, then as time passed protein content of macromolecules gradually seeped as well. In summary, the drug destroyed the cell membrane structure of the *E. coli*.

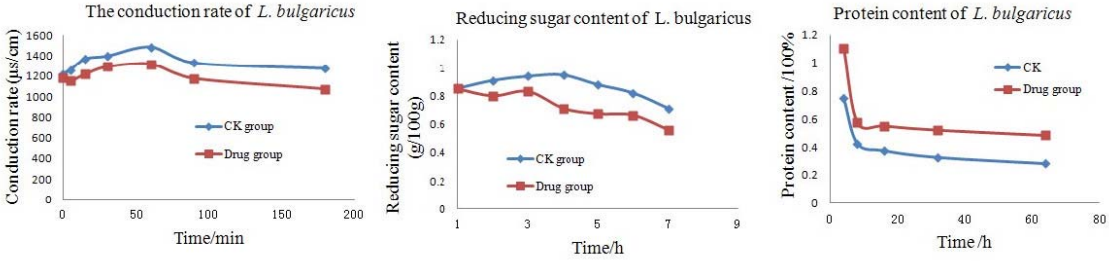

Fig. 5: The permeability changes of *L. bulgaricus* membrane before and after drug added.

The Fig. 5 shows that after drugs were added, the cells wall seepage velocity of small molecular, the reducing sugar content and the protein content were slower than the normal *L. bulgaricus*. In summary, the increased mechanism of the *Schisandra chinensis* extract could be deduced as follows: drugs keep the cell membrane structure of the *L. bulgaricus*, which can reduce the permeability of the cell membrane or the formation of membrane pores, leading to intracellular material intact and finally the enrichment of cells.

This article researchs the antimicrobial activity and explains that how the extract from *Schisandra chinensis* could inhibit the *E.coli* growth and increase the *L.bulgaricus* growth. Maybe, it's why in some herb books, *Schisandra chinensis* (wu wei zi) can treat chronic gastritis, gastric and duodenal ulcer, qi-stagnating abdominalgia and indigestion [13].

4. Acknowledgements

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