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Article

Antimicrobial Activity of the Extracts from *Coriandrum sativum*

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Abstract: Freeze-dried coriander was extracted by petroleum ether, 95% ethanol, and water, respectively. Antibacterial experiment indicated that only water extract presented significant antimicrobial activity and the minimum inhibition concentration (MIC) was below 10% of original extract. The inhibition effect of coriander extracts on microorganisms and the effects of pH, temperature and NaCl concentration on its antimicrobial activities were evaluated. The results showed that antibacterial activity of coriander extracts was stable under heating and had the best antibacterial effects at pH 6 with 2.0% NaCl concentration.

Keywords: coriander; *Coriandrum sativum*; antimicrobial activity; extract.

1. Introduction

Food preservation is an old topic, and people have been using high-salted, high molasses system, acid, alcohol, smoking, under-water, underground storage and so on to extend food shelf life. With the industrial development, chemical preservatives are widely used in the food processing industry. However, with the development of food industry, as well as great attention of people to food safety, people have a higher demand to food preservation method, and try to find more secure and efficient preservatives (Zhou et al., 2001). In order to meet the requirements of food safety, it should reduce and avoid the use of chemical preservatives. It becomes a research hotspot to find preservative from natural resources.

Coriander (*Coriandrum sativum*), first is called "coriandrum". The countries of its origin are the shores of the Mediterranean and Central Asia, and it is cultivated all over the world. Coriander can be

used as a medicine and widely useful a spice. It contains rich vitamins, decanal, nonanal, linalool and fragrant beans fine substances (Du, 1999; Li et al., 2002; Song et al., 2003; Zhao et al., 2011). Coriander have been widely studied and reported about its fruit in domestic and foreign. The essential oil is mainly extracted from its fruit, and there are relatively few research reports about physiological functions of stems and leaves including antiseptic efficiency (Dai et al., 2009; Guo et al., 2001). Coriander can be used as condiment, at the same time it has preservative function. It has a good prospect of application as a natural food preservative. In this paper, we studied its antibacterial activity against different microorganisms under the different conditions, and provided certain basis for coriander as a new secure natural food preservative.

2. Materials and Methods

2.1. Materials

Coriander was purchased from the market. *E. coli, Bacillus subtilis*, S*accharomyces cerevisiae and P. ericillinm* were obtained from Microbial Basic Laboratory, School of Bioengineering, Sichuan University of Science & Engineering.

2.2. Media

Bacteria are grown in beef extract peptone medium. Yeast is grown in glucose beef extract peptone medium, and mold is grown in potato medium (Zhou, 2006).

2.3. Coriander Lyophilized

The coriander was lyophilized after the pretreatment. The freeze-drying conditions were the material quality 1260 g per square meter and the drying time 12 h.

2.4. Coriander Extract Material Preparation

Freeze-dried coriander was crushed in order to more than 60 meshes. The coriander was extracted with the petroleum ether, 95% ethanol and water as non-polar, semi-polar, polar solvents, respectively. The mixture was centrifuged, and the supernatant was obtained. The 1 mL stock solution contained 1 g coriander lyophilized extract (Li et al., 2006). The extraction conditions are material liquid ratio 1:30, temperature 40 ℃, water bath shaker for 130 r/min and time 12 h.

2.5. Preparation of Bacterial Suspension

The tested original strains are activated formerly with the corresponding medium slant, and small cell are picked to a 10 mL of sterile saline solution in a test tube by inoculation loop and then vortexed and oscillated into the bacterial suspension standby.

2.6. Inhibition Experiments

The filter paper method is used for the determination of the antibacterial effect of the extract (Liu, 2002). The diameter 6 mm filter papers were placed in water extract liquid, 95% ethanol extract liquid, petroleum ether extract liquid, water extraction blank, 95% ethanol extract blank and petroleum ether extract blank to soak 4 h, respectively, then at 120 ^{o}C under moist heat sterilization standby. The tested bacteria liquid from each 0.5 mL is made of paper plate containing bacteria with the corresponding culture medium, and the paper pieces were placed into the surface of a medium with a pair of tweezers. Each experiment was repeated thrice. The bacteria were cultured for 24 h at 37 ^{o}C, and yeast and mold were cultured for 48 h at 28 ^{o}C. Then, the antibacterial circle diameter was accurately measured.

2.7. Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) refers to minimum extract concentration of the complete absence of bacteria growth. The best inhibitory effect of the extraction solution using sterile water was configured to be 25, 20, 15, 10, 5 and 2.5% concentration series, then the antibacterial circle diameter in different concentration was measured by the filter paper method.

2.8. Effects of Different pH Value, Temperature, Concentration of NaCl on the Bacteriostatic Activities of Extracts

The effect of different pH values on the antibacterial activities of the extract was tested using *Escherichia coli* and *Bacillus subtilis* as the test bacteria (Qi et al., 2003), and the water extraction liquid and pH adjustment liquid were injected into the oxford cup, so that the final concentration reached 10% of the stock solution. With different pH of the blank solution as a control, then the size of the zone of inhibition was measured after thermostatic incubation.

The effect of different temperature on the antibacterial activity of extracts was tested. The 10% stock solution was treated at different temperatures for 30 min, and 0.2 mL of the solution was taken to inject into the oxford cup, then the size of the zone of inhibition was measured after 24 h culture.

The effect of different NaCl concentration on the antibacterial activity of the extracts was tested. *E. coli* and *Bacillus subtilis* were used as the test bacteria to study the antibacterial activity of 10% stock liquid in different NaCl concentration gradient. According to the general food salt content, salt concentration was arranged to be 1, 1.5, 2, 2.5 and 3%.

3. Results and Discussion

3.1. Effects of Different Extraction Solvents on Antibacterial Activities of Coriander

The effects of different extraction solvents on antibacterial activities of coriander are shown in Table 1. Seen from Table 1, coriander water extract had very strong inhibition to *E. coli* and *Bacillus subtilis,* and no significant inhibitory effect to yeast and molds. It had more strong inhibitory effect to *E. coli* than *Bacillus subtilis*, which indicated the water extract had better inhibitory effect to the gram-negative bacteria. The antibacterial effects of ethanol and petroleum ether extract were not obvious. Thus, effective antibacterial ingredients in coriander could be water soluble substances.

Table 1. Effects of different extraction solvents on antibacterial activities of coriander

Strains	Inhibition zone diameter (mm)					
	water extract	95% ethanol extract	petroleum ether extract	water extraction blank	95% ethanol extraction blank	petroleum ether extract blank
E. coli	18.3	-	-	-	-	-
Bacillus subtilis	16.6	-	-	-	-	-
Saccharomyces cerevisiae	±	-	-	-	-	-
P. ericillinm	±	-	-	-	-	-

Note: ± inhibitory effect but not obvious, - no significant inhibitory effect. The inhibition zone diameter was average diameter of three duplicates, the same below.

3.2. Determination of Filter Paper Immersion Time

Filter paper of the same diameter of 6 mm was taken into the aqueous extract and soaked for 1, 2, 4, 6 and 8 h. The bacteriostatic effect on *Escherichia coli* was observed after sterilization. The results are shown in Table 2. The size of bacteriostasis zone increased with immersion time before 4 h, and bacteriostatic ring size no longer increased after 4h, which indicated that antibacterial substances on the paper could be saturated. Thus, 4 h was selected as the optimal paper soak time.

Table 2. Effects of soaking time on the antimicrobial activity of coriander extracts

Soaking time (h)	1	2	4	6	8
Zone of inhibition size (mm)	13.58	14.00	17.88	17.90	17.82

3.3. Determination of Minimum Inhibitory Concentration

The aqueous extract was diluted with sterile water to different concentrations, then minimum inhibitory concentration (MIC) was determined by comparison with Cephradine. The results are shown in Table 3. The minimum inhibitory concentration of coriander water extract to *E. coli* was 8%, and to *Bacillus subtilis* was 10% from Table 3.

Table 3. The minimum inhibition concentration (MIC) of water extracts

Strains	Inhibition zone diameter (mm)						
	extract concentration %						Cephradine
	Liquid	25	10	8	5	2.5	50 μg/kg
E. coli	17.40	13.60	10.12	9.32	-	-	19.60
Bacillus subtilis	16.45	12.30	9.68	±	-	-	17.20

3.4. Effects of pH on the Antibacterial Activity of Coriander Extract

The influences of pH on the antibacterial activity of coriander extract were preliminary studied at the level of pH 4, 5, 6, 7 and 8, respectively. The results showed that the bacteriostatic action was the best at pH 6 (Table 4). The results from Table 4 showed that the increase in acidity of the sample solution led to better antibacterial effect at the pH values 4-5, but the ratio of R_{sample}/R was very small, indicating that the antibacterial activity were relatively poor under acidic conditions. Similarly, the ratio of R_{sample}/R was the highest at pH 6, and antibacterial effect of the extracts in this condition was better.

Table 4. Effects of pH value on the antimicrobial activity of coriander extracts

Strains	pH value	R_{sample}	R	R_{sample}/R
E.coli	4	2.10	2.07	1.01
	5	1.23	1.23	1.00
	6	1.16	1.00	1.16
	7	1.14	1.00	1.14
	8	1.10	1.00	1.10
Bacillus subtilis	4	1.83	1.87	0.98
	5	1.26	1.18	1.07
	6	1.10	1.00	1.10
	7	1.12	1.00	1.12
	8	1.05	1.00	1.05

Note: R_{sample} - the ratio of sample solution of the inhibition zone diameter and the outside diameter of oxford cup; R- ratio of control the liquid zone of inhibition and the outside diameter of the oxford cup (Below).

3.5. Effects of Different Temperature on the Antibacterial Activity of Extracts

When the temperature level of 40, 60, 80, 100 and 121 ^{o}C was selected, the inhibitory effect is observed at short-term dealing with the sample solution for 30 min. The results were shown in Table 5.

Seen from Table 5, heat treatment does not influence the antibacterial activity of the extract under 80 ^{o}C. But after treating at 100 ^{o}C or above, a small amount of suspended solids would appear, and a small amount of macromolecules might be present in the extract of coriander. The coriander fragrance became thinning. The antibacterial activity of extracts from the data of the table showed that the effects were still better, which illustrated the heat stability of the main antibacterial substances from the coriander extract was good.

Table 5. Effects of temperature on R_{sample}/R with strains

Strains	R_{sample}/R				
	40 ^{o}C	60 ^{o}C	80 ^{o}C	100 ^{o}C	121 ^{o}C
E. coli	1.14	1.14	1.14	1.12	1.09
Bacillus subtilis	1.12	1.12	1.12	1.11	1.08

3.6. Effects of NaCl on the antibacterial activity of the coriander extract

Most foods contain certain salinity, therefore the antibacterial activity of extracts is studied at different concentrations of NaCl, and the results are shown in Table 6. The R_{sample}/R is the highest under the 2.0% salt concentration. At low salt condition, the antibacterial activity of the extract is actually strengthened. This may be synergistic effect with the presence of salt, and the specific reasons need further be studied. As the salt concentration increased, the antibacterial effect became better, but the R_{sample}/R reflected that high osmotic pressure led by the salt concentration could play a major role at this condition.

Table 6. Effects of NaCl concentration on the antimicrobial activity of coriander extracts

Strains	NaCl concentration (%)	R_{sample}	R	R_{sample}/R
E. coli	1.0	1.14	1.00	1.14
	1.5	1.18	1.00	1.18
	2.0	1.19	1.00	1.19
	2.5	1.23	1.10	1.12
	3.0	1.26	1.14	1.11
Bacillus subtilis	1.0	1.12	1.00	1.12
	1.5	1.15	1.00	1.15
	2.0	1.16	1.00	1.16
	2.5	1.20	1.08	1.11
	3.0	1.23	1.12	1.10

4. Conclusions

We have preliminary studied coriander antibacterial effect to provide certain scientific basis for coriander as a natural food preservative. Freeze drying coriander was extracted with petroleum ether, 95% ethanol and water, respectively. The results showed that the aqueous extracts displayed obvious inhibition of different bacteria, and the minimum inhibitory concentration is less than or equal to 10% solution. The bacteriostatic effect is good under the conditions: 10% solution, 1.5% NaCl and pH 6.

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