# ANTIMICROBIAL ACTIVITIES OF SOME SPICE EXTRACTS AGAINST FOUR SPECIES OF COMMON MEAT SPOILAGE ORGANISMS

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## Introduction

Synthetic preservatives have traditionally been used in food processing; however, increasing consumers' awareness of the potential side effects of synthetic compounds had led to reduced acceptance of their use in foods (Namiki, 1990). Many kinds of Oriental spice plants have long been used to inhibit spoilage microorganisms in households, and increasing evidence suggests that natural substances from some plants, e.g., herbs and spices, may possess antimicrobial and antioxidant activities. In a variety of plants, antimicrobial compounds exist to provide a natural defense mechanism, which leads to the suggestion that they are used as natural preservatives in food (Farag et al., 1989). Indeed, antimicrobial activity has been identified in cassia, clove, garlic, sage, oregano, pimento, thyme, rosemary, scutellaria, and forsythia suspensa (Thunb) (Shelef et al., 1980, Djenanea et al., 2003). The objective of this study was to examine the antimicrobial activity of clove, cassia bark, rosemary, liquorice, aniseed, prickly ash, pepper and dahurian angelica root extracts against four meat spoilage organisms on agar media. Four bacteria that could potentially contaminate chilled meat and precooked ham slices, i.e., *Listeria monocytogenes*, *Pseudomonas fluorescens*, *Lactobacillus sake*, and *Escherichia coli*, were tested.

## Materials and Methods

Spices were purchased from Baofeng herbal and spice companies in Harbin, China. Aliquots (50 g) of pulverized and dried spices were mixed into 400 mL of 95% edible ethanol in enclosed flasks and agitated for 12 h (100 rpm). After filtration with a Whatman No. 2 filter paper, the residue was re-extracted and then filtered. The combined filtrates were subsequently concentrated on a rotary evaporator (50°C) with a vacuum pump, and the extracts were dried in a vacuum drier at 50°C and 0.07 MPa. *Lactobacillus sake* (strain AS1.80) was obtained from Microbial Research Institute of Chinese Academy of Science (Beijing, China). *Escherichia coli*, *Pseudomonas fluorescens*, and *Listeria monocytogenes* were from the in-house laboratory stock.

The antimicrobial activity of spice extracts was examined in triplicate using the well diffusion test (Kim et al., 1995). Sterile micro steel cylinders (7.8 dia. × 10 ht., mm) were vertically set on the agar in the plates. The control was 0.1 mL of 95% edible ethanol only. The inhibitory effect was assessed by measuring the disk diameter of the inhibition zone (clear zone) around the extract-containing steel cylinder by means of a vernier caliper. The minimal inhibitory concentration (MIC) was determined by means of concentration gradient test tubes method. To each duplicate test tube containing 9 mL of sterile broth medium was added a 20 μl aliquot of bacterial suspension at $10^6$ CFU/mL and a test spice extracts preparation (final concentration of 10.00, 7.50, 5.00, 3.75, 2.00, 1.25, or 0.63 mg/mL). Each test was performed in duplicate and repeated twice.

Analysis of variance (AOV) was done to determine the significance of the main effects. Significant differences ($P < 0.05$) between means were identified using Least Significant Difference procedures.

## Results and Discussion

*The antimicrobial effect of individual spice extracts in agar media.* For each of these spice extracts, the zone of inhibition was significantly different ($P < 0.05$) from each other between the lower and higher concentrations on the tested bacteria (Table 1). Increasing the spice extract concentration generally resulted in a greater inhibition of the growth of the test bacteria. In particular, *P. fluorescens* and *L. monocytogenes* were more sensitivity to the spice extracts than *E. coli* and *L. sake*. The differences in susceptibility for these bacteria to the antimicrobial spice extracts may be explained by the differences in their cell wall composition and/or inheritance of antimicrobial-resistance genes on plasmids that can easily be transferred (Şengűl et al., 2005).

*The MIC of individual spice extracts.* The MIC of nearly all of the spice extracts was less than 1% (10 mg/mL) on all the tested bacteria (Table 2). Generally, the 1% concentration of the spice extracts completely inhibited the test bacterial. The growth of *L. monocytogenes* was completely inhibited by clove, rosemary and liquorice at 0.63 mg/mL; the growth of *P. fluorescens* was completely inhibited by clove at 1.25 mg/mL; the growth of *E. coli* was completely inhibited by clove and rosemary at 1.25 mg/mL; and the growth of *L. sake* was completely inhibited by clove, rosemary and cassia bark at 1.25 mg/mL. Evaluation of the bacterial growth inhibition by means of concentration gradient test confirmed the particular sensitivity of *P. fluorescens* and *L* *monocytogenes* to these eight kinds of test spice extracts. Clove, rosemary and cassia bark exhibited the strongest inhibition activity against these four common meat spoilage organisms.

**Table 1.** Antimicrobial activity of individual ethanol extracts of spices at various concentrations

| Strains | Concn (%) | Diameter of inhibition zone (mm)[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Clove | Rosemary | Cassia bark | Liquorice | Aniseed | Prickly Ash | Pepper | Dahurian angelica root |
| *E. coli* | 0.5 | 12.3e | 12.9d | 7.8e | 7.8 | 7.8e | 7.8c | 7.8 | 7.8 |
| | 1.0 | 13.2d | 13.9c | 13.5d | 7.8 | 10.4d | 7.8c | 7.8 | 7.8 |
| | 2.0 | 18.3c | 16.3b | 15.3c | 7.8 | 12.3c | 7.8c | 7.8 | 7.8 |
| | 4.0 | 20.1b | 16.6b | 16.1b | 7.8 | 13.8b | 9.7b | 7.8 | 7.8 |
| | 8.0 | 26.7a | 18.4a | 17.1a | 7.8 | 15.3a | 10.9a | 7.8 | 7.8 |
| *P. fluorescens* | 0.5 | 11.2e | 9.4e | 15.2e | 11.4c | 7.8b | 11.1e | 7.8c | 12.0e |
| | 1.0 | 13.5d | 11.5d | 16.2d | 11.7c | 7.8b | 12.0d | 7.8c | 13.0d |
| | 2.0 | 17.1c | 13.1c | 17.4c | 13.2b | 7.8b | 13.3c | 7.8c | 13.8c |
| | 4.0 | 20.3b | 17.7b | 20.0b | 14.1a | 7.8b | 15.5b | 15.2b | 15.3b |
| | 8.0 | 22.1a | 19.2a | 26.9a | 14.4a | 12.3a | 19.5a | 16.0a | 20.0a |
| *L. monocytogenes* | 0.5 | 7.8e | 12.1e | 7.8c | 14.2e | 7.8c | 7.8c | 7.8c | 7.8c |
| | 1.0 | 11.3d | 13.8d | 7.8c | 12.3d | 7.8c | 7.8c | 7.8c | 7.8c |
| | 2.0 | 16.7c | 16.8c | 7.8c | 15.8c | 7.8c | 7.8c | 7.8c | 7.8c |
| | 4.0 | 17.8b | 17.5b | 10.7b | 17.4b | 9.8b | 9.9b | 9.9b | 10.7b |
| | 8.0 | 20.8a | 20.8a | 15.7a | 20.8a | 13.4a | 12.0a | 10.a | 15.4a |
| *L. sake* | 0.5 | 7.8d | 9.3e | 7.8e | 7.8 | 7.8 | 7.8 | 7.8 | 7.8b |
| | 1.0 | 7.8d | 9.9d | 9.7d | 7.8 | 7.8 | 7.8 | 7.8 | 7.8b |
| | 2.0 | 9.4c | 10.9c | 10.8c | 7.8 | 7.8 | 7.8 | 7.8 | 7.8b |
| | 4.0 | 12.8b | 12.4b | 14.9b | 7.8 | 7.8 | 7.8 | 7.8 | 7.8b |
| | 8.0 | 15.9a | 16.9a | 16.8a | 7.8 | 7.8 | 7.8 | 7.8 | 10.4a |

[1]The diameter of inhibition zone for control (95% ethanol) was 7.8 mm.[a-e] Means in each column and for each spice extract with different superscript letters differ significantly ($P < 0.05$).

**Table 2.** The minimal inhibitory concentration (MIC) of spices extracts against four test bacteria strains.

| Strains | MICs (mg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Clove | Rosemary | Cassia bark | Liquorice | Aniseed | Prickly ash | Pepper | Dahurian angelica root |
| *E. coli* | 1.25 | 1.25 | 5.00 | >10.00 | 5.00 | 10.00 | 10.00 | 10.00 |
| *P. fluorescens* | 1.25 | 2.50 | 2.50 | 5.00 | 5.00 | 3.75 | 3.75 | 7.50 |
| *L. monocytogenes* | 0.63 | 0.63 | 2.50 | 0.63 | 2.50 | 2.50 | 2.50 | 2.50 |
| *L. sake* | 1.25 | 1.25 | 1.25 | 10.00 | 10.00 | >10.00 | 10.00 | 5.00 |

## Conclusions

The study demonstrated antimicrobial activities of selected spices in agars, suggesting that they may serve as potential, natural antimicrobial agents for the inhibition of spoilage and pathogenic microorganisms in foods. The spices extract may be useful and economically feasible as antibacterial agents to prevent the deterioration of meat products caused by bacteria provided that organoleptic effects are also acceptable.

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