

ANTIMICROBIAL ACTIVITY OF SOME SPICES USED IN THE MEAT INDUSTRY

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Abstract

The antimicrobial activity of some food additives used in meat products such as cumin, cinnamon, cloves, crushed red pepper, fennel, and anise against some microorganisms was investigated. For this purpose, the diethyl ether-treated extracts of spice samples were tested *in vitro* with *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* FML 5, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 15753, *Mycobacterium smegmatis* CCM 2067, *Micrococcus luteus* A 2971, and *Candida albicans* ATCC 60192 as test strains. The disc diffusion method was applied in the trial. Cinnamon was found to be the most effective spice against tested microorganisms. The weakest antimicrobial activity was displayed by fennel. Crushed red pepper and anise were found to be ineffective against the test strains.

Key words: meat products, spice, antimicrobial activity.

In the Turkish Food Codex (3), a spice is defined as a natural compound, or a mixture of natural compounds that is extracted from the seeds, fruits, flowers, or trunks (skins, roots, leaves) of several plants, and added to food in order to provide colour, taste, smell, or flavour.

Spices are used as substances that increase the taste and variation of food (9, 12). Furthermore, some spices are reported to have bactericidal or bacteriostatic activities. The inhibitory effects of spices are mostly due to the volatile oils present in their composition (1, 4, 7, 15, 19, 20, 22, 33, 36).

The main factors that determine the antimicrobial activity are the type and composition of the spice, amount used, type of microorganism, composition of the food, pH value, temperature of the environment, and proteins, lipids, salts, and phenolic substances present in the food environment (32).

Gram-negative bacteria are more resistant to the antibacterial activity of essential oils than Gram-

positive bacteria (28, 34). In many studies (21, 28, 29), essential oils that contain carvacrol and eugenol have been shown to exhibit the strongest antimicrobial activity. Barbosa-Canovas *et al.* (6) showed that cinnamon and cloves, had a strong inhibitory activity against microorganisms, cumin a moderate one, and red pepper had a weak inhibitory activity.

In different studies, the inhibitory effect of cumin on *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (1, 13); cinnamon on *Escherichia coli* and *Klebsiella pneumoniae* (28); cloves on *Candida albicans* (4, 16), *Enterococcus faecalis* (14, 16), *Escherichia coli* (14, 16, 21, 30, 31), *Klebsiella pneumoniae* (14, 16), *Micrococcus luteus* (14), *Pseudomonas aeruginosa* (14, 16, 21), and *Staphylococcus aureus* (21, 27, 30); fennel on *Candida albicans* (26), and red pepper on *Staphylococcus aureus* (27, 35) was observed.

In the present study, the antimicrobial activity of some commonly spices used in Turkish meat industry, including cumin, cinnamon, cloves, crushed red pepper, fennel, and anise, was investigated.

Material and Methods

Spice materials. Six different types of spices, widely used in meat products (cumin, cinnamon, cloves, fennel, red crushed pepper, and anise), constituted the material for the study. The samples were obtained from a wholesaler or retail spice-sellers in the amounts of 500 g each. The samples were kept in closed containers after being chopped into small pieces (1 mm) by the laboratory grinder (17).

Tables 1 and 2 show the botanic features and volatile oil components of the spice samples used in the study.

Microorganisms. The following bacterial strains, that cause food poisoning or food spoilage, and a yeast culture were used as test strains: *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* FML 5,

Pseudomonas aeruginosa ATCC 27853, *Enterococcus faecalis* ATCC 15753, *Mycobacterium smegmatis* CCM 2067, and *Micrococcus luteus* A 2971 obtained from the Culture Collection of the Department of Microbiology, Faculty of Medicine, Yüzüncü Yıl University, and *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 60192 provided by the Istanbul University Faculty of Medicine, Department of Microbiology and Clinical Microbiology, Culture Collections, the Society of Culture Collections, Industrial Microbiology and Biotechnology (KÜKEM). Trypticase Soy Broth (Difco-0369-01-4) for the activation of bacterial cultures, Saboraud Dextrose Agar (Difco-210950) for yeast cultures, and Mueller-Hinton Agar (Oxoid CM 337) for antimicrobial activity trials were used.

Preparation of extracts. For the preparation of sample extracts, the method reported by Hanafy and Hatem (17) was used. For this purpose, 500 ml of diethyl ether was added into 200 g of chopped spices and the mixture was left for 6 h. The mixture was periodically agitated during this period (15 min). Afterwards, it was filtered and the ether was vaporized

in an evaporator (60°C). The dark coloured oily extract obtained at the end of these processes was used in a non-diluted form for the analyses. Antimicrobial activity tests were started on the same day. The sample extracts were kept in the refrigerator (4°C) until the analyses were accomplished.

Antimicrobial tests. The Disc diffusion method was used to determine the antimicrobial activity of the spices. A volume of 0.1 ml of the tested microorganisms grown in liquid growth media (at 37°C for 24 h, 10^8 - 10^9 cells/ml), was inoculated on Mueller-Hinton growth media, and then spread on the entire surface of the dish using a sterile Drygalski spatula. Then, sterile paper discs (Whatman: 1.6 mm) with absorbed spice extract (30 µl/disc) were placed onto the agar at certain intervals by pressing gently. After the plates were incubated at $35 \pm 0.1^\circ\text{C}$ for 48 h, the inhibition zones around the discs where no growth occurred, were measured in millimetres. The experiments were repeated in duplicate for all of the test strains (2).

Table 1
Botanic features of the spice samples (8, 11, 23)

Spices	Family	Botanical names of the plant	Name of plant part used as spice
Cumin	<i>Umbelliferae</i>	<i>Cuminum cyminum</i> L	Leaf, seed
Cinnamon	<i>Lauraceae</i>	<i>Cinnamomum zylancium</i>	Leaf, bark
Cloves	<i>Myrtaceae</i>	<i>Eugenia caryophyllata</i> Thunb	Flower stalk, bud
Fennel	<i>Umbelliferae</i>	<i>Foeniculum vulgare</i> Mill	Leaf, twig, root, seed
Crushed red pepper	<i>Solanaceae</i>	<i>Capsicum annuum</i> L	Fruit
Anise	<i>Umbelliferae</i>	<i>Pimpinella anisum</i> L	Leaf, seed

Table 2
Volatile oil components of the spice samples (11, 23, 25)

Spices	Volatile oil (%)	Substances and type of substances
Cumin	1-4	Carvone, dihydro carvone, limonene, carvacrol
Cinnamon	1-2	Cinnamic aldehyde, hydrocinnamic aldehyde, eugenol, caryophyllene, phellandrene, cymene, pinene, linalool, furfural, benzaldehyde, cumaldehyde
Cloves	15-20	Eugenol, eugenol acetate, caryophyllene, methyl-n-amyl carbinol, methyl-n-heptyl carbinol, methyl benzoate, benzyl alcohol, vanillin
Fennel	2.5-3.5	Anethole, fenchone, methylchavicol
Crushed red pepper	Trace amount	Capsaicine
Anise	1.5-3	Trans-anethole, methylchavicol-isoanethole, anisaldehyde, dianethole

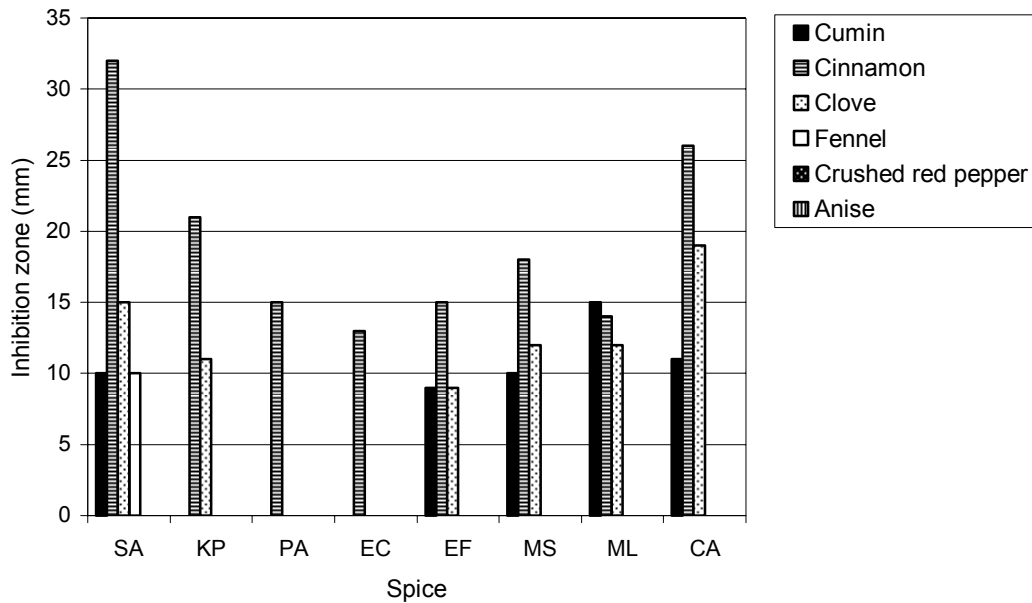


Fig. 1. Antimicrobial activity of the tested spices.
SA: *S. aureus*, KP: *K. pneumoniae*, PA: *P. aeruginosa*, EC: *E. coli*, EF: *E. faecalis*,
MS: *M. smegmatis*, ML: *M. luteus*, CA: *C. albicans*.

Results

The antimicrobial test results of the spice samples are shown in Fig. 1. At the end of the analyses, only cinnamon was found to have an inhibitory effect against all of the test strains. The most susceptible bacteria to cinnamon were *S. aureus* and *C. albicans*.

Cumin had an inhibitory effect against five of the test strains (*S. aureus*, *E. faecalis*, *M. Smegmatis*, and *C. albicans*), whereas cloves was effective against six of them (*S. aureus*, *K. pneumoniae*, *E. faecalis*, *M. smegmatis*, *M. luteus*, and *C. albicans*), and fennel was effective only against *S. aureus*. Crushed red pepper and anise were ineffective against any of the test strains.

In Fig. 1, it can be suggested that *P. aeruginosa* and *E. coli* were the most resistant strains to spice samples, and that they were susceptible to cinnamon only. *S. aureus* was found to be susceptible to cumin, cinnamon, cloves, and fennel, *K. pneumoniae* to cinnamon and cloves, and *E. faecalis*, *M. smegmatis*, *M. luteus*, and *C. albicans* to cumin, cinnamon, and cloves. All the test strains were resistant to crushed red pepper and anise

Discussion

According to the tests, cinnamon was found to be, with varying degrees, the most effective spice against tested microorganisms. When data in Fig. 1 are assessed, it can be seen that the most susceptible species to this spice was *S. aureus*, followed by *C. albicans*, *K. pneumoniae*, and *M. smegmatis*. Cinnamon was detected

to exhibit a similar inhibitory effect against *P. aeruginosa* and *E. faecalis*, and its weakest activity was against *E. coli* and *M. luteus*. The antimicrobial activity of cinnamon may be explained by its volatile oil components. The most important active substances found in cinnamon oil are cinnamic aldehyde and eugenol (Table 2). A number of investigators (5, 10, 24) reported that cinnamon inhibited the growth and toxin production of some mould species, with an activity emerging from cinnamic aldehyde and eugenol. In another study (28), eugenol was shown to have a stronger bactericidal activity against *E. coli* and *K. pneumoniae* than some antibiotics. The results of the present study concur to the results of the study mentioned before.

Cumin was shown to have an inhibitory effect against *S. aureus*, *E. faecalis*, *M. smegmatis*, *M. luteus*, and *C. albicans*. However, a similar effect against other microorganisms (*K. pneumoniae*, *P. aeruginosa*, and *E. coli*) was not found. According to the results of the tests, *M. luteus* is the most susceptible strain to cumin. In a study on antimicrobial activity of volatile oils of some spices, Çon *et al.* (13) demonstrated that cumin had an inhibitory effect against *S. aureus* and *M. luteus*. In a similar investigation, Akgül and Kıvanç (1) reported that cumin exhibited an inhibitory effect against *S. aureus*, *K. Pneumoniae*, and *P. aeruginosa*. The results of the present study are similar to those of Çon *et al.* (13), except the results of inhibitory effect against *E. coli* and *P. aeruginosa*. It can be suggested that the inhibitory effect of cumin might be due to carvone and carvacrol contained in its volatile oil as reported by Ouattara *et al.* (28).

Cloves exhibited similar results as cumin. This spice was effective against *S. aureus*, *E. faecalis*, *M. smegmatis*, *M. luteus*, and *C. albicans*; however, in contrast to cumin, it was found also to be effective against *K. pneumoniae*. The obtained results of the present study are comparable to the results of other reports (4, 14, 16, 21, 27, 30, 31). The antimicrobial effect of cloves may be explained by the action of eugenol and eugenol acetate contained in its volatile oil, as many investigators have reported (5, 10, 18, 21, 24, 27, 29-31).

The weakest antimicrobial effect was that of fennel. Fennel showed an inhibitory effect only against *S. aureus*. This result is inconsistent with the findings of Nkanga and Uraih (26). The inhibitory effect of fennel is possibly due to anethole found in its volatile oil.

Crushed red pepper and anise showed no antimicrobial effect against test strains. These findings concerning crushed red pepper are not in accordance with findings of other investigations (27, 35). The differences detected for fennel and crushed red pepper may be due to the different climates in which the plants of the spice are grown and the varying methods of extraction (1).

Four spices (cumin, cinnamon, cloves, and fennel) were shown to have an inhibitory effect against *S. aureus*, which is an important pathogen in food poisoning. The most effective activity against this bacterium was exhibited by cinnamon, cloves showed less activity; and the effects of cumin and fennel were the lowest. The most effective spice against *M. luteus* was cumin, and the most effective spice against *K. pneumoniae*, *E. faecalis*, *M. smegmatis*, and *C. albicans* was cinnamon. The other test strains, *P. aeruginosa* and *E. faecalis* were affected only by cinnamon.

Cinnamon was found to be the most effective spice against all the test strains except *M. luteus*. Cloves, cumin, cinnamon, and fennel exhibited the weakest antimicrobial effect towards this bacterium. The susceptibilities of microorganisms to spice extracts are different. In the present study, Gram-positive bacteria (*S. aureus*) are found to be more susceptible to spice samples. This may be explained by the fact that Gram-positive bacteria, due to their structural features, are more susceptible to phenolic compounds than Gram-negative bacteria (28,34,35).

In conclusion, cinnamon, cloves, and cumin, were found to have important antimicrobial activity against the test strains. In this regard, the use of spices and their volatile compounds as natural preservatives in food products; may be an alternative to the use of chemical additives.

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