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Antimicrobial and Biochemical Analysis of Some Spices Extract against Food Spoilage Pathogens

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Abstract: The antimicrobial activity of some food additives used in meat products such as Cumin, Cinnamon, Cloves, Fennel, Red Crushed Pepper, Mustard, Cardomon, Ginger, Poppy 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, Klebsiella pneumonia*e, *Pseudomonas aeruginosa, Enterococcus faecalis, Micrococcus luteus, Escherichia coli* and *Candida albicans* as test strains. The disc diffusion method was applied in the trial. Cinnamon was found to be the most effective spice against all the test strains except *M. luteus.* The weakest antimicrobial activity was displayed by Cloves, cumin, cinnamon and fennel towards this bacterium.

Key words: Meat products, spice, disc diffusion, antimicrobial activity.

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Introduction

In the Turkish Food Codex (Anonim, 2000), 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. The "Spice" is a culinary term, not a botanical category it does not refer to a specific kind of plant or plant part (Farrell, 1990). Each spice has a unique aroma and flavour, which derive from compounds known as phytochemicals or secondary compounds. These chemicals evolved in plants to protect them against herbivorous insects, vertebrates, fungi, pathogens, and parasites (Walker, 1994). Spices are used as substances that increase the taste and variation of food (Bulduk, 2004).

Naturally occurring compounds in spices such as, sulphur compounds, terpenes and terpene derivatives, phenols, esters, aldehydes, alcohols and glycosides have shown antimicrobial functions (Russel and Gold, 1991; Davidson and Baren, 1993; Deis, 1999). 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 (Sagdic, 2003).

Spices are used as substances that increase the taste and variation of food (Ceylan, 1997 and Bulduk, 2004). For thousands of years, aromatic plant materials have been used in food preparation and preservation, as well as for embalming, in areas

where the plants are native, such as Hindustan and the Spice Islands (Govindarajan, 1985, Dillon and Board, 1994). 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 (Arora-Dlijit and Kaur, 1999). In the present study, the antimicrobial activity of some commonly spices used in meat industry, including cumin, cinnamon, cloves, crushed red pepper, fennel, and anise, was investigated.

Material and Methods

Spice materials. Ten different types of spices, widely used in meat products (Cumin, Cinnamon, Cloves, Fennel, Red Crushed Pepper, Mustard, Cardomon, Ginger, Poppy and Anise) constituted the material for the study. The samples were obtained from retail spice-sellers in the amounts of 50 g each. The samples were kept in closed containers after being chopped into small pieces (1 mm) by the laboratory grinder (Hanafy and Hatem, 1991).

Microorganisms. The following bacterial strains, which cause food poisoning or food spoilage, and a yeast culture, were used as test strains *viz., Staphylococcus aureus, Klebsiella pneumonia*e, *Pseudomonas aeruginosa, Enterococcus faecalis, Micrococcus luteus, Escherichia coli* and *Candida albicans* provided by Easma Institute of Technology, Aravakurichy, Karur district of Tamil Nadu, India.

Preparation of extracts. For the preparation of sample extracts, the method reported by Hanafy and Hatem (1991) was used. For this purpose, 50 ml of diethyl ether was added into 20 g of chopped spices and the mixture was left for 6 hours. The mixture was periodically agitated during this period (15 min). Afterwards, it was filtered and the ether was vaporized in an evaporator (60℃). The dark colored oily extract obtained at the end of these processes was used in a non diluted form for the analysis. Antimicrobial activity tests were started on the same day. The sample extracts were kept in the refrigerator (4℃) until the analysis was accomplished.

Bacterial isolation and pure culture. There are different types of media were used for bacterial isolation. Like Zobell's Marine Agar 2216, Mac Conkey's Agar (MAC), Vibrio agar, Nutrient Agar, Nutrient Broth were used for the isolation and identification of some Gram- Positive and Gram - Negative bacteria.

The Gram Stain. A heat fixed bacteria smear was covered completely with a few drops of crystal violet solution. After 30- 60 sec the smear was rinsed with water by squirting the slide above the smear and letting the water wash over it until the water runs clear. Several drops of iodine (mordant) was applied to cover the smear and left for 60 sec then rinsed again. A few drops of Isopropanol-acetone mixture was added at a time until it become colorless, then the slide was rinsed again. Aqueous safranin was applied for 30- 60 sec followed by a rinse. The smear was blotted to remove excess water, using absorbent paper. The slide was then air dried and observed under a microscope.

Motility. Motility Test Medium was used to check the motility of the bacterium. Bacterial motility can be shown using different types of motility medium. The composition of these preparations gives freedom of movement comparable to that of broth culture. Motile bacteria were identified by the presence of growth away from the line of inoculation whereas non – motile organisms grow only the initial stab line.

Biochemical tests

Catalase test. This test was conducted to detect the presence of the enzyme Catalase. A capillary tube was dipped into 3% H_2O_2 and the colony was touched.

Citrate utilization. The organisms were streaked onto Simmons Citrate agar plate and incubated at 37℃ for 24hours. The results were interpreted based on the change of color from initial green to deep blue if it was positive.

Indole test. Tryptone agar was inoculated and incubated at 37^{o} C for 48 hours and added Kovacs reagent and read immediately. They results were interpreted based on the change of color from yellow to pink.

Methyl red test (MR). Buffered glucose broth was inoculated and incubated at 37^{o} C for 48 hours. A few drop of methyl red solution was added to culture and the results were read immediately. The results were interpreted based on the change of color from yellow to red.

Voges Proskauer test (VP). The organisms were inoculated in buffered glucose broth and were incubated at 37^{o} C for three

Table. 1. Botanical name of spices and used plant parts

S. No	Name of Spices	Family	Botanical Name	Plant Part Used
1	Cumin	Umbelliferae	*Cuminum cyminum*	Seed
2	Cinnamon	Lauraceae	*Cinnamomum zylancium*	Bark
3	Cloves	Myrtaceae	*Eugenia caryophyllata*	Flower bud
4	Fennel	Umbelliferae	*Foeniculum vulgare*	Seed
5	Crushed red pepper	Solanaceae	*Capsicum annuum*	Fruit
6	Anise	Umbelliferae	*Pimpinella anisum*	Seed
7	Mustard	Brassicaceae	*Brassica nigra*	Seed
8	Cardamon	Fabaceae	*Trigonella foenum*	Seed
9	Ginger	Zingiberaceae	*Zingiber officinale*	Rhizomes
10	Poppy	Paparveraceae	*Papaver somniferum*	Seed

days and 3 ml of alpha napthol was added followed by 1 ml of 40% KOH. It was mixed well and allowed to stand for 30 min. The results were interpreted based on the change of yellow color to pink.

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℃ for 24 hrs, 108-109 cells/ml), was inoculated on Mueller- Hinton growth media, and then spread on the entire surface of the dish using a sterile 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±2℃ for 48 hours, the inhibition zones around the discs where no growth occurred were measured in millimeters. The experiments were repeated in duplicate for all of the test strains.

Results

Crude extract. Diethyl Ether extract of spices samples viz, Cumin yielded a total amount of 5.4g of crude extract, Cinnamon yielded 3.8g, Cloves yielded 5.04g of crude extract. Fennel yielded 3.3g, Red Crushed Pepper yielded 4.7g, Mustard yielded5.15 g, Cardamon yielded 3.5g, Ginger yielded5.06g, Poppy yielded 4.75g, and Anise yielded 3.65g.

Antimicrobial activity. The antimicrobial test results of the spice samples are shown in (Fig.1& Fig.2). 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. Mustard was showed activity against *S. aureus,* Cardamon was showed activity against *K. pneumoniae*, Ginger was showed activity against *E. faecalis*, *M. smegmatis*, poppy was showed activity against *M. luteus*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

Fig. 1. Antibacterial activity of species against human pathogens

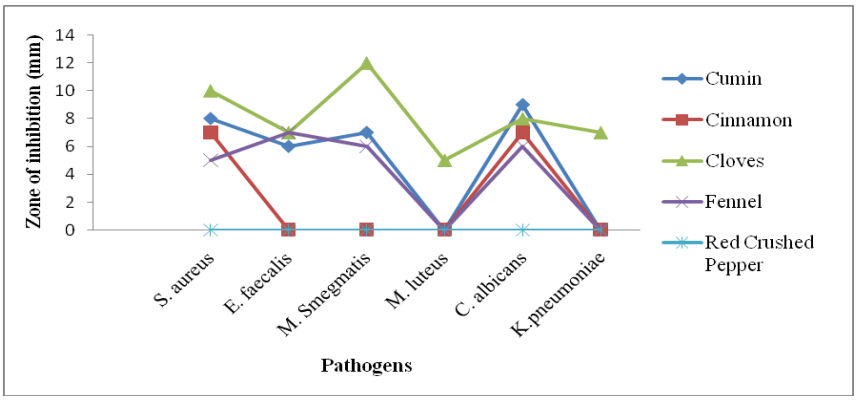
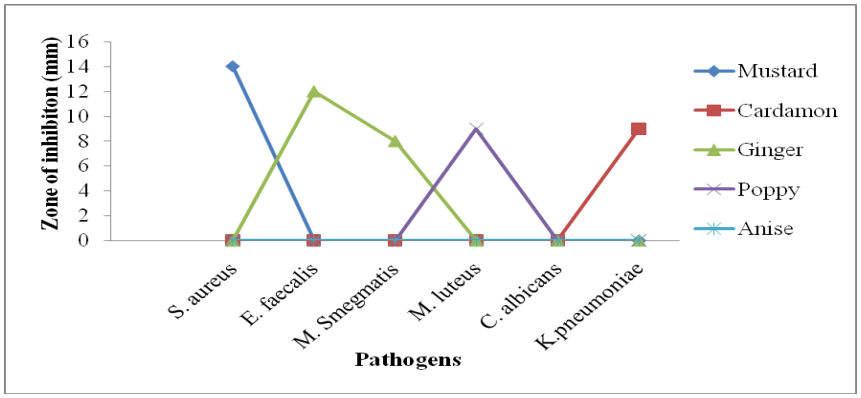


Fig. 2. Antibacterial activity of species against human pathogens



Discussion

According to the tests, cinnamon was found to be, with varying degrees, the most effective spice against tested microorganisms. When data 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* reported by Mabrouk and Shayeb, (1980). Ramadan *et al.* (1972) investigated the eugenol shown to have a stronger bactericidal activity against *E. coli* and *K. pneumoniae*. The results of the present study also good activity against human pathogens. Previously, Barbosa-Canovas *et al.* (1998) showed that cinnamon and cloves had a strong inhibitory activity against microorganisms.

Whereas in the case of Cumin showed inhibitory effect against *S. aureus*, *E. faecalis*, M. *smegmatis*, *M. luteus* and *C. albicans*. There is no activity was found against *K. pneumoniae*, *P. aeruginosa* and *E. coli.* Previously Con *et al.* (1998) demonstrated that cumin had an inhibitory effect against *S. aureus* and *M. luteus*. Similarly, Akgul and Kıvanc, (1989) reported that cumin exhibited an inhibitory effect against *S. aureus*, *K. Pneumoniae* and *P. aeruginosa*.

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*. Katayama and Nagai, 1960 reported the antimicrobial effect of cloves may be explained by the action of eugenol and eugenol acetate contained in its volatile oil.

In the present study fennel showed inhibitory effect only against *S. aureus*. This result is inconsistent with the findings of Minija and Thoppil, 2002. The inhibitory effect of fennel is possibly due to anethole found in its volatile oil. Whereas the 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 (Nkanga and Uraih, 1981). Four spices such as cumin, cinnamon, cloves and fennel were showed inhibitory effect against *S. aureus*, which is an important pathogen in food poisoning. 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. In the present study, Gram-positive bacteria (*S. aureus*)

was 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 (Ouattara *et al.,* 1997).

Conclusion

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 and it may be an alternative to the use of chemical additives.

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