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Antibacterial activity of garlic, cinnamon and chitosan against food related pathogens

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ABSTRACT

Antibacterial activity of aqueous extracts and aqueous infusions of garlic, cinnamon and chitosan was investigated on food pathogens, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Natural antibacterial substances can be used to inhibit such pathogens and also to increase shelf life and safety of food products. All spices and herbs under study exhibited strong antibacterial activity against food related bacteria. Results suggested that among the spices investigated garlic had excellent antibacterial activity followed by cinnamon and chitosan. This indicates practical importance of these natural substances in increasing shelf life and decreasing the risk of contamination of food without any undesirable side effects which are commonly observed with the use of chemical preservatives.

1. Introduction

Food borne pathogens such as diarrheagenic serotypes of *Salmonella spp*, *Escherichia coli*, *Staphylococcus aureus* are widely distributed in nature, causing considerable mortality and morbidity in the population.[1] These bacteria cause major infections transmitted via food ingestion causing gastroenteritis, diarrhoea, vomiting, abdominal cramps, enteric fever, septicaemia and in severe cases death.[2] Recently used chemical food additives are being questioned due to increased awareness of safety and increased ability to evaluate safety. Thus, there is currently great interest in antibacterial compounds naturally present in foods and food ingredients. Spices are frequently used as an active ingredient in certain medicines and reported to possess a number of pharmacological effects to treat different human ailments.[3] Several investigations have been directed towards their antibacterial properties. For example allicin in garlic and onion, lysozyme in eggs are studied for enhanced microbial stability of some foods.[4] Garlic (*Allium sativum*) has traditional dietary and medicinal application as an antimicrobial agent mostly due to presence of allicin that inhibit various thiol-dependent enzymatic system of bacteria.[5] Chitosan is obtained from deacetylation of chitin, which is a natural polymer found in the exoskeleton of arthropods, such as insects and crustaceans. Chitosan exhibit antitumoural, immunoadjuvant, hypolipidemic, haemostatic, antioxidant and bactericidal activities.[6,7] The currently accepted antimicrobial mechanism is based on the interaction of the positively charged chitosan with the negatively charged residues on bacterial cell surface. It is believed that this charge interaction alters bacterial surface morphology and damages the membrane to induce membrane permeability that causes leakage of intracellular substances like electrolytes, proteins, nucleic acids, glucose etc.[8] Chitosan is mostly applied as a food additive or preservative, and as a component of packaging material, not only retard microorganism growth in food, also to improve the quality and shelf life of food.[9,10] A number of investigators reported that cinnamon inhibited the

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growth and toxin production of some mould species, with an activity emerging from cinnamic aldehyde and eugenol. Both these compounds significantly contributed to antibacterial properties. The present study gives an access on the antibacterial effect of aqueous decoction of garlic, cinnamon and chitosan against food-related bacteria.

2. Material and Methods

Antibacterial activity testing using agar well method.

2.1. Test organisms used

Staphylococcus aureus, *Salmonella typhi*, *Escherichia coli* that are associated with food poisoning or food spoilage, were used as test strains.

2.2. Collection of spices

All samples of spices viz., garlic, cinnamon, chitosan were purchased from the local market of Kalyan and were thoroughly washed with water.

2.3. Preparation of aqueous decoction

Aqueous decoction of garlic, cinnamon and chitosan were prepared by steeping 10 g in 100 ml sterile distilled water and these flasks were kept over low flame for 15 minutes. The flask were then plugged and removed from heat and allowed to cool. After cooling the content of flasks were filtered.

2.4. Antibacterial activity testing using agar well method

Sterile nutrient agar was used for this purpose. The selected strains of bacteria (i.e. *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*) were inoculated into 10 ml of sterile nutrient broth and incubated at 37^0C for 16-18 hours. The culture density were adjusted to 10^6 cells/ml. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile nutrient agar plates. Agar wells were prepared with the help of sterile cork borer with 10 mm diameter. Using a Pasteur pipette, 100 μl of different spices extracts were added to different wells in the plate. All the experiments were performed in triplicates.

2.5. Antibiotic sensitivity testing

The test microorganisms were also tested for their sensitivity against the antibiotics ciprofloxacin (5 μg), streptomycin (10 μg) and tetracycline (30 μg) by disc diffusion method. The cultures were enriched in sterile nutrient broth for 6-8 hours at 37^0C. Using sterile cotton swabs, the cultures were aseptically swabbed on the surface of sterile Mueller-Hinton (MHA) agar plates. Using an ethanol dipped and flamed forceps, the antibiotics discs were aseptically placed over the seeded MHA plates sufficiently separated from each other to avoid overlapping of the inhibition zones. The plates were incubated at 37^0C for 24 hours and the diameter of inhibition zones was measured in mm.

All the media used in the present investigation were obtained form Hi-media Laboratories Ltd., Mumbai, India. All the experiments were performed in triplicates.

2.6. Determination of efficacy of chitosan as preservative by inoculation into fruit juice

This test was used to find out the efficacy of chitosan to use as preservative.

2.7. Method

Sterile native chitosan was added to orange juice (70 mg in 100 ml). The juice was stored at 4^0C for 10 days. Aliquots were withdrawn at the interval of 5 days and 10 days, diluted and total viable counts were determined by plating on PCA, MRS and Sabouraud agar plates.

3. Results and Discussion

According to the tests, garlic cinnamon and chitosan were found to be very effective against tested micoorganisms. Garlic was found to exhibit maximum antibacterial activity followed by cinnamon and chitosan. As indicated from Table 1 the most susceptible species to this spice was *Staphylococcus aureus*. This result concur with those obtained by.[11,12] The antibacterial effect of garlic is especially, due to allicin that affect the growth of bacteria by inhibiting their DNA and proteins synthesis partially and also by inhibiting RNA synthesis as primary target. The antimicrobial activity of cinnamon may be explained by its volatile oil components.[13] In another study, eugenol was shown to have a stronger bactericidal activity against *Escherichia coli* and Klebsiella pneumoniae than some antibiotics.[14] The results of the present study concur the results of the study mentioned by.[12,15] The diameter of the zone of inhibition obtained against various spices by agar well method was compared to those obtained against commonly used antibiotics (Table 2). It was observed that Garlic extract presented higher diameter of inhibition zones than all the three antibiotics used for this study (Table 2). *S. aureus* was found to be more susceptible to spice samples. Similar results were found by.[16,17]

This may be explained by the fact that the Gram positive bacteria, due to their structural features, are more susceptible to phenolic compounds than gram negative bacteria.[6] It was observed that chitosan reduced the viable count as obtained on MRS, PCA and Sabouraud's media over a period of ten days (Tables 1, 2 and 3). The extension of the quality of the orange juice, especially in parameters such as browning and reduction of aerobic counts.[18]

Table 1: Antibacterial activity of different concentrations of garlic, cinnamon and chitosan extracts against food related bacteria.

Organism	Diameter of inhibition zone (in mm) against various concentration of species extract		
	Garlic	Cinnamon	Chitosan
Escherichia coli	23	21	19
Staphylococcus aureus	29	18	20
Salmonella typhi	20	20	23

Table 2: Antibacterial activity of different antibiotics against food related bacteria.

Organism	Diameter of inhibition zone (in mm) against various antibiotics		
	Streptomycin	Tetracycline	Ciprofloxacin
Escherichia coli	23	22	25
Staphylococcus aureus	19	20	22
*Salmonella typh*i	16	23	28

Determination of efficacy of chitosan as preservative by inoculation into fruit juice.(Tables 3, 4 and 5)

Table 3: As obtained on MRS medium

No. of days	Control (log cfu/ml)	Sample containing chitosan (log cfu/ml)
0	10.9541	10.9021
5	11.7829	10.0120
10	11.8369	9.6222

Table 4: As obtained on PCA medium

No. of days	Control (log cfu/ml)	Sample containing chitosan (log cfu/ml)
0	11.5888	10.4470
5	11.1200	10.1200
10	11.3132	10.0000

Table 5: As obtained on sabouraud's agar

No. of days	Control (log cfu/ml)	Sample containing chitosan (log cfu/ml)
0	10.9555	10.6970
5	11.0633	10.1365
10	11.2405	9.0580

4. Conclusion

Garlic, cinnamonc and chitosan 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.

5. Source of Funding

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6. Conflicts of Interest

The authors declare that they have no conflicts of interest.

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