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Efficacy of Aqueous Extract And Essential Oil of Cinnamon in Decontaminating Chicken Meat

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ABSTRACT

Cinnamon *(Cinnamomum zeylanicum)* of the family Lauraceae is a commonly used spice in India. This study was designed to evaluate the antimicrobial efficiency of aqueous extract and essential oil of cinnamon on food borne pathogens. Essential oil of cinnamon (Minimal Inhibitory Concentration (MIC) of 1:1000) was effective against reference strains of *Salmonella typhimurium, Escherichia coli* (MTCC 452), *Staphylococcus aureus, Escherichia coli O157 and Bacillus cereus,* whereas, aqueous extract of cinnamon even at 100 per cent concentration did not produce significant zone of inhibition against the test organisms such as *Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium, E. coli O157.* Dilutions of essential oil of cinnamon at 1:150 and 1:250 concentrations brought more reduction in bacterial counts compared to 1:500. The concentration of 1:150 was more efficient in decontaminating chicken meat surface among the concentration tried. Aqueous extract of cinnamon at 100 and 75 per cent concentrations did not have any effect in decreasing the viable bacterial pathogens.

Key words: *Cinnamon, chicken, pathogens, antimicrobial.*

INTRODUCTION

Spices are roots, barks, seeds, buds, leaves or fruits of aromatic plants that have been in use for centuries, incorporated in food as flavoring agents. Scientific evidences with respect to the antimicrobial property of the spices and their components were reported since the late nineteenth century (Shelef, 1983). Cinnamon is the bark of *cinnamonum zylanicum* tree, which the Egyptians used for embalming after recognizing the antibacterial efficacy. The antimicrobial activity of cinnamon has been recognized against several food spoilage organisms as well as food borne pathogens of public health importance (Shelef, 1983)

In India consumers generally prefer fresh meat without refrigeration. The initial microbial load in the chicken carcass is usually high due to unhygienic processing prevailing in retail shop, which along with the lack of cold chain puts the consumers at high risk. The consumers are now more concerned about the safety of the food they consume and majority is against chemical preservatives. Progressive removal of chemical preservatives and adoption of natural alternatives is the of food safety (Brull and Coote, 1999). Although the herbal spices have been well known for their medicinal, preservative and anti oxidant properties, they have been used with primary purpose of enhancing flavor of foods rather than extending shelf life. Scarce information is available regarding their use as antimicrobial agents in meat industry. Hence this study has been designed to measure the efficacy of cinnamon as antimicrobial agent in chicken meat system.

MATERIALS AND METHODS

Preparation of extracts of cinnamon: Aqueous extract (AE) of cinnamon was prepared as per the method outlined by Indu *et al.*, (2006). The fresh dried cinnamon was obtained from the local market and cleaned. In order to obtain the spices

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extract, about 100g of cinnamon was powdered in sterile blender with 100 ml of sterile distilled water. The extract was then sieved through a fine sterile muslin cloth and sterilized using a membrane filter (0.45-micron sterile filter). This sterile aqueous extract obtained was considered as 100 per cent concentrate and diluted to make it 75 per cent concentrate. The essential oil (EO) of cinnamon was obtained from M/S Plants lipids Ltd, Cochin, Kerala. The various dilutions (1:150 to 1:1300) of EO were prepared using ethanol.

Bacterial count: Total viable count (TVC) and count of different bacterial pathogens in meat was enumerated as per the method given by Mortan (2001). The reference strains of *Escherichia coli* (MTCC 452), *Escherichia coli* O157 (MTCC 452), *Staphylococcus aureus* (MTCC3103), *Salmonella typhimurium (*MTCC 1251), *Bacillus cereus* (MTCC), *Bacillus subtilis* (MTCC), *Klebsiella pneumoniae* (MTCC) were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh. The cultures were maintained at 4°C in Brain Heart infusion agar slants and were tested for purity and subcultured every 15 days.

Testing antibacterial sensitivity: The bacterial pathogens *viz., Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, E. coli* O157, *Bacillus cereus, Bacillus subtilis, Klebsiella pneumoniae* were inoculated into Brain Heart infusion broth and incubated at 37°C overnight. The inoculum was centrifuged at 8000 rpm for 10 min. The supernatant was discarded and the cell pellet was mixed in a sterile normal saline and centrifuged at 8000 rpm for 10 min. The cells were washed twice with normal saline and the concentration of cells was matched to the Nephelometer tube number 4 which gave a cell concentration of 10^9 cells/ ml of the culture. This culture was used as inoculum for the seeded plates in determining antimicrobial activity.

Antimicrobial activity of aqueous extract: The disc diffusion method was used to determine the antibacterial activity of the AE of Cinnamon. The test organisms grown in liquid growth medium of 0.1 ml was transferred to the surface of Muller Hinton agar and spread uniformly on the entire surface of agar medium using a sterile glass spreader. Then sterile filter discs of 8 mm diameter (HiMedia) with 25µl absorbed AE of cinnamon was placed over the agar by pressing gently. The plates were incubated at 35 ± 1° C for 48 hr. After the incubation, the inhibition zones were measured in millimeter. The sensitivity of the AE was classified based on the diameter of inhibitory zone as per the procedure of Moreira *et al.* (2005). Results were interpreted as below:

Non Sensitive: Diameter less than 8mm; Sensitive: Diameter between 9- 14mm; Very Sensitive: Diameter between 15- 19mm; Extremely Sensitive: Diameter more than 20mm

Minimum inhibitory concentration: Agar diffusion assay was used to determine MIC of the cinnamon extracts by following the procedure of Moreira *et al.*, (2005). Fresh bacterial culture of 10 ml was added to 100 ml of molten Tryptic soya agar maintained at 45°C in a water bath and give a final concentration of 10^6 cells /ml of medium and the culture was thoroughly mixed. Sterile molten nutrient agar was maintained at 45°C was poured to sterile petriplates and was allowed to solidify and placed in a refrigerator for 10 min. Wells of 8 mm diameter were punched in agar into which 25 µl of the EO of spices was placed after sealing the bottom of the well with a drop of sterile agar of 1 per cent to ensure that radial diffusion occurred from the well in order to get clear and easily measurable zone of inhibition. The plates were then incubated at 37° C for 24 hr. The inhibitory zones were measured after the incubation period. The results were interpreted as follows

- Less than 8mm; + 8-9 mm; ++ 10-13 mm; +++ 14-16 mm

***Cinnamon for decontaminating chicken carcass*:** The chicken whole legs and breast (n=3) were obtained from market and surface swab (4 cm X 4 cm) of chicken leg and breast collected from chicken carcasses were put into the tube containing 9 ml of sterile diluent and agitated for five minutes so as to transfer the bacteria attached to the cotton swab into the diluents. The AE (100% and 75%) and EO (1:150. 1:250, and 1: 500) were

applied over the different samples in the form of dip with a contact time of 2 min. The samples drawn from different treatments were subjected for microbial analysis *viz.,* TVC, *E. coli,* Salmonella and *Staphylococcus aureus*

Statistical Analysis: The data obtained in the study were analyzed statistically for significance as per the procedure outlined by Snedecor and Cochran (1994).

RESULTS & DISCUSSION

Antimicrobial activity of aqueous extract of cinnamon on bacterial pathogens: The in vitro antimicrobial activity of aqueous extract of cinnamon expressed in terms of zone of inhibition (mm) for different concentration were presented in Table 1. The results revealed that aqueous extract of cinnamon even at 100 per cent concentration did not produce significant inhibition against the test organisms such as *Bacillius cereus, Staphylococcus aureus, Salmonella typhimurium, E. coli O157.* The results obtained were in agreement with Indu *et al. (2006)* who observed no antibacterial activity in cinnamon extract against *E. coli,* Salmonella, *Listeria. monocytogenes and Aeromonas hydrophila.* However, Srinivasan and Lakshmanaperumalsamy (1993) and Suresh *et al.* (2004) reported moderate activity of cinnamon extract against food borne pathogens.

Essential oil of cinnamon against variousbacterial pathogens: The MIC of essential oil of cinnamon against various bacterial pathogens is presented in Table 2. Among the reference organisms, *Staphylococcus aureus, Salmonella typhimurium, E. coli (MTCC 452), E. coli O157* and *Klebsiella pneumoniae* were found to be sensitive to essential oil of cinnamon at a minimum inhibitory concentration of 1:1000, whereas, *Bacillius cereus, Bacillius subtilis* were sensitive at a minimum inhibitory concentration of 1:700. In case of field isolates, *Salmonella and E. coli* were found to be less sensitive at MIC of 1:700, however, *Staphylococcus aureus* was found to be sensitive at this concentration level. Results of this study revealed that EO of cinnamon was found to have better activity against food borne pathogens compared to its aqueous extract. This

Table 1: In vitro antimicrobial activity of aqueous extract of cinnamon on various bacterial pathogens determined by Disc Diffusion Assay (Zone of Inhibition in mm) (mean±SE) *

Bacterial Pathogens (cfu/g)	Concentration of aqueous extract of cinnamon	
	100 %	**75 %**
Salmonella typhimurium	6.05±0.42 [a]	-
Escherichia coli	7.2±0.22 [a]	6.11±0.34[b]
Staphylococcus aureus	6.12±0.42 [a]	-
Escherichia coli O157	7.01±0.20 [a]	6.06±0.51 [b]
Bacillus subtilis	9.06±0.70 [a]	-
Bacillus cereus	10.5±0.19 [a]	8.09±0.14 [b]
Klebsiella pneumoniae	6.12±0.47 [a]	-

* Figures bearing different super scripts in a row vary significantly (P<0.05)

Table 2: Minimum Inhibitory Concentration (MIC) of essential oil of Cinnamon against various bacterial pathogens determined by Agar Diffusion Assay

Bacterial pathogens	Concentration of essential oil of cinnamon					
	1: 150	**1:250**	**1: 500**	**1: 700**	**1: 1000**	**1: 1300**
	Inhibition zone					
Salmonella typhimurium	++	++	++	+	+	——
Escherichia coli	++	++	++	+	+	——
Staphylococcus aureus	++	++	++	+	+	——
Escherichia coli O157	++	++	++	+	+	——
Bacillus subtilis	++	++	+	+	——	——
Bacillus cereus	++	++	++	+	——	——
Klebsiella pneumoniae	++	++	+	+	+	——
Salmonella(F)	++	++	+	——	——	——
Escherichia coli (F)	++	+	+	——	——	——
Staphylococcus aureus (F)	++	++	++	+	——	——

—— Less than 8 mm + 8 to 9 mm ++ 10 to 13 mm +++ 14 to 16 mm

Table 3: Effect of aqueous extract (AE) & essential oils (EO) of cinnamon in reducing pathogens of chicken meat in the form of a dip (Mean ± SE)

Type of cinnamon extracts	Concentration of cinnamon extracts	Treatment effect	TVC (cfu/g)	*E. coli* (cfu/g)	Salmonella (cfu/g)	Staphylococcus (cfu/g)
AE of Cinnamon	**100 %**	Before Treatment	5.9 ± 0.03	3.92± 0.01	3.30 ± 0.18	4.76 ± 0.07
		After Treatment	5.85 ± 0.03	3.80 ± 0.03	3.24 ± 0.19	4.69 ± 0.07
	75 %	Before Treatment	5.95 ± 0.05	3.83 ± 0.06	3.21 ± 0.21	5.02 ± 0.38
		After Treatment	5.90 ± 0.04	3.74 ± 0.06	3.05 ± 0.17	4.94± 0.21
EO of cinnamon	**1:150**	Before Treatment	5.65 ± 0.17	3.77 ± 0.04	3.18 ± 0.31	5.16 ± 0.41
		After Treatment	5.06 ± 0.024	2.88 ± 0.01	2.06 ± 0.18	4.47 ± 0.14
	1:250	Before Treatment	5.81 ± 0.05	3.79 ± 0.07	3.18 ± 0.15	4.11 ± 0.18
		After Treatment	5.31 ± 0.13	2.92 ± 0.22	2.64 ± 0.05	3.71 ± 0.30
	1:500	Before Treatment	5.856± 0.04	3.76± 0.02	3.42 ± 0.03	4.44± 0.03
		After Treatment	5.51 ± 0.05	3.04 ± 0.05	2.89 ± 0.05	4.51 ± 0.18

might be due to the fact that the active principle of cinnamon, cinnamic aldehyde was extracted better in oils than in aqueous extracts (Shelef, 1983).

Aqueous extracts and essential oils of cinnamon as decontaminating agent: Results of efficacy of decontamination of AE and EO of cinnamon on chicken carcass are given in Table 3. Essential oil of cinnamon at 1:150 and 1:250 concentrations brought more reduction in bacterial counts compared to 1:500. Based on the results it was observed that 1:150 concentration was more effective in reducing microbial load. This is in agreement with the reports of Ouattara *et al.* (1997) and Koidis *et al*. (2000), who reported the antimicrobial activity of cinnamon against several food spoilage organisms and *E. coli*. The aqueous extract of cinnamon at 75 and 100 percent concentrations did not bring any significant reduction in pathogenic bacteria level.

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