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Ginger Extract as an Antimicrobial Agent: A Case Study in Chicken Meat†

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ABSTRACT

The extracts of many spices and herbs have become popular in food industry in the recent years for their antimicrobial and antioxidant properties. Ginger *(Zingiber officinale roscae*) of family *Zingiberaceae* is a commonly used spice in India. This study was conducted to examine the antimicrobial efficacy of ginger on few meat borne pathogens in fresh chicken. *Staphylococcus aureus* was most sensitive to ginger oil extract followed by *Salmonella typhimurium, Escherichia coli O157, Bacillus cereus, Bacillus subtilis and Klebsiella pneumoniae.* Aqueous extract of ginger had no effect on bacterial counts whereas oil extract of ginger decreased bacterial counts significantly ($P \leq 0.01$).

Key words: *Ginger, antimicrobial effect, chicken meat, food borne pathogens.*

INTRODUCTION

Broiler meat industry in India has shown tremendous growth in production from 1.08 million tons in 2000 to 2.68 million tons in 2009 (FAOSTAT, 2008). Chicken meat became very popular and it was attributed primarily to its taste, health concerns and nutritional value followed by freedom from religious taboos, comparatively less price and easy availability (Fairoze, 2001).

Incorporation of antibiotics, chemical preservatives, and antimicrobial compounds *viz*., trisodium polyphosphate, lactic acid, acetic acid and salt and storage treatments such as low temperature, heat and irradiation processes have been tried for reducing the bacterial load in meat (Bin Jasass, 2007). Increasing incidences of some pathogens connected to food borne illness acquiring antibiotic resistance has been a worry (Shan *et al*., 2007). This perspective has put pressure on the food industry for progressive removal of chemical preservatives and adoption of natural alternatives to achieve the goal concerning microbial food safety (Brull and Coote, 1999).

Herbs and spices have been added to foods since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives (Buchart, 2001). In addition to imparting characteristic flavors, certain spices and herbs have proved to prolong the shelf life of foods by preventing rancidity through their antioxidant activity and also through their bacteriostatic or bacteriocidal activities (Buchart and Golden, 1989). Herbs and spices and their components are generally recognized as safe, either because of their traditional use without any documented detrimental impact or as a result of dedicated toxicological studies (Smid and Gorris, 1999).

Ginger is used as a spice in many Asian foods, especially in Indian cuisine along with garlic. A number of researchers have investigated antibacterial activity of ginger. Mascolo *et al.* (1989) reported that the hydro ethanolic extract of ginger have potent antibacterial activity against Gram positive and Gram negative bacteria and Salzer (1982) reported inhibition of *E.coli, Streptococcus faecalis, Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringes* by the use of ginger extracts in meat products. Hence this experiment was designed to study the effect of marination with ginger extracts on the bacterial load of chicken meat.

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MATERIALS AND METHODS

Preparation of extracts of ginger: Aqueous extract (AE) of ginger was prepared as per the method outlined by Indu *et al.,* (2006). The fresh ginger was obtained from the local market and cleaned. In order to obtain the aqueous spice extracts, about 100g of ginger was made into paste 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 thus obtained was considered as the 100 per cent concentration of the extract. The essential oil (EO) of ginger was obtained from M/S Plants lipids Ltd, Cochin, Kerala. The EO of ginger was diluted with ethanol.

Processing and dilution of samples: All swab samples collected from each area of carcass and surface (4 cm X 4 cm) was put into the tube containing nine ml of sterile diluent and agitated for five minutes so as to extricate the bacteria attached to the cotton swab into the diluents. Similarly, five grams of meat sample was taken and triturated in a sterilized mortar and pestle and transferred to 45 ml of sterile diluents, separately.

Bacterial count: Different bacterial pathogens in fresh chicken meat were enumerated as per the method given by APHA (1992). The reference strains of *Escherichia coli* (MTCC 452), *Staphylococcus aureus* (MTCC3103), *Salmonella typhimurium (*MTCC 1251), *Escherichia coli* O157 (MTCC 452), *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^{o}C in Brain Heart Infusion broth and were tested for purity, morphology and biochemical characters every 15 days.

Testing antibacterial sensitivity Preparation of bacterial cultures: The bacterial pathogens *viz.,Escherichia coli, Staphylococcus aureus, Salmonella typhimurium Escherichia coli* O157, *Bacillus cereus, Bacillus subtilis, Klebsiella pneumoniae* were inoculated into Brain Heart Infusion broth and incubated at 37^{o}C overnight. The inoculum was centrifuged at 8000 rpm for 10 min. The supernatant was discarded and the 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 No.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.

Test to determine antibacterial activity of AE of Ginger: The disc diffusion method was used to determine the antibacterial activity of the both AE and EO of ginger. 0.1 ml (approximately 10^{9}cells/ ml) of the tested microorganisms grown in liquid growth media at 37^{o}C was inoculated on Muller Hinton agar and then spread uniformly on the entire surface of petri dish using a glass spreader. Then sterile filter discs of 8 mm diameter (HiMedia) with 25µl of AE and EO of ginger were placed by pressing gently. The plates were incubated at 35 ± 1^{o}C for 48 hr. After the incubation the inhibition zones were measured in millimeter. The sensitivity of the AE and EO was classified based on diameter of inhibition zone as per the procedure of the Moreira *et al.* (2007). The experiment was repeated in duplicate and the results were interpreted as below:

Non Sensitive: diameter less than 8 mm;
Sensitive: diameter between 9- 14 mm

Very Sensitive: diameter between 15- 19 mm;
Extremely Sensitive: diameter more than 20 mm

Test to determine Minimum Inhibitory Concentration of (MIC) EO of Ginger: As per the procedure followed by Moreira *et al.* (2005), agar diffusion assay was used to determine MIC of the ginger extract. 10 ml of fresh bacterial culture was added to 100 ml of tryptic soya agar maintained at 45^{o}C in a beaker to give a final concentration of 10^{7} cells /ml of medium and the culture was thoroughly mixed. In fresh petri plates, nutrient agar was poured and was allowed to solidify and placed in a refrigerator for 10 min. Holes of 8 mm diameter were punched in to agar to create wells into which 25 µl of the EO of ginger was placed after sealing the bottom of the well with a drop of sterile agar of 1 per cent to

ensure that radial diffusion from the well gave a clear and easily measurable zone of inhibition. The plates were then incubated at 37° C for 24 hr. The inhibition zones were measured after the incubation period. The results were interpreted as follows:

- less than 8 mm; + 8-9 mm; ++ 10-13 mm; +++ 14-17 mm

Evaluation of ginger as decontamination agent in chicken carcass

To evaluate the efficacy of ginger as decontamination agent in chicken meat, two concentrations of aqueous extract (100 and 75 percent) and three concentration of essential oils(1:50, 1:100 and 1:250) of ginger were selected based on the MIC and antimicrobial activity. Chicken whole leg and breast samples were procured from the market individually for each of the treatment. Initial microbial counts of the samples were assessed. The same samples were then dipped into different concentrations of aqueous and essential oils of ginger and were allowed a contact time of 3 minutes (contact time was standardized based on the efficacy of the extracts to reduce bacterial counts and the sensory evaluation). Then samples were drawn from each of the treated sample and microbial counts were evaluated and expressed as $\log_{10}$ cfu/g of meat sample. The difference in log values before and after treatment was used as a guide to assess the antimicrobial and decontamination ability of spice extracts. All the experiments were repeated thrice.

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

In vitro antimicrobial activity of aqueous extract (AE) of ginger on various bacterial pathogens: The mean ± SE values of antibacterial activity in terms of zone of inhibition (mm) for different concentrations of AE of ginger are presented in Table 1. Results revealed that aqueous extract of ginger even at 100 per cent concentration did not produce significant zone of inhibition against the test organisms like *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Escherichia coli* O157, *Bacillus cereus, Bacillus subtilis* and *Klebsiella pneumoniae.* The results were in close agreement with Indu *et al.,* (2006), who found that ginger extract did not show any antibacterial activity against *Escherichia coli, Salmonella, L. monocytogenes and Aeromonas hydrophila.* However, the results recorded in the present study were on contrary to the findings of Suresh *et al.,* (2004) and Lakshmanaperumalswamy and Srinivasan (1993) who observed that ginger extract had moderate anti-microbial activity against common food borne pathogens.

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

Bacterial Pathogens	100 per cent	75 per cent
Salmonella typhimurium	——	——
Escherichia coli	——	——
Staphylococcus aureus	07**±0.58**	2.5**±0.50**
Escherichia coli O157	——	——
Bacillus subtilis	——	——
Bacillus cereus	03**±0.73**	——
Klebsiella pneumoniae	6.75**±0.48**	——

Minimum Inhibitory Concentration (MIC) of oil extract of ginger against various bacterial pathogens determined by Agar Diffusion Assay: The MIC of essential oil of ginger against various bacterial pathogens is presented in Table 2. Among the reference organisms, *Staphylococcus aureus* was found to be most sensitive at a MIC of 1:1300, followed by *Salmonella typhimurium, Escherichia coli, Bacillus cereus, Bacillus subtilis and Klebsiella pneumoniae* at a MIC of 1:1000. *E.coli O157* and field isolates of *Salmonella, Staphylococcus aureus* and *E.coli* were found to be less sensitive at a MIC of 1:700. Depending upon the results obtained, two major clusters of bacterial pathogens were found. One cluster consisted of *Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Bacillus cereus, Bacillus subtilis,* and *Klebsiella pneumoniae,* which were more sensitive to the action of EO of ginger. The second cluster comprised of *E.coli O157* and field isolates of *Salmonella, Staphylococcus aureus* and *E.coli,* which were comparatively less sensitive to the action of EO of ginger. Similarlrly Dorman and Deans, (2000) and

Kalemba and Kunicka (2003) opined that *Zinger officinaleis was* effective against Gram positive and Gram negative bacteria.

Evaluation of AE and EO of ginger as decontamination agent: Results of efficacy of decontamination by AE & EO of ginger are given in Table 3. Analysis of variance revealed that AE of ginger at 100 per cent and 75 per cent concentrations did not have any effect in terms of viable log reduction with respect to the bacterial pathogens, whereas, essential oil of ginger had resulted in a highly significant (P<0.01) reduction of bacterial count in different dilutions with respect to TVC, *Staphylococcus aureus*, *E.coli* and *Salmonella* counts. Dilutions of EO of ginger at 1:150 and 1:250 concentrations brought about a significant (P<0.01) decrease in bacterial count compared to 1:500. Based on the results it was observed that 1:150 concentration was the best among the different treatments used. Negbenebor *et al.*, (1995) reported that the initial psychrotropic aerobic counts of beef patties were not

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

Bacterial pathogens	Essential Oil of Ginger					
	1: 150	1:250	1: 500	1: 700	1: 1000	1: 1300
Salmonella typhimurium	+++	++	++	+	+	——
Escherichia coli	+++	++	++	+	+	——
Staphylococcus aureus	+++	++	++	++	+	+
Escherichia coli O157	++	++	+	——	——	——
Bacillus subtilis	++	++	++	+	+	——
Bacillus cereus	+++	++	++	+	+	——
Klebsiella pneumonia	++	++	++	+	+	——
Salmonella(F)	++	++	+	——	——	——
Escherichia coli (F)	++	++	++	——	——	——
Staphylococcus aureus(F)	++	++	++	+	+	——

—— Less than 8 mm + 8 to 9 mm ++ 10 to 13 mm +++ 14 to 17 m

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

		TVC	E. coli	Salmonella	Staphylococcus
100 Per cent AE of ginger	Before Treatment	5.68 ± 0.02	3.35 ± 0.05	3.12 ± 0.17	4.63 ± 0.04
	After Treatment	5.65 ± 0.02	3.32 ± 0.05	3.09 ± 0.17	4.59 ± 0.05
	log reduction	**0.03 ± 0.01**[a]	**0.03 ± 0.01**[a]	**0.03 ± 0.01**[a]	**0.03± 0.01**[a]
75 Per cent AE of ginger	Before Treatment	5.67 ± 0.03	3.50 ± 0.04	3.19 ± 0.15	4.59 ± 0.02
	After Treatment	5.65 ± 0.03	3.42 ± 0.05	3.18 ± 0.15	4.57± 0.02
	log reduction	**0.01 ± 0.04**[a]	**0.02 ± 0.01**[a]	**0.02 ± 0.01**[a]	**0.02 ± 0.05**[a]
EO of ginger 1:150 conc.	Before Treatment	5.65 ± 0.02	3.33 ± 0.02	3.04 ± 0.17	4.87 ± 0.12
	After Treatment	5.14 ± 0.04	3.13 ± 0.06	2.91± 0.17	4.30 ± 0.13
	log reduction	**0.49± 0.05**[b]	**0.22 ± 0.05**[b]	**0.13 ± 0.01**[b]	**0.57± 0.03**[b]
EO of ginger 1:250 conc.	Before Treatment	5.66 ± 0.03	3.45 ± 0.06	3.24 ± 0.02	4.63 ± 0.05
	After Treatment	5.30 ± 0.03	3.34 ± 0.07	3.17± 0.02	4.16 ± 0.05
	log reduction	**0.037 ± 0.01**[bc]	**0.12± 0.01**[a]	**0.07 ± 0.01**[a]	**0.47 ± 0.04**[bc]
EO of ginger 1:500 conc.	Before Treatment	5.72 ± 0.02	3.55± 0.02	3.26± 0.24	4.70 ± 0.03
	After Treatment	5.51 ± 0.06	3.45± 0.01	3.19 ± 0.24	4.51 ± 0.05
	log reduction	**0.21± 0.03**[b]	**0.09 ± 0.01**[a]	**0.07 ± 0.01**[a]	**0.19 ± 0.03**[b]

a,b,c significant at Pd<0.01

significantly affected by the addition of ginger extract, however after 6 days of storage at 5-7 ^{0}C, the ginger treated samples had mean log bacterial counts of 6.8 CFU/g compared to control with 8.2 CFU/g.

Therefore it was concluded that the EO of ginger decreased bacterial count significantly where as AE of ginger had not much effect. This might be due to the fact that active principle of ginger Di- allyl- di-sulphide and gingerols are insoluble in water and are extracted only during solvent extraction process (Shelef, 1983)

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