J.Meat Sci., 2015, 10(2) : 1 - 6

Inhibitory Effect of Certain Spices and Herbs Powders and their Extracts on Normal and Pathogenic Meat Microflora

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ABSTRACT

The antibacterial activity of clove, green tea, ginger, garlic, fenugreek and coriander seeds against microflora (heterogeneous) isolated from fresh and spoiled mutton as well as, *Escherichia coli* (food isolate, biochemically identified) and *Staphylococcus aureus* (MTCC-96) was evaluated. Both powder as well as ethanolic extract of the spices and herbs were used to establish and compare the inhibitory effect by semi-quantitative and cell enumeration (quantitative) methods. Of the spice and herb powders evaluated (by semiquantitative test), clove exhibited higher degree of inhibition against both fresh and spoiled mutton micro-flora. Green tea, ginger and garlic powders were effective against fresh meat micro-flora, while for spoiled meat micro-flora, green tea exhibited limited inhibitory effect and ginger and garlic were not effective. Fenugreek and coriander were found to be least effective for both fresh and spoiled meat micro-flora at the levels used. Ethanolic extracts equivalent to 1 g and 2 g clove powder exhibited complete inhibition in case of fresh mutton flora (SPC 5.81 log cfu/g) and spoiled mutton flora (SPC 8.91 log cfu/g) respectively. Other spices/ herbs viz., green tea, garlic and ginger exhibited comparatively lesser degree of inhibition for the same concentrations as compared to that in case of clove. With respect of pathogens, clove exhibited 100% inhibition where as other spices/herbs showed comparatively lower inhibitory activities. These spices/ herbs were found to be useful in extending the shelf life of raw mutton since significant ($p<0.01$) inhibitory effect is noticed with respect to heterogeneous mutton micro-flora including certain pathogens.

Keywords : *Inhibitory activity, Spices and herbs, Meatmicro-flora, Pathogens, Shelf life extension*

Received : 18-6-2014 Accepted: 23-8-2014

INTRODUCTION

In spite of the improvements in slaughter hygiene and food production techniques, food safety still remains an important public health issue (WHO 2002a).There is a need for newer methods for preservation as well as for reduction or elimination of foodborne pathogens using natural preservatives, since the consumer's preference for foods prepared with preservatives of chemical origin is on the decline. The western society appears to be experiencing a trend of 'green consumerism' desiring fewer synthetic food additives with a minimum impact on the environment (Tuley del Silva 1996; Smid and Gorris 1999). There is a scope for methods which render the food safe with a natural or'green' image. One such possibility is the use of spices or their essential oils as antibacterial agents to improve the shelf life of perishable foods like meat. In Indian culinary, certain spices like clove, cinnamon, ginger, garlic etc., are extensively used to provide distinctive flavour. Spices have been reported to exhibit suppressing action on many foodborne pathogens (Smith-Palmer *et al*. 1998; Aureli *et al*.1992) in addition to inhibition of food spoilage organisms (Wan *et al*. 1998; Abd-Alla-Magda *et al*. 2000). The antimicrobial properties of onion and garlic have been observed and recorded since the time of Pasteur (Hoffman and Evans 1911). Majority of the published literature is restricted to the study of antibacterial property of spices in pure cultures. The antimicrobial activity of spices/herbs has been reported to be due to essential oils present in them.

Most of these studies, revealed the antimicrobial activity of a wide variety of herbs and spices against specific microorganisms (Krisch *et al*. 2010; Tajkarimi *et al.* 2010), have not applied this property to preserve fresh mutton which contains heterogeneous microflora (normal flora) at ambient temperature conditions. There has been little work on standardization of test procedures for use of anti-bacterial agents in food. Keeping this in view, in the present study, the effect of clove, greentea, ginger, garlic, fenugreek and coriander both in the powder form as well as in the form of ethanolic extracton fresh and spoiled mutton microflora as well as on *E. coli* and *S. aureus* has been evaluated and reported as quantitative inhibitory effect. The work was taken up with view to explore the potential of these spices/herbs in extending the shelf life of fresh mutton.

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MATERIALS AND METHODS

Materials

a) Mutton: Deboned fresh mutton (sheep meat) from 18-20 month sold 'Bannur' Sheep, (a local breed) 6-7 h after post-mortem , was purchased from a known source in four different batches. In each batch, one portion of the mutton was deliberately spoiled by exposing to 25°C (in an incubator) for 30h to increase the microbial load. Duplicate samples were taken from each batch and subjected to analysis.

b) Spice powder: Powdered spices, clove *(Eugenia caryophyllus),* ginger *(Zingeber officinale),* garlic *(Ailium sativurn),* fenugreek *(Trigenell afenunzgraecum),* coriander *(Coriandrurn sativurn)* and greentea *(Camellia sinensis)* were procured from retail shop and sterilized in an autoclave at 15 psi for 15 min before using.

c) Microbiological media: All microbiological media/analytical ingredients were obtained from Hi-media, Mumbai.

d) Test organism: Two test organisms, *E. coli* (Food isolate) and *S. aureus* (MTCC-96) were used for experimental purposes.

Methods

In each batch three sets of experiments were conducted to evaluate the efficacy of spices and herbs.

Experiment 1: To test the efficacy of powdered whole spices/ herbs on fresh and spoiled mutton micro-flora.

a) Preparation of inoculum: Microflora from 50 g each of fresh and spoiled mutton were extracted separately in 100 ml sterilzed distilled water by shaking for 10 min. The viable cell concentration in the inoculum was enumerated using the standard procedure.

b) Preparation and inoculation of plate count agar (PCA) plates containing spice powder : PCA was prepared as per the standard procedure and autoclaved at 121 °C for 15 min. To 20 m1 of molten agar, 2 g spice/herb powders were added individually, and poured into a sterile petridish. One control (medium) was maintained. The plates were allowed to set, surface dried in previously sterilized laminar air flow chamber and marked into four segments. Using sterile platinum loop (0.01 ml), both fresh and spoiled mutton inoculums were deposited separately in two segments each. The plates were incubated at 37 °C for 24 h and observed for growth.

c) Evaluation of the plate for in hibitory activity (Semi-quantitative): The growth on the plates was graded depending on the visual opacity/thickness. The growth on the medium control plate (for both fresh and spoiled mutton inoculum) was taken as maximum, and graded as 10+. However the value of 10+, for fresh mutton flora control may not mean the same for spoi1ed mutton flora control in quantitative terms. Compared with the growth on respective control plates, the herbs were prepared according to the method described by Wendakooo and Sakaguchi (1992) powdered spices (20g each) viz., clove, ginger garlic and green tea were added separately to 100 ml of 95 % ethanol, left overnight and filtered to obtain the extracts (Fenugreek and coriander were excluded, since from the previous experiment (1), it was observed that they exhibited least inhibitory activity against mutton flora, at the levels used). Ethanol was evaporated to dryness and the residue was made up to 10 ml with 95% ethanol, so that the final concentration of spice/herb is 2 g/ml.

b) Effect of fresh mutton micro-flora: From the previous experiment (1), it was observed that 2 g spice/herb powder inhibited the fresh mutton flora to different levels as mentioned in the text. So in these experiments, ethanolic extracts of each spice/herb equivalent to 2 g and less than 2 g i.e. 1.0 ml, 0.75 ml and 0.5 ml equivalent to 2.0 g, 1.5 g and 1.0 g respectively and inoculated to PCA medium. There alcohol blanks, each with 1.0 ml, 0.75 ml and 0.5 ml (without spice) and one medium blank (without spice and alcohol) were also included. The plates were marked into four segments. One loop full (0.01ml) of fresh mutton innoculum was deposited in each of the four segments (replicates) and incubated as per standard procedure.

c) Effect on spoiled mutton micro-flora: From the experiment (1), it was observed that 2 g spice/herb powder was not sufficient to inhibit spoiled mutton flora except in case of clove. So in the present study, ethanolic extract equivalent to 2 g and more than 2 g i.e., 1.0 ml, l.5 m1 and 2.0 m1 equivalent to 2 g, 3 g and 4 g spice/herb respectively were added separately to sterile molten PCA and poured into sterile petridishes. Three separate alcohol blanks, each containing 1.0 ml, 1.5 m1 and 2.0 m1 alcohol (without spice/herb) and one medium control were also inoculated. To the set plates, one loopful (0.01 ml) of spoiled mutton inoculum was deposited in each of 4 segments (replicates) and incubated.

Experiment 3: Inhibitory activity of ethanolic extract from spices and herbs as determined by enumeration (quantitative) method. One ml of inoculum from both fresh and spoiled mutton was serially diluted and pour-plated separately with each of the spice/herb ethanotic extracts containing PCA as per the standard procedure.

To test the efficacy of ethanolic extract from spices/herbs against *E. coli* and *S. aureus* by enumeration (quantitative) method:

a) Preparation of the culture: Stock cultures of *E. coli* and *S. aureus* were streaked on the nutrient agar slants and incubated

at 37°C for 24h. A loopful each of fully-grown cultures was inoculated individually into 9 ml of nutrient broth and incubated. The cell density of each organism was established by serial dilution of the stock with peptone water as diluent and plating on PCA plates for incubation.

b) Establishing the inhibitory activity of spice/herb extracts: The inhibitory activity of spice/herbs extracts on both *E. coli* and *S. aureus* was established using enumeration method. One ml from each 10^1 to 10^9 dilutions was placed in petri-dishes. One ml of ethanolic extract of the spice/herb equivalent to 2 g (prepared as per 2.4a) was added to PCA medium and incubated.

Statistical analysis: Data obtained were analyzed for statistical significance using analysis of variance (ANOVA) and the significance was established at $p < 0.01$.

RESULTS AND DISCUSSION

Table 1 shows the effect of powders from whole spices/herbs on fresh (5.81 log cfu/g) and spoiled mutton micro-flora (8.91 log cfu/g). This semi-quantitative method of testing was conducted as a preliminary check for anti-microbial activity prior to the detailed studies, to see the effect of 2 g of spice/herb powder. It is seen from the table that, except fenugreek and coriander all the other spice/herb powders were very effective in inhibiting the fresh mutton flora. Clove was effective in inhibiting the spoiled mutton flora as well, while green tea was comparatively less effective than clove. Ginger, garlic, fenugreek and coriander were not effective in inhibiting spoiled mutton micro-flora at 2 g levels. For further studies, fenugreek and coriander were excluded since they were not found to be effective in inhibiting the mutton micro-flora at the levels used. A weak activity of fenugreek against *S. typhimurium*, *B. cereus*, *E. coli* and *A. flavus* has been reported (Packyasothy and Kyle 2002). Anti-microbial activity of fractions of coriander essential oil has been reported (Delaquis *et al.* 2002). However, the whole powder did not seem to inhibit the heterogeneous mutton micro-flora.

Table 1: Inhibitory effect of whole spice and herb powders on fresh and spoiled mutton micro-flora (n=6)

Sample	Fresh meat flora (SPC 5.81 log cfu/g)	Spoiled meat flora (SPC 8.91 log cfu/g)
Control	10+	10+
Clove	1+	1+
Green Tea	1+	5+
Ginger	1+	10+
Garlic	1+	10+
Fenugreek	10+	10+
Coriander	10+	10+

Table 2 depicts the effect of ethanolic extracts from spices/herbs on fresh mutton micro-flora using semi-quantitative method. As seen from the growth opacity (visual) alcohol blanks of 0.5 m1, 0.75 m1 and 1.0 ml showed inhibition of 20 %, 30 %, and 40 % respectively, as compared to the medium blank. Clove and green tea extracts were able to inhibit the growth totally (100%), even at the lowest levels used while between ginger and garlic extracts, ginger was more effective. Ginger extract was able to show an inhibition of 80 %, 90 % and 95 % as against garlic extract (70 %, 80 % and 90 %) for respective volumes of extract, when compared to medium blank. This semi quantitative test was more convenient to perform to scan the inhibitory effect of ethanolic extracts, as compared to the enumeration technique (quantitative but laborious). Enumeration test (Fig. 1) also showed clove as the most effective spice, which caused complete inhibition of micro-flora at all levels. Green tea extract was able to cause complete inhibition at 1.0 ml levels whereas at 0.75 ml and 0.5 ml levels, there was a reduction by more than 3 logs in SPC as compared to respective alcohol blanks. Ginger and garlic extracts also exhibited inhibitory effect though too lesser extent. The inhibitory effect in case of all the four extracts was highly significant ($P<0.01$) as compared to respective alcohol blanks. With respect to fresh mutton micro-flora, the inhibitory effect was in the order clove> green tea> ginger> garlic.

Fig.1: Enumeration test for the inhibitory effect of ethanolic extracts from spices and herbs on fresh mutton micro-flora (SPC 5.81 log cfu/g). (n=8)

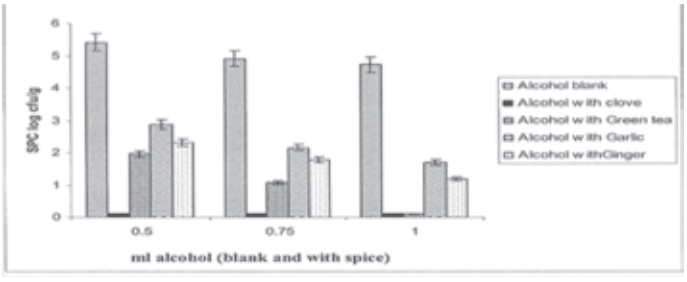


Table 2: Inhibitory effect of ethanolic extracts of spices and herbs on the fresh mutton micro-flora as estimated by semi-quantitative method (n=8)

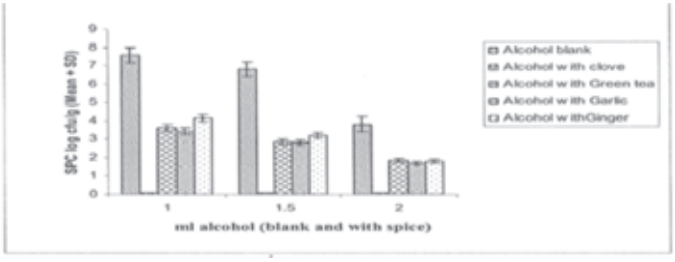
Sample	Volume of ethyl alcohol (ml)	Equivalent quantity of spices and herbs (g)	Growth opacity (visual) Semi-quantitative)
Control (media blank)	No alcohol	No spice	10+
Alcohol blank	0.5	No spice	8+
	0.75	No spice	7+
	1.0	No spice	6+
Clove	0.5	1.0	-ve
	0.75	1.5	-ve
	1.0	2.0	-ve
Green Tea	0.5	1.0	-ve
	0.75	1.5	-ve
	1.0	2.0	-ve
Garlic	0.5	1.0	3+
	0.75	1.5	2+
	1.0	2.0	1+
Ginger	0.5	1.0	2+
	0.75	1.5	1+
	1.0	2.0	<1+

Table 3: Inhibitory effect of ethanolic extracts of spices and herbs on the spoiled mutton micro-flora as estimated by semi quantitative method (n=8)

Sample	Volume of ethyl alcohol (ml)	Equivalent quantity of spices and herbs (g)	Growth opacity (visual) Semi-quantitative)
Control (media blank)	No alcohol	No spice	10+
Alcohol blank	1.5	No spice	8+
	1.5	No spice	6+
	2.0	No spice	3+
Clove	1.0	2.0	-ve
	1.5	3.0	-ve
	2.0	4.0	-ve
Green Tea	1.0	2.0	4+
	1.5	3.0	<1+
	2.0	4.0	-ve
Garlic	1.0	2.0	3+
	1.5	3.0	<1+
	2.0	4.0	-ve
Ginger	1.0	2.0	5+
	1.5	3.0	1+
	2.0	4.0	-ve

The extent of inhibition exhibited by extracts from spices and herbs in case of spoiled mutton micro-flora by semi-quantitative method has been depicted in Table 3. Alcohol blanks of 1.0 ml, 1.5 m1 and 2.0 ml exhibited an inhibition of 20 %, 40 % and 70 % respectively as compared to medium blank. Clove exhibited complete inhibition even at the lowest concentration levels (2 g) as evaluated by visual growth observation. Between green tea, garlic and ginger, the inhibitory effect of garlic was found to be higher. The percentage of inhibition was 60 %, 95 % and 100 % for green tea extract, 70 %, 95 % and 100 % for garlic extract and 50 %, 90 % and 100 % for ginger extract for 1.0 ml, 1.5 ml and 2.0 ml respectively. In the enumeration test (Fig. 2), between the four extracts, clove exhibited maximum inhibitory activity, followed by garlic, green tea and ginger. Green tea and garlic extracts exhibited more or less similar inhibitory activity. Ginger extract had a comparatively lesser inhibitory effect at lower levels while at higher levels, the inhibitory effect was as that for green tea, garlic extracts. A highly significant inhibitory effect ($P < 0.01$) was observed with respect to all the four extracts as compared to their respective alcohol blanks. The inhibitory activity with respect to spoiled mutton micro-flora was in the order of clove> garlic> green tea> ginger.

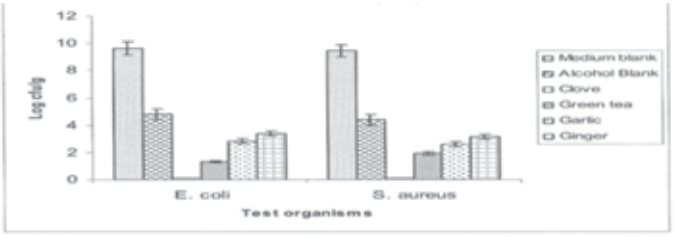
Fig.2: Enumeration test for the inhibitory effect of ethanolic extracts from spices and herbs on spoiled mutton micro-flora (SPC 8.91 log cfu/g). (n=8)



The proportions of different organisms in the fresh and spoiled micro-flora on the carcasses held at ambient temperature have been reported earlier (Rao and Murthy 1985). The authors reported S*taphylococcus* 48%, *Micrococcus* 19 %, *Acenitobactar* - like 4 %, *Pseudomonas* 3 %, *Escherichia* 12 %, *Enterobacter* 6 %, *Serratia* 1 %, *Flavobacterium* 1 % and *Brochotrix* 3 % in fresh mutton. When the mutton was spoiled, the counts of *Escherichia* increased to 28 % and that of *Enterobacter* 16%, *Acenitobacter-like* 22%, *Staphylococcus* 18% and *Pseudomonas* 16%. These findings suggest that spoilage of meat at ambient temperature (28±2 °C) is mesophilic in nature. The idea of using spoiled meat flora in the present investigation was to determine as to what extent these spice/herbs would be of help in reducing such a heavy microbial load of heterogeneous nature, if the meat was contaminated accidentally before it

could cause any deteriorative sensory changes. The quantitative inhibitory effect of extracts from various spices and herbs on *E. coli* (9.63 log cfu/g) and *S. aureus* (9.44 log cfu/g) has been depicted in Fig 3. One ml of alcohol (alcohol blank) was able to cause a reduction by nearly 5 log numbers both with respect to *E. coli* and *S. aureus*. While clove extract was able to inhibit both *E. coli* and *S. aureus* completely, green tea and garlic extracts caused nearly 8 and 7 log decrease respectively. Ginger extract had comparatively lesser inhibitory (6 log reduction) effect. The inhibitory effect was significant ($p<0.01$) as compared to alcohol blanks in case of clove, green tea and garlic extracts. The level of inhibition was in the order of clove > green tea> garlic > ginger. The inhibitory activity of clove is due to the presence of eugenol, an important constituent of the essential oil of clove (Deans and Ritchie 1987). Most of the published literature reports the inhibitory properties of essential oil from clove on the individual cultures. But in the present study, the whole powder as well as the ethanolic extract from clove was found to be very effective in inhibiting both the pathogens as well as the heterogeneous mutton microflora.

Fig.3. Enumeration test for the inhibitory effect of extracts from spices and herbs on *E. coli* and *S. aureus*. (n=8)



Knobloch *et al.*(1989) indicated that the essential oils act on cell proteins embedded in the cytoplasmic membrane. The inhibitory activity could be due to the action of essential oils on the enzymes involved in the energy regulation or synthesis of structural components (Connor and Beuchat 1984). The hydro-phobicity of essential oils enables these to cause partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable (Sikkeme *et al.* 1994).

The most important compound (polyphenols) in tea leaves is flavanols i.e. catechins. The inhibitory effect of green tea polyphenols against several food borne bacteria has been reported (Bong-Jeun An *et al.* 2004). The polyphenols present in tea seems to inhibit the growth of *E. coli* and *S. aureus* (Kim *et al.* 1998; Kong 1995). The anti-microbial effect of garlic is due to the presence of organo-sulphur compounds like diallyl shipside, diallyl disulphide etc. (Mei-Chin Yin and Wen-Shen Cheng 2003). Allicin, the most important constituent of garlic has boon reported to possess pronounced inhibitory activity against a range of bacteria and fungi (Beuchat 1994). The inhibitory effect of garlic powder observed in the present study could also be due to the presence of organo-sulphur compounds. These compounds readily permeate through phosphor-lipid membrane and are thought to act by reacting with critical thiol groups in the cell, affecting several physiological processes including respiration and RNA synthesis (Miran *et al.* 2000).

Ginger, a pungent spice used in Indian culinary and as a medicine, has gingerone and gingerol as active components, which are known to possess anti-oxidant, anti-microbial and sporo-static activities (Kenji Hirasa and Mitsuo Takemaso 1998; A1-Khayat and Blank 1985). The antimicrobial activity of ginger and garlic against gram negative bacteria and yeasts has been reported by Won-Doe-Ji *et al*. (1997). In the present study the extent of inhibition by ginger was higher than garlic in case of fresh mutton micro-flora. But with respect to spoiled mutton flora and pathogens, ginger was less effective than other spices tested, at the levels used.

The spices and herbs used in the present investigation are basically a part of the culinary practices and hence they generally pose neither any health hazards nor regulatory and sensory constraints for their usage to extend the shelf life of fresh mutton since they are traditionally a part of the curry preparations and are not preservatives per se. From the present study, it was observed that ethanolic extracts from spices/herbs seem to exhibit higher inhibitory activity than the whole powder. Though alcohol itself is able to inhibit the micro-flora to certain levels, the cumulative inhibitory effect of alcohol and the spice is found to be greater.

CONCLUSION

It is concluded from the present investigation that the results of the visual semi-quantitative method were almost comparable with those from the enumeration method (quantitative) when ethanolic extracts from the spices/herb were used. This semi-quantitative method of evaluation can be adopted as a simple and reliable technique to assess the antimicrobial activity of spices. However, to accurately assess the exact level of inhibition, a more laborious enumeration method can be followed.

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