Antimicrobial Activity of Some Common Spices

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ABSTRACT

Objective: Antibiotic toxicity and multi drug resistant pathogens are the two greatest challenges that today's medical world has been facing. As a consequence of the haphazard use of antimicrobials, the spread of antimicrobial resistance is now a global issue. This study aimed to investigate antimicrobial activity of some common spices.

Methods: During the study period Five commonly used spices were collected from local market of Lagankhel, Lalitpur. The antimicrobial activity of selected naturally grown spices was done against two gram positive and three gram-negative pathogenic bacteria. The extracts of the spices were obtained by using absolute ethanol (99.9%) to carry out the antibacterial susceptibility assay using agar well diffusion method.

Results: The result of agar well diffusion method showed Clove and Cinnamon were found to possess relatively higher antimicrobial activities by preventing the growth of all 5 tested bacteria. Gram positive bacteria were found to be more sensitive to spices than Gram negative bacteria.

Conclusion: The finding of this study showed that extract of spices can be alternative to synthetic drugs to control infectious diseases.

Key words: Spices, absolute ethanol, dimethyl sulfoxide, antibacterial susceptibility assay, agar well diffusion method

INTRODUCTION

Spices have been used for many centuries by various cultures to enhance flavor and aroma of our foods as our ancestors have recognized the usage of spices in food preservation and in treatment of clinical ailments and there are several reports on development of antibiotic resistance in diverse bacterial pathogens (Gold and Moellering 1996). There is no particular definition of spices, mostly because they are derived from different parts of the plants, such as clove from flower bud, pepper from fruit, cinnamon from bark or ginger from rhizome. Spices and aromatic vegetable materials have long been used in food not only for their flavour and fragrance qualities and appetizing effects but also for their preservative and medicinal properties. Since the ancient times, they have been used for preventing food spoilage and deterioration and also for extending the shelf life of foods (Shan et al 2007).

The antioxidant and antimicrobial property of spices are very important to preserve the quality of food material and at the same time provide safety to consumer (Singh et al 2007).

The present study aimed to investigate the antibacterial properties of locally available spice extracts of *Zingiber officinale, Circuma longa, Piper nigrum, Syzygium aromaticum* and *Cinnamonum verum* using agar well diffusion method against some gram negative bacteria (*Escherichia coli, Pseudomonas* sps*, Salmonella* sps) and gram positive bacteria (*Staphylococcus aureus, Bacillus* sps). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased (Bernhoft 2010). The use of alternatives and natural sources of antimicrobials hence becomes important to tackle with the current situation.

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Present investigation was carried out to report antimicrobial activity of the extract of commonly used spices against microorganisms that can cause infectious disease.

METHODS

In this study five commonly used spices were randomly sampled from the local market of Lagankhel, Lalitpur. Clean and dirt free spices were dried in laminar flow biological safety cabinet. Among the wet spices, turmeric and ginger were skinned. They were oven dried at 55^0C.The spices extract was prepared by ethanol extraction method. The sticky mass of the extract was dissolved in an aliquot of 1ml of 10% v/v Dimethyl sulfoxide (DMSO). The phytochemical yield was estimated according to the method described by Prasani et al (2005).

Five microorganisms, two gram positive (*Staphylococcus aureus* and *Bacillus* sps) and three gram negative (*Pseudomonas* sps. *Escherichia coli* and *Salmonella* sps) were used for antimicrobial susceptibility test. All strains were hospital isolates preserved at Department of Microbiology, D.A.V. College. For bacterial growth nutrient agar and nutrient broth (Himedia Laboratories Ltd. Mumbai, India) were used. Muller Hinton agar (Himedia Laboratories Ltd. Mumbai, India) was used for sensitivity assay and Dimethylsulphoxide (DMSO) (HiMedia Laboratories Ltd. Mumbai, India) was used for solvent for tested extracts.

The crude extracts of plants were screened for its antimicrobial activity against the organisms by agar well diffusion method. The bacterial culture was taken from NA and inoculated in nutrient broth and incubated at 37°C for 3 hours and turbidity was compared with 0.5 McFarland standard dilutions having cell suspension of about $1.5x10^8$ CFU/ml. This was used to inoculate by carpet culture on the surface of MHA plates. The 6mm sterile cork borer was used to make wells. Sample extract of 50µl was used in 6mm well and DMSO as a negative control. The plates were then left for half an hour with the lid closed. Then the plates were incubated at 37°C for 24 hrs then observed for the zone of inhibition which is suggested by the clear area around the well (WHO 1991).

Determinations of MICs of the spice extracts were determined by well diffusion and agar dilution techniques and the concentrations of the extracts used were 0.25, 0.05, 0.1 and 0.2 mg/ml. The lowest concentration that did not permit any visible growth when compared with the control was considered as the minimum inhibitory concentration. MBC was determined for those extract which showed the antibacterial activities by two-fold dilution method.

RESULTS

There existed a difference in the percentage yield of the extracts obtained from different spices. Among the spices, the yield percentage of the spices was obtained from *Cinnamonum verum* (21%) and the lowest percentage of spice extract was obtained from *Piper nigrum* (12.85%) (Figure 1). Stalikas (2007) in his study found that extraction efficiency is affected by the chemical nature of phytochemicals, the extraction method used, sample particle size, the solvent used, as well as the presence of interfering substances.

Among the five spices, clove and cinnamon were found to be effective against tested bacteria (Table 1). It was found during the present investigation that the spice extract of *Syzygium aromaticum* was the most effective spice among the five chosen spices. It had the maximum zone of inhibition against *E. coli* (20.67mm). The result of *Syzygium aromaticum* supports the results obtained by Agaoglu (2006) and Agnihotri and Vaidya (1995). In case of MIC (Table 2) the spices performed, C4 (Clove) showed the lowest concentrations in which no growth was observed in comparison to all the other spices. The MIC ranged from 0.025 mg/ml to 0.2 mg/ml in case of ethanol extracts. Black Pepper (C3) showed turbidity in most of the concentrations observed and hence it was considered to be the least effective in inhibiting the microorganisms at low concentration of the extract.

The MBC test (Table 3) results were found to be comparable to the MIC test results. The MBC ranged from 0.025 mg/ml to 0.2 mg/ml in case of ethanol extracts. Clove showed highest bactericidal activity even at lowest concentrations being the most effective among all the spices. Black pepper didn't show bactericidal activity even athigherconcentrations used in the study and showed bacterial growth on the culture media.

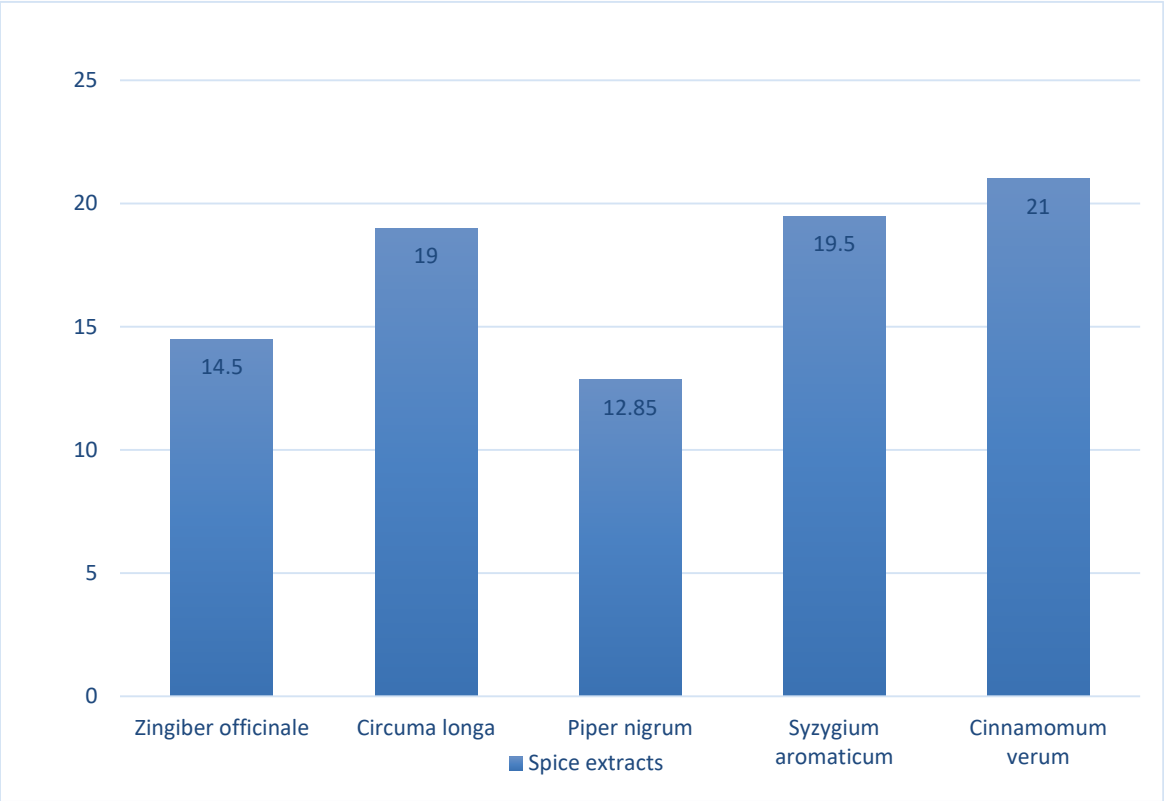

Figure 1: Percentage yield of extracts

Table 1: Antibacterial activity of spice extract against selected bacterial strains

Spices	**Minimum inhibitory concentration of spice extract (in mg/ml)**				
	E. coli	***S. aureus***	***Pseudomonas* sps**	***Salmonella* sps**	***Bacillus* sps**
Ginger	>0.2	0.2	0.2	>0.2	>0.2
Turmeric	0.05	0.025	0.2	0.1	0.05
Black pepper	>0.2	0.2	>0.2	>0.2	>0.2
Clove	0.05	0.025	0.05	0.05	0.025
Cinnamon	0.05	0.1	0.05	0.05	0.025

Table 2: Minimum inhibitory concentration of spice extracts

Spices	Zone of Inhibition(in mm)				
	E. coli	***S . aureus***	***Pseudomonas* sps**	***Salmonella* sps**	***Bacillus* sps**
Ginger	-	11.67	12	-	-
Turmeric	12	12	11.67	11.33	11.67
Black pepper	-	12.33	-	-	-
Clove	20.67	18.33	19.67	18.33	20
Cinnamon	13	12.67	15	11.33	11

Table 3: Minimum bactericidal concentration of spice extracts

Spices	Minimum bactericidal concentration of spice extract (in mg/ml)				
	E. coli	***S . aureus***	***Pseudomonas* sps**	***Salmonella* sps**	***Bacillus* sps**
Ginger	>0.2	0.2	0.2	>0.2	>0.2
Turmeric	0.1	0.05	>0.2	0.1	0.05
Black pepper	>0.2	0.2	>0.2	>0.2	>0.2
Clove	0.05	0.025	0.1	0.05	0.025
Cinnamon	0.1	0.1	0.2	0.1	0.025

DISCUSSION

This study was carried out with the aim that spices possess antimicrobial agents. In this study, commonly used spices was taken and analysed for the phytochemicals. Among the five spices used for study Cinamon (*Cinnamonum verum*) was found to be the most efficient. For the study the ethanol extraction method was used which has the capacity to extract the secondary metabolites for the antimicrobial phytochemicals like flavonoids, polyphenyl, tannis, terpenoids and alkaloids. (Tiwari et al 2011)

Antimicrobial activity of the extract also tallies with the above results as in case of cinnamon showed highest microbial activity and its MIC value also less in compare to others.

From this it ws aobserved that if proper extraction was done we can extract the active phytochemicals from the spices and use it to treat the patients with infectious diseses.

CONCLUSION

The present study provides an important basis of antimicrobial activity of these spices extracts. This guided that pure form of extract is more effective agent and can be used as alternative for the treatment of infections associated with the studied microorganisms.

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