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Research Article

A Study of Antimicrobial Activity of Some Spices

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Abstract

The prominent threat that the medical world, today, is facing are Antibiotic toxicity and Multi-drug resistivity. In my present study of antimicrobial activity of spices, they (spices) have been investigated as an alternative to antibiotics in order to summon these dares. To determine the antimicrobial activity of some selected important naturally grown spices against gram positive and gram-negative pathogenic bacteria, the study was done. The extracts of the spices were obtained by using absolute ethanol to carry out the antibacterial susceptibility assay using agar well diffusion method. Of the different spices tested Clove and Cinnamon were found to possess relatively higher antimicrobial activities. Extract of Clove showed broad spectrum of inhibition against all tested bacteria while extract of Cinnamon inhibited 80%. The MBC value ranged from 0.025 to 0.2 mg/ml. The lowest MBC (Minimal Bactericidal Concentration) value was given by extract of Clove against *S. aureus* and *Bacillus* sps and by *Cinnamon* against *Bacillus* sps. Gram positive bacteria were found to be more sensitive to spices than Gram negative bacteria. The research conducted suggests that these spice extracts have instrumental antibacterial roles that can be employed to control and forbid bacterial growth.

Keywords: Spices; Absolute Ethanol; Dimethyl Sulfoxide; Antibacterial Susceptibility Assay; Agar Well Diffusion Method

Introduction

From decades spices are being harnessed in our foods as flavoring agent or as an additive to increase the taste and texture of food along with its lifespan. They are also being utilized in treatment of clinical ailments as per reports on development of antibiotic resistance in diversified pathogens. There has been no any peculiar definition of spices as they are derived from different parts of the plants, such as flower bud, fruit, bark or rhizome. These aromatic materials are in use in food from very long time not only for their zest and incense qualities and succulent effects but also for diverse properties of preserving foods from spoilage and medical values. Since the ancient times, they have been used for preventing food spoilage and deterioration and also for extending the shelf life of foods [1]. These spices have antimicrobial and antioxidant properties as well that provide consumers food safety.

This research work is 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). The resistivity of microorganisms against the drugs have been escalating instead of development of number of numerous new antibiotics in Pharmacological Industries. The use of alternatives and natural sources of antimicrobials hence becomes important to tackle with the current situation.

Materials and Methods

The research work was carried out in microbiological laboratory, Department of Microbiology, DAV College.

Test organisms used

A total of five microbes were used, among which two microorganisms were gram positive (*Staphylococcus aureus* and *Bacillus)* and three were gram negative (*Pseudomonas, Escherichia coli* and *Salmonella).*

Preparation and extraction of the powder of spices

The fresh spices were collected from the local market. Firstly, all the spices were cleaned in order to remove any dirt or debris by tap water, and later using sterile distilled water. Afterwards, they were dried in laminar flow biological safety cabinet. Among the wet spices, turmeric and ginger were scraped. After this, they were dried at 55 degree Celsius in oven and after complete drying, the wet as well as the dry spices were finely crushed into coarse powder using blender and were preserved at room temperature (RT) for future use. The spices were procured by using absolute ethyl alcohol (20gm fine powder mixed with 200ml ethanol and the mixture kept at 30° Celsius for 72 hours along with constant agitation and the extract obtained was then filtered through Whattman No. 1 filter papers. Then the filtrate was centrifuged at 4000rpm for 15minutes. The solution obtained was then vaporized at 50 degree Celsius down to get a sticky mass which was weighed and dissolved in an aliquot of 1ml of 10% v/v Dimethyl sulfoxide (DMSO).

Inoculum preparation for bacteria

The loop full of 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.

Assessment of antibacterial activities of extracts

Screening and assessment of antibacterial activity

The unpurified extracts of plants were screened for their antimicrobial activities against the organisms by agar well diffusion method. Into the prepared inoculums the sterile cotton swab was dipped and seeded all over the MHA plate by rotating through an angle of 60° after each swabbing finally the swab was passed round the edges of the agar surface and left to dry for few minutes at room temperature with lid closed. Later, wells were made in the inoculated plate by using sterile cork borer (no. 6), and labeled. 50µl of the working suspensions which were spices extract was dispensed in the respective wells through micropipette. The plates were then left for half an hour with the lid closed to diffuse. And then the plates were incubated at 37°C. After incubation of 24 hrs the plates were observed for the zone of inhibition which was suggested by the clear zone around the well.

Determination of Minimum Inhibitory concentration (MIC) and Minimal Bactericidal concentration (MBC)

The assurance of MICs of the spice extracts was done through 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 least concentration that inhibited any visible growth of microbes after an overnight incubation when compared with the control was considered as the minimum inhibitory concentration. MBC persisted for those extract that represented the germicidal activities by bifold dilution method.

Results, Discussion and Conclusion

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 minimal percentage of spice extract was obtained from *Piper nigrum* (12.85%). Stalikas [2] 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.

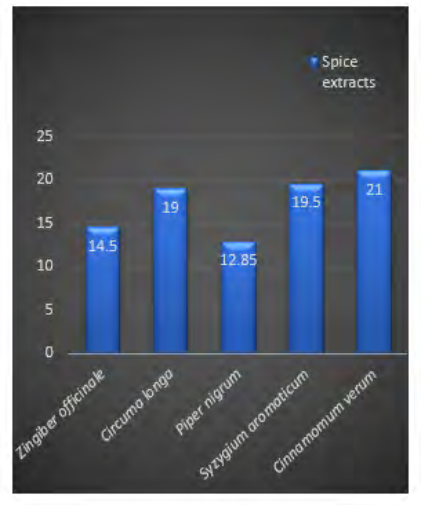

Figure 1: Percentage yield of extracts.

Table 1 summarises the antimicrobial activities of the ethanol extracts of spices.

Among the five spices, it was discovered that clove and cinnamon were potent at odds with the tested microbes. It was found during the present investigation that the spice extract of *Syzygium aromaticum* was the most effective spice among the five chosen one. It had the utmost zone of inhibition opposed to *E. coli* (20.67 mm). The result of *Syzygium aromaticum* supports the results obtained by Agaoglu [3] and Agnihotri and Vaidya [4]. *Syzygium aromaticum* was efficacious against both gram positive and gram-negative bacteria and was detected to have a broad spectrum.

The crude extract of *Zingiber officinale* was recognized to be effective against gram positive bacteria, *S. aureus, with* zone of inhibition 11.67 mm and gram-negative bacteria, *Pseudomonas* spp with zone of inhibition 12 mm. Similar finding was observed from the study by Karuppiah and Rajaram [5], who reported the highest zone of inhibition exhibited by spice extract of *Zingiber officinale* was against *Pseudomonas* spp (13 mm) and *S. aureus* (11 mm). *Circuma longa* (C2) was found to be effective against all the five organism chosen with highest zone of inhibition against *E. coli* and *S. aureus* (12 mm). Chandrana., *et al*. [6] who studied antimicrobial activity of *Circuma longa* reported that it was effective against *E. coli* and *S. aureus* and suggested that the activity is due to presence of curcuminoid, a phenolic compound. The maximum zone of inhibition shown by spice extract was against *S. aureus* (12.33 mm). *Piper nigrum* showed no zone of inhibition against *E. coli, Pseudomonas* spp*, Salmonella* spp and *Bacillus* spp. According to Singh., *et al.* [7] and Pradhan., *et al.* [8], *Piper nigrum* was the spice that is discovered to be effectual against *S. aureus* which completely tally with the result of present study. According to the study carried out by Maharajan., *et al.* [9], *Cinnamonum verum* was shown to be broad spectrum inhibiting all the bacteria similar to the present study.

Combined antibacterial spices are preferred as microbial tolerance is less likely to develop against substances having more than one type of modes of action [10]. It was the necessary to check the antibacterial activity of the spices in combinations. The study was progressed to witness the synergistic effect of commonly used spices. The highest zone of inhibition was shown by the combination of *Syzygium aromaticum* and *Cinnamonum verum* against *Bacillus* spp (20 mm) and the least zone of inhibition was shown by the combination of *Zingiber officinale* and *Circuma longa* against *Pseudomonas* spp and *Salmonella* spp (8 mm), also the combination of *Zingiber officinale* and *Cinnamonum verum* showed the least zone against *Pseudomonas* spp (8mm).

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

Organism	Diameter of zone of inhibition (in mm)					
	G+T	**G+C**	**G+Ci**	**T+Ci**	**T+C**	**C+Ci**
E. coli	12	14	14	14	13	17
S. aureus	12	18	13	12	12	17
Pseudomonas sps	8	16	8	13	12	19
Salmonella sps	8	9	14	15	12	11
Bacillus sps	11	17	13	14	16	20

Table 2: Synergistic effect of commonly used spices against common pathogens.

Among the MIC of the spices performed, C4 (Clove) exhibited the minimum concentrations in which no growth was observed in comparison to all the other spices. The MIC arrayed 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.

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 3: Minimum Inhibitory Concentration of spice extracts.

The MBC test results were found to be comparable to the MIC test results. The MBC encompassed 0.025 mg/ml to 0.2 mg/ml in case of ethanol extracts. Clove showed highest bactericidal activity even at minutest concentrations being the most effective among all the spices. Black pepper didn't show bactericidal activity even at higher concentrations used in the study and showed bacterial growth on the culture media [11-13].

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

Table 4: Minimum Bactericidal Concentration of spice extracts.

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