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Antibacterial Activity of Acetone and Ethanol Extracts of Cinnamon (*Cinnamomum zeylanicum*) and Ajowan (*Trachyspermum ammi*) on four Food Spoilage Bacteria

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Abstract

The in vitro antibacterial activities of two spices cinnamon bark (Cinnamonum zeylanicum) and Ajowan fruits (Trachyspermum ammi) ethanol and acetone extracts has been evaluated against two gram negative food spoilage bacteria Pseudomonas sp., Escherichia coli and two gram positive bacteria Bacillus subtilis and Staphylococcus aureus. The in vitro antibacterial activity was performed by disc diffusion method. Ethanol extract of cinnamon and ajowan revealed an antibacterial activity against Pseudomonas sp., whereas acetone extract of spices exhibited highest activity against Escherichia coli. Acetone extract of cinnamon and ajowan showed no activity against Staphylococcus aureus and Bacillus subtilis. The results obtained in the present study suggest that the ethanol extract of cinnamonum zeylanicum and trachyspermum ammi revealed a significant scope to develop a novel broad spectrum of antibacterial herbal formulation and can be used for cooked food preservation.

Keywords: Cinnamon, c*innamomum zeylanicum,* ajowan, *trachyspermum ammi,* crude extracts, pathogenic bacteria.

Introduction

Natural preservatives are the chemical agents derived from plants that prevent the decomposition of products by any means[1]. The mode of action of these natural preservatives is inhibition of microbial growth, oxidation and certain enzymatic reactions occurring in the food stuffs. Spices offer a promising alternative for food safety[2]. This study limelight naturally derived constituents of spice extracts contained high levels of phenolics and exhibited antibacterial activity against food borne pathogens. Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects[3]. Food conservation for nutrition and superior shelf life can be obtained by controlling the growth of food borne pathogenic microorganisms and food spoilage. This could be achieved by suppressing one or more factors that are essential for microbial survival[4]. Suppression might be possible by adding suitable chemical substances and by controlling physical factors for the growth of microbes[5, 6].

Material and Methods

Plant Material: The spice cinnamon bark (*Cinnamomum zeylanicum*) and ajowan fruits (*Trachyspermum ammi*) were purchased from local market of Indore city, Madhya Pradesh of India in September 2011. The spices were botanically identified.

Preparation of Extract: The spices were cleaned with de-ionized water and dried first in sunlight for two days and then in an oven at 40^0C for about 24 hours. Finally the dried materials were pulverized into fine powdered substance by a grinder. 20 grams of powder of cinnamon was weighed with the electric balance and transferred into two separate 100 ml conical flasks. Then 40ml of ethanol in one flask and 40 ml of acetone in another was added. The conical flasks were closed by foil paper and put on dark place for maximum seven days. The crude ethanol and acetone extracts were then filter by passing the extracts through Whatman No.1 filter paper and then concentrated under vacuum at 40^0C by using a rotary evaporator. The powder of Ajowan fruits was also extracted separately with ethanol and acetone. The residual extracts were stored in refrigerator at 4^0C in small and sterile plastic bottles.

Tested Bacteria: Antibacterial activity of spices powder extracts was investigated against two gram negative bacteria *Escherichia coli* and *Pseudomonas Sp.*, and two gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* which were obtained from the Microbiology department of Holkar Science College, Indore, India. The tested bacteria were cultured on Nutrient Agar (Himedia, Mumbai) at 37^0C for 24 hrs. The cultures were sub-cultured regularly (every 30 days) and stored at 4^0C.

Inoculums Preparation: 10 ml of distilled water was taken into the screw cap tube and pure colony of freshly cultured bacteria was added into the tube and vortex was done. The OD (optical density) was measured with the colorimeter and microbial population was confirmed to be within in 10^7ml^{-1} to 10^8ml^{-1}. This suspension was used as inoculums.

Antimicrobial Bioassay: The *in vitro* antibacterial activities of the test samples were carried out by disc diffusion method[7]. In the disc diffusion method, nutrient agar (Himedia, Mumbai) was used as culture media and the discs were placed aseptically over the bacterial culture on nutrient agar plates and incubated at 37^0C for 24 hrs. After incubation for 24 hrs, the zone of inhibition around the discs was measured by millimeter scale. Discs were impregnated with each treatment and control was assayed on duplicate agar medium plate for *Pseudomonas sp., Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus.* The diameter of zone of inhibition mean of two replicates ± SD as indicated by clear area which was devoid of growth of microbes was measured to determine antibacterial activity. The experiment was replicated two times to confirm the reproducible results. Sterile blank paper discs impregnated with only sterile ethanol and acetone served as negative control each time. Standard erythromycins (15µg/disc) and ciprofloxacin (5µg/disc) were used as positive control for comparison of the antibacterial activity. Minimum inhibitory concentration (MIC) value of the extracts of the cinnamon and ajowan was determined in present study following the serial dilution technique according to Reiner[8].

Statistical Evaluation: The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of duplicates ±SD of two replicates.

Results and Discussion

Antimicrobial Activities: Ethanol extract of cinnamon were found sensitive to *Pseudomonas sp*., *E. coli, Bacillus subtilis* and *Staphylococcus aureus.* Crude ethanol extract produced zone of inhibition 11 mm and 9 mm against *Pseudomonas sp.*, and *Bacillus subtilis* respectively. Crude ethanol extract produced zone of inhibition of 8 mm and 7 mm against *Staphylococcus aureus*, and *E. coli* respectively (table-1). However, it exhibited highest zone of inhibition (11 mm) against *Pseudomonas sp*. The MIC value was also determined against all the tested bacteria. The MIC values of ethanol extract were found to be 64µg/ml against *Bacillus subtilis*, *Staphylococcus aureus* and *E. coli*. Against *Pseudomonas Sp*., the MIC values were found 32µg/ml (table-2). Negative control (disc containing only ethanol) showed no zone against any bacteria. All the positive controls showed antibacterial activity against tested bacteria.

Acetone extract of cinnamon produced zone of inhibition against *Pseudomonas sp.*, *E. coli*, *Bacillus subtilis. Staphylococcus aureus* was not sensitive to acetone extract of ajowan. Crude acetone extract produced zone of inhibition 8 mm against both *Pseudomonas sp.* and *Bacillus subtilis.* Crude acetone extract produced highest zone of inhibition 9mm against *E. coli* (table-3). The MIC value was also determined against the sensitive bacteria *Pseudomonas sp.*, *E. coli* and *Bacillus subtilis.* The MIC values of acetone extract were found to be 64µg/ml against both *Pseudomonas sp.*, and *Bacillus subtilis.* Against *E. coli,* the MIC values were found 16µg/ml (table-4). Negative control (disc containing only acetone) showed no zone against any bacteria. All the positive controls showed antibacterial activity against tested bacteria (table-3).

Ethanol extract of ajowan was found sensitive to *Pseudomonas sp., E. coli*, *Bacillus subtilis* and *Staphylococcus aureus.* Crude ethanol extract of ajowan produced zone of inhibition 7 mm against both *Bacillus subtilis* and *Staphylococcus aureus.* Crude ethanol extract produced zone of inhibition of 8mm and 9mm against *E. coli* and *Pseudomonas sp.* respectively (table-5). However it exhibited highest zone of inhibition (9 mm) against *Pseudomonas sp*. The MIC value was also determined against all the selected bacteria. The MIC values of ethanol extract were found to be 64µg/ml against both *Pseudomonas sp.,* and *Bacillus subtilis*. Against both *E. coli* and *Staphylococcus aureus,* the MIC values were found 32µg/ml (table-6). Negative control (disc containing only ethanol) showed no zone against any bacteria. All the positive control showed antibacterial activity against tested bacteria (table-5).

Acetone extract of ajowan produced zone of inhibition against *Pseudomonas sp., E. coli* and *Bacillus subtilis. Staphylococcus aureus* was not sensitive to acetone extract of ajowan. Crude acetone extract of ajowan produced zone of inhibition 7mm and 8mm against *Pseudomonas sp.* and *Bacillus subtilis* respectively. However, it exhibited highest zone of inhibition (9mm) against *E. coli* (table-7). The MIC value was also determined against the sensitive bacteria *Pseudomonas sp., E. coli* and *Bacillus subtilis.* The MIC values of acetone extract were found to be 128µg/ml against both *Pseudomonas sp.,* and *Bacillus subtilis.* Against *E. coli,* the MIC values were found 64µg/ml (table-8). Negative control (disc containing only acetone) showed no zone against any bacteria. All the positive control showed antibacterial activity against tested bacteria (table-7).

Table-1
Activity of crude ethanol extract of Cinnamon on *Pseudomonas sp., E. coli*, *B. subtilis* and *S. aureus*

Bacteria	DIZ	Negative control	Positive control; mm	
			CIP(5µg/ml)	E(15µg/ml)
Pseudomonas sp.	11	+	11	22
B. subtilis	9+0.50	+	9	20
S. aureus	8+0.25	+	17	16
E. coli	7+0.23	+	11	22

`*DIZ*' Diameter of zone of inhibition in mm (mean ± SD), '+'= No zone formation

Table-2
Comparison of minimum inhibitory concentration (MIC) values of ethanol extract of Cinnamon against *Pseudomonas sp*., *E. coli*, *B. subtilis* and *S. aureus*

Bacteria	Ethanol extract of Cinnamon (µg/ml)									
	512	**256**	**128**	**64**	**32**	**16**	**8**	**4**	**2**	**0**
Pseudomonas sp.	-	-	-	-	-	+	+	+	+	+
B. subtilis	-	-	-	-	+	+	+	+	+	+
S. aureus	-	-	-	-	+	+	+	+	+	+
E. coli	-	-	-	-	+	+	+	+	+	+

'+' = No zone formation, '-' =Formation of inhibition zone

Table-3
Activity of crude acetone extract of Cinnamon on *Pseudomonas sp., B. subtilis, S. aureus,* and *E. coli*

Bacteria	DIZ	Negative control	Positive control; mm	
			CIP(5µg/ml)	E(15µg/ml)
Pseudomonas sp.	8+0.62	+	23	13
B. subtilis	8+0.34	+	18	10
S. aureus	+	+	18	15
E. coli	9+0.23	+	18	20

'*DIZ*' Diameter of zone of inhibition in mm (mean ± SD), '+'= No zone formation

Table-4
Comparison of minimum inhibitory concentration (MIC) values of acetone extract of Cinnamon against *Pseudomonas sp., E. coli,* and *B. Subtilis*

Bacteria	Acetone extract of Cinnamon (µg/ml)									
	512	**256**	**128**	**64**	**32**	**16**	**8**	**4**	**2**	**0**
Pseudomonas sp.	-	-	-	-	+	+	+	+	+	+
B. subtilis	-	-	-	-	+	+	+	+	+	+
E. coli	-	-	-	-	-	-	+	+	+	+

'+'= No zone formation, '-' = Formation of inhibition zone

Table-5
Activity of crude ethanol extract of Ajowan on *Pseudomonas sp., E. coli*, *B. subtilis* and *S. aureus*

Bacteria	DIZ	Negative control	Positive control; mm	
			CIP(5µg/ml)	E(15µg/ml)
Pseudomonas sp.	9+0.71	+	10	20
B. subtilis	7+0.25	+	8	18
S. aureus	7+0.5	+	18	15
E. coli	8+0.62	+	10	20

'*DIZ*' Diameter of zone of inhibition in mm (mean ± SD), '+'= No zone formation

Table-6
Comparison of minimum inhibitory concentration (MIC) values of ethanol extract of Ajowan against *Pseudomonas sp., E. coli*, *B. subtilis* and *S. aureus*

Bacteria	Ethanol extract of Ajowan (µg/ml)									
	512	**256**	**128**	**64**	**32**	**16**	**8**	**4**	**2**	**0**
Pseudomonas sp.	-	-	-	-	+	+	+	+	+	+
B. subtilis	-	-	-	-	+	+	+	+	+	+
S. aureus	-	-	-	-	-	+	+	+	+	+
E. coli	-	-	-	-	-	+	+	+	+	+

'+'= No zone formation, '-' =Formation of inhibition zone

Table-7
Activity of crude acetone extract of Ajowan on *Pseudomonas sp., B. subtilis*, *S. aureus* and *E. coli*.

Bacteria	DIZ	Negative control	Positive control; mm	
			CIP(5µg/ml)	E(15µg/ml)
Pseudomonas sp.	7+0.34	+	23	13
B. subtilis	8+0.21	+	18	10
S. aureus	+	+	18	15
E. coli	9+0.64	+	18	20

`*DIZ*' Diameter of zone of inhibition in mm (mean ± SD), '+' = No zone formation

Table-8
Comparison of minimum inhibitory concentration (MIC) values of acetone extract of Ajowan against *Pseudomonas sp., E. coli, B. subtilis* and *S. aureus*

Bacteria	Ethanol extract of Ajowan (µg/ml)									
	512	256	128	64	32	16	8	4	2	0
Pseudomonas sp.	-	-	-	+	+	+	+	+	+	+
B. subtilis	-	-	-	+	+	+	+	+	+	+
E. coli	-	-	-	-	+	+	+	+	+	+

'+' No zone formation, '-' = Formation of inhibition zone

Thymol, the major phenolic compound present in Ajowan, has been reported to be a germicide, antispasmodic, and antifungal agent[9]. Micro-Organisms are the concealed enemies to the mankind. They are small but cause a very profound damage in human body as well as other living organisms. The agents, which have the capacity to kill the microbes or arrest the multiplication, are called the antimicrobial agents. There are a lot of antimicrobial agents of which some are discovered or established and some are hidden in the nature. Hence, the last decade witnessed an increase in the investigations on plants as a source of human disease management and more natural antimicrobials have driven scientists to investigate the effectiveness of inhibitory compounds such as extracts from plants. Several studies have been focused on the application of individual EOs derived from plants. Some studies showed whole EOs have more antimicrobial activity compared to the mixture of major components[10]. However, information on the effects of these natural compounds in combination and or as crude extracts against food-borne micro-organisms is limited[11, 12]. Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens and can be used as preservative of cooked food.

Researchers in different parts of the world have studied the antimicrobial activities of indigenous herbs and spices for over a century. Zaika[13] has reviewed the antimicrobial effectiveness of spices and herbs. Recent results of one Indian study[14] indicated that cinnamon have potent antimicrobial activities against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas sp.*

In the present work, the antibiotic potential of the ethanol and acetone extracts of the cinnamon and ajowan has been determined against, *Pseudomonas sp., E. coli, Bacillus subtilis* and *Staphylococcus aureus.* In this study, crude ethanol extracts of cinnamon and ajowan are found to be effective in inhibiting the growth of *Pseudomonas sp., Bacillus subtilis* and *Staphylococcus aureus and E. coli.* On the other hand, crude acetone extract of cinnamon and ajowan showed antimicrobial activities against *Pseudomonas sp., Bacillus subtilis* and *Escherichia coli.* Acetone extract of cinnamon and ajowan showed highest inhibitory activity against *Escherichia coli* whereas ethanol extract of two spices showed highest activity against *Pseudomonas sp.* The extracts of cinnamon and ajowan have been reported to possess antibacterial activity. Blank disc produced no zone of inhibition of *Pseudomonas sp., Escherichia coli,* indicating that the solvents ethanol and acetone did not possess any antimicrobial effect on the pathogen. MIC value was also determined against all bacteria. The MIC values against these organisms were between 16-128 µg/ml.

Conclusion

The extracts of *Cinnamomum zeylanicum* and *Trachyspermum ammi* L. were found to be effective antibacterial agents against human pathogens. This study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect. To conclude we can use studied spices extract for cooked food preservation to extend shelf life of food and to save economy.

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