INDIAN SPICES AND ITS ANTIFUNGAL ACTIVITY

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Abstract

Antibiotic toxicity and multi drug resistant pathogens are the two greatest challenges being faced by today's medical world. In the present study, the antimicrobial activity of spices has been investigated as an alternative to antibiotics in order to tackle these dangers. In search of bioactive compound, methanol and acetone extract of 5 Indian spices were screened for antibacterial property. The choice of spice as an alternative is based on two basic reasons: firstly, plants have been the model source of medicine since ancient times and secondly, the increasing acceptance of herbal medicines by general population methanolic and acetone extracts were used to determine antifungal properties of the spices. The antifungal activity of five common Indian spices namely clove, ajwain, turmeric, dalchini and black pepper against two bacteria Aspergillus niger and Trichoderma sp. The results revealed that the methanol extracts of spices (MIC values of 20- 100 µl/ml) have high antimicrobial activities on all test organisms (range of inhibition, 6- 16 mm) as compare to acetone extracts of spices in same concentration. Results concluded that these spices contain high amount of secondary metabolites due to these metabolites they have high antimicrobial activity and it can be used as good bio- preservater and it can also use for medicinal purpose.

Keywords: *Antibacterial Properties, Secondary Metabolites, Multi Drug Resistant Pathogens.*

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1. INTRODUCTION

Plants have been a valuable source of natural products for a long period of time to maintain human health, especially with more intensive studies in the last decade for natural therapies (Gislene *et al.*, 2000). Spices have been used for not only flavor and aroma of the foods but also to provide antimicrobial properties (Nanasombat *et al.*,2002). Spices may contribute piquancy of foods and beverages (Praveen *et al.*, 2006). In addition to these spices are some of the most commonly used natural antimicrobial agents in foods. Some of the natural compounds found in various spices possess antimicrobial. (Hatha *et al.*, 2006). Therefore, actions must be taken to control this problem by using the plant extracts containing phytochemical having antimicrobial properties. (Agaoglu *et al.*, 2007). Keeping in view this fact the present study was conducted to find out the antimicrobial activity of five spices including Clove (*Eugenia caryophyllus*, family Mytraceae), Cinnamon (*Cinnamomum zylancium*, family Lauraceae), Black pepper (*Piper nigrum* L. family Piperaceae) Turmeric (*Curcuma longa* family Lauraceae,) and Ajwain (Trachyspermum ammi, family Apiaceae) against pathogenic fungal spices have been recognized for their value of preserving foods and medicinal values due to the presence of bioactive antimicrobial compounds. (Shelef, 1983, Papp *et al.*, 2007). Ethno pharmacological studies on spices its anti oxidant, anti-inflammatory (Hirasa *et al.*, 1998).

2. MATERIALS & METHODS

The spices namely cinnamon (*Cinnamomum zeylanicum*), black pepper (*Pepper nigrum*), clove (*Syzygium aromaticum*), turmeric (*Curcuma domestica*), ajwain (*Trichospermum ammi)* were used for the present study collected from the local market.

2.1 Preparation of Spice Extract

Extract of each spice was prepared by 30g of dry spice in 300ml acetone and methanol for 48hrs at room temperature for spice extract preparation.

2.2 The Microorganism

Two microbial strains were selected for the experiment on the basis of their pathogenic activity in human being. *Aspergillus niger* and *Trichoderma* sp.

2.3 Preparation of Bacterial Culture:

The stock culture of each of the bacteria used was sub cultured at 37^0C for 24 hours.

2.4 Assay for Antimicrobial Activity:

Antimicrobials are agents that kill microorganisms or inhibit their growth. The antimicrobial effects of the plant extracts are sufficient in a way to cater the healing effect. In vitro fungus activities of different plant extract were tested *Aspergillus*

niger and *Trichoderma* sp. 0.2 ml of overnight grown cultures of each organism was dispensed into 20 ml of sterile potato dextrose broth (PDB) and incubated for 48 hrs at 27°C.

2.5 Minimum Inhibitory Concentration (MIC)

2.5.1 Antifungal Activity:

The PDA broth was prepared. Each fungal strain was sub culture in separate broth and named it. All the inoculants broth were then incubate at 26-28^{0}C for 72hrs.Potato Dextrose Agar medium were prepared for performing antifungal tests against all the fungal strain. Agar well diffusion assay method was used for antifungal activity of methanolic and acetone extract of spices. The activity was determined after 72 hrs of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

3. RESULTS AND OBSERVATIONS

In present studies antifungal activity of five spices clove, ajwain, cinnamon, turmeric and black pepper were done. **Table -1a, 1b, Graph -1a, 1b, 2a and 2b and fig -1 and2 ,** shown the antimicrobial activity of spices extracted in acetone against *Aspergillus niger*, in turmeric extract maximum zone of inhibition is 12mm at 100(µl/ml) and cinnamon showed maximum zone of inhibition is 11mm at 250(µl/ml) concentration. Similarly the antimicrobial activity of spices extracted in methanol against *Aspergillus niger*, in ajwain extract, maximum zone of inhibition is 15mm in concentration 100(µl/ml) and cinnamon shown maximum zone of inhibition is 12mm at 250(µl/ml) concentration.

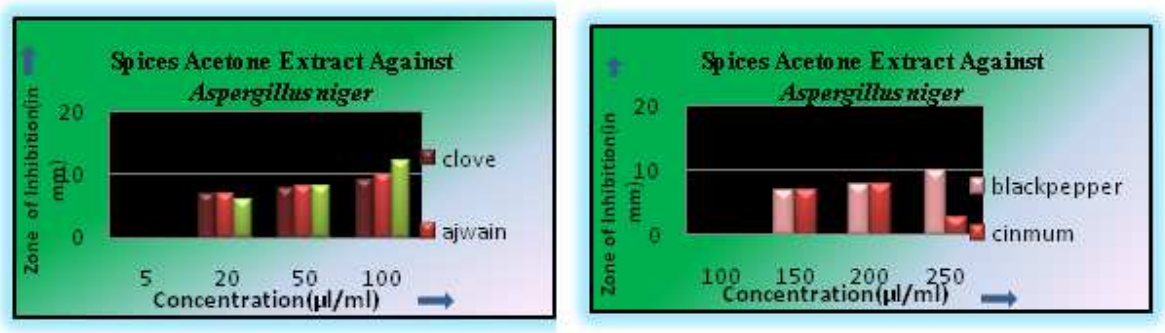
Table-2a, 2b graph-3a, 3b,4a and 4b and fig- 3 and 4, shown the antimicrobial activity of spices extracted in acetone against *Trichoderma* sp**.,** in clove extract, maximum zone of inhibition is 17mm in concentration 100(µl/ml), and cinnamon maximum zone of inhibition is 12mm at 250(µl/ml) concentration and antimicrobial activity of spices extracted in methanol against *Trichoderma sp.* in ajwain extract, maximum zone of inhibition is 16mm in concentration 100(µl/ml), and cinnamon maximum zone of inhibition is 14mm at 250(µl/ml) concentration.

Table 1 (A): Zone of inhibition of spice extracts against *Aspergillus niger* in well diffusion assay

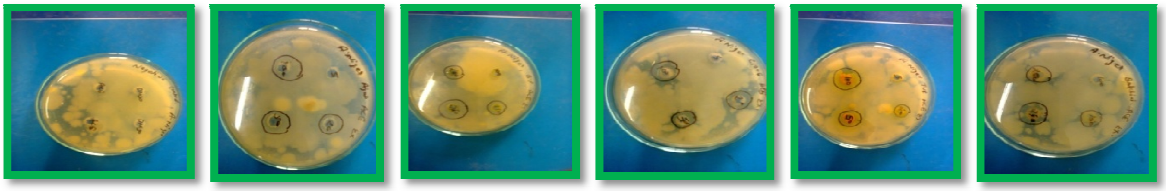
Plant Extract	**Dilution of Plant Extract.(µl/Ml)**								
	Acetone extract				**Methanol extract**				**Negetive Control**
Conc / Extract	5	20	50	100	5	20	50	100	
	Zone of Inhibition (Mm)								
Clove	-	7	8	9	-	7	10	13	-
Ajwain	-	7	8	10	6	7	9	15	-
Turmeric	-	6	8	12	7	8	9	10	-

Table 1 (B): Zone of inhibition of spice extracts against *Aspergillus niger* in well diffusion assay

Plant Extract	**Dilution of Plant Extract.(µl/Ml)**								
	Acetone extract				**Methanol extract**				**Negetive Control**
Conc / Extract	100	150	200 200	250	100	150	200	250	
	Zone of Inhibition (Mm)								
Blackpepper	-	7	8	10	6	7	8	9	-
Dalchini	-	7	8	11	7	10	11	12	-

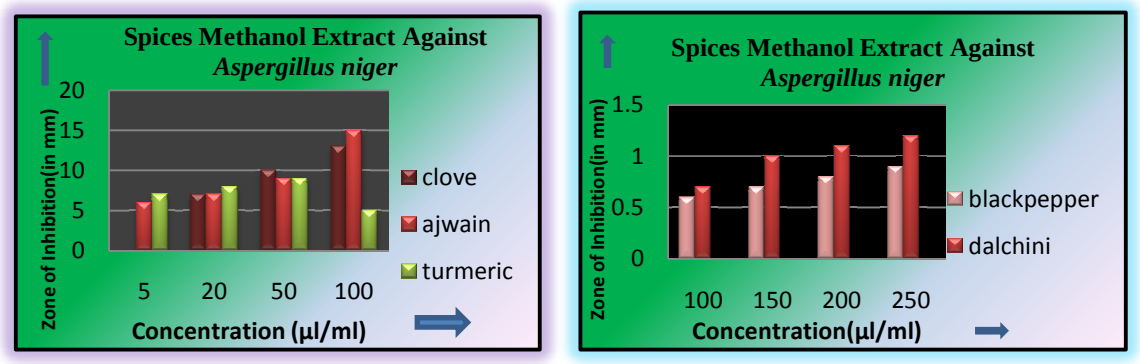


Graph 1(a) and (b): showing zone of inhibition (mm) of acetone extract of spices

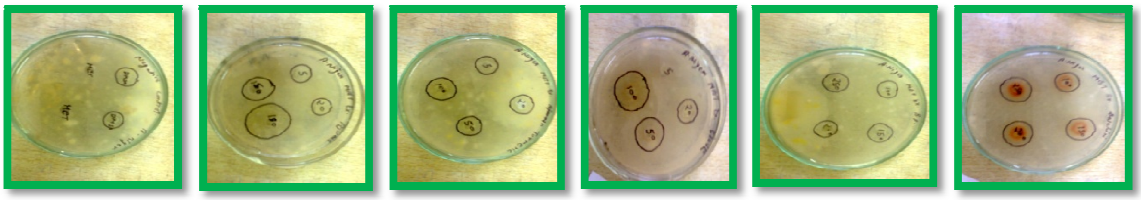


Control | **With ajwain Extract** | **With turmeric Extract** | **With clove Extract** | **With Black pepper Extract** | **With dalchini Extract**

Fig 1: Inhibition zone photographs of Gram-negative bacteria *Aspergillus niger* based on agar well diffusion assay for the various extracts of spices.



Graph 2 (a) and (b): showing zone of inhibition (mm) of methanol extract of spices at different concentration in µl/ml on the *Aspergillus niger*.



Control | **With ajwain Extract** | **With turmeric Extract** | **With clove Extract** | **With Black pepper Extract** | **With dalchini Extract**

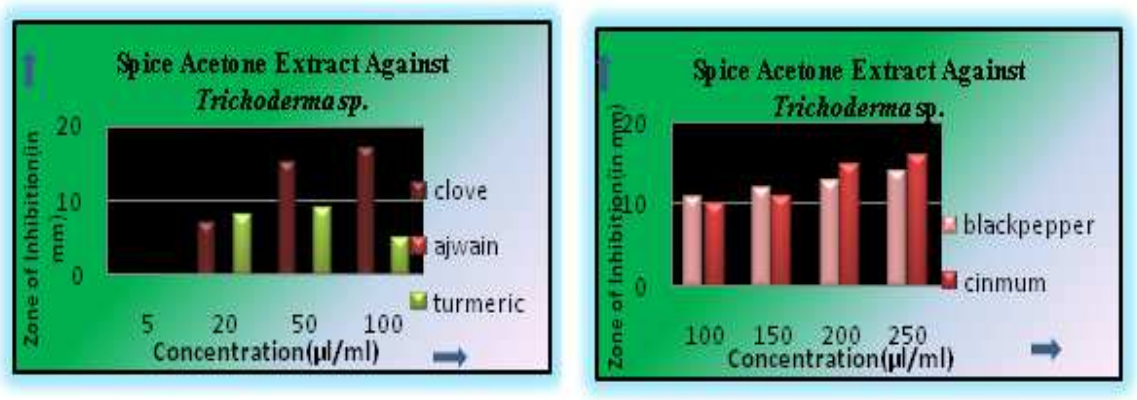
Fig 2: Inhibition zone of the *Aspergillus niger* based on agar well diffusion for the methanol extracts of spices.

Table 2 (A): Zone of inhibition of spice extracts against *Trichoderma* sp.

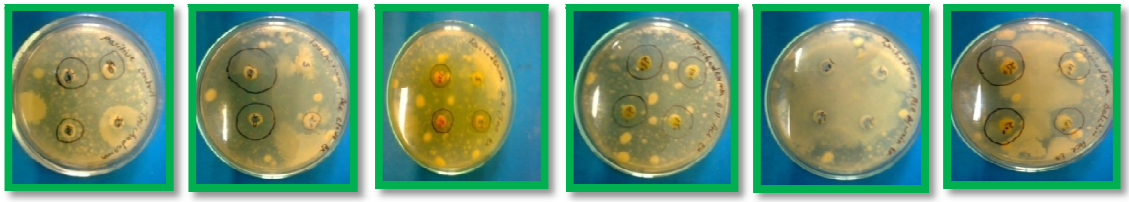
Plant Extract	**Dilution of Plant Extract.(µl/Ml)**								
	Acetone extract				**Methanol extract**				**Negetive Control**
Conc Extract	5	20	50	100	5	20	50	100	
	Zone of Inhibition (Mm)								
Clove	-	7	15	17	11	12	13	14	-
Ajwain	-	-	-	-	10	11	15	16	-
Turmeric	-	8	9	10	11	12	13	14	-

Table 2(B): Zone of inhibition of spice extracts against *Trichoderma* sp.

Plant Extract	**Dilution of Plant Extract.(µl/Ml)**								
	Acetone extract				**Methanol extract**				**Negetive Control**
Conc Extract	100	150	200	250	100	150	200	250	
	Zone of Inhibition (Mm)								
Blackpepper	-	-	5	9	-	6	7	8	-
Dalchini	7	10	11	12	10	12	13	14	-

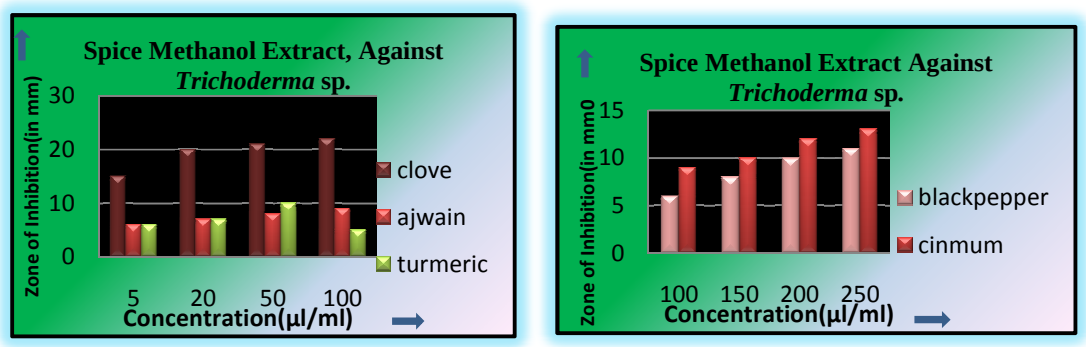


Graph 3(a) and (b): showing zone of inhibition (mm) of acetone extract of spices.

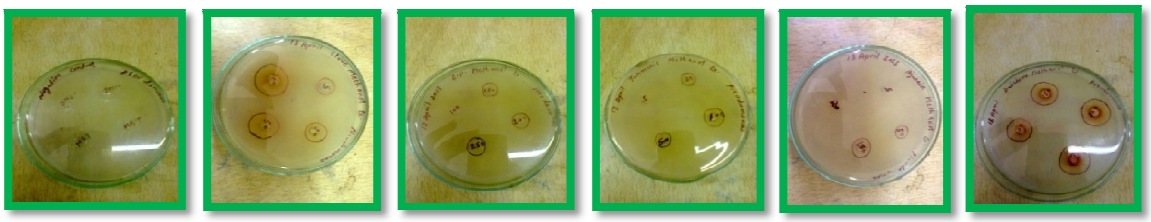


Control | **With clove Extract** | **With turmeric Extract** | **With black pepper Extract** | **With ajwain Extract** | **With dalchini Extract**

Fig 3: Inhibition zone photographs of bacteria *Trichoderma* sp. based on agar well diffusion assay for the acetone extracts of spices.



Graph 4(a) and (b): showing zone of inhibition (mm) of methanol extract of spices at different concentration.



Control | **With clove Extract** | **With turmeric Extract** | **With black pepper Extract** | **With ajwain Extract** | **With dalchini Extract**

Fig 4: Inhibition zone photographs of bacteria *Trichoderma* sp. based on agar well diffusion assay for the methanol extracts of spices.

4. DISCUSSION

The data supports the hypothesis that some common Indian spices have an inhibitory effect on the growth of certain food borne pathogens in tissue culture. The results suggest that turmeric, clove, pepper and ajwain and dalchini powder, they produced significant antimicrobial effects. In the antimicrobial study of spices , antimicrobial activity against Aspergillus niger maximum activity was shown in methanol extract of ajwain, and minimum activity was shown in both acetone and methanol black pepper extract. Antimicrobial activity against Trichoderma sp. maximum activity was shown in methanol extract of ajwain, and minimum activity was shown in both acetone and methanol extract of turmeric and black pepper.
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. Shamsuddeen et al., (2009). 9 crude ethanolic extracts and 11 essential oils were selected to determine the minimum inhibitory concentrations (MICs) using micro broth dilution test. (Nanasombhand and Lohasupthawee, 2005). Previously investigated that extract and essential oil of clove (Syzygium aromaticum) as natural antibacterial agents. (Sabahat Saeed and Perveen Tariq 2008).
Present studies showed that methanol extracts of spices (clove, ajwain, turmeric, black pepper and dalchini) given high antifungal activity against different fungi (Aspergillus niger and Trichoderma sp) shown similarity with the previous results (Abhishekh. Seth, 2010).

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