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COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY OF INDIAN SPICES

BHAWANA PANDEY[a1] AND SHABINA KHAN[b]

[ab]Department of Microbiology & Biotechnology, Bhilai Mahila Mahavidyalaya, Bhilai, C.G., India

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 antibacterial properties of the spices. The antibacterial activity of five common Indian spices namely clove, ajwain, turmeric, dalchini and black pepper against two bacteria *Escherichia coli* and *Pseudomonas fluroscence*. 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

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 and Nazia, 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 bacteria.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 and Takemasa, 1998; Abhishek, 2011).

MATERIALS AND 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.

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. .

The Microorganism

Two microbial strains were selected for the experiment on the basis of their pathogenic activity in human being. *Escherichia coli* and *Pseudomonas florescence*.

Preparation of Bacterial Culture

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

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. The antimicrobial effect of spices extracts also helps to prevent diseases in many forms. In antimicrobial activity, Minimum inhibition concentrations were determined for each extract. The bacteriostatic and bactericidal effects were determined.

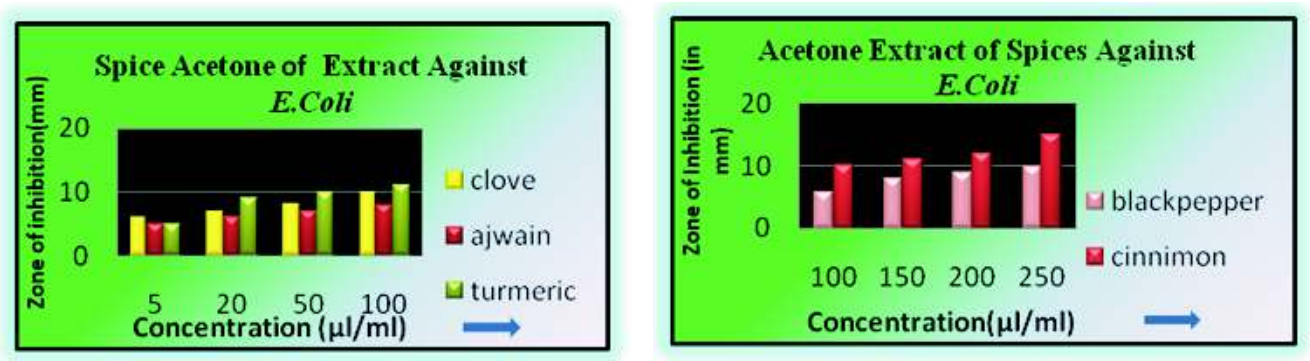
[1]**Corresponding author**

Table 1 (A): Zone of Inhibition of Spice Extracts Against *E.coli* in Well Diffusion Assay

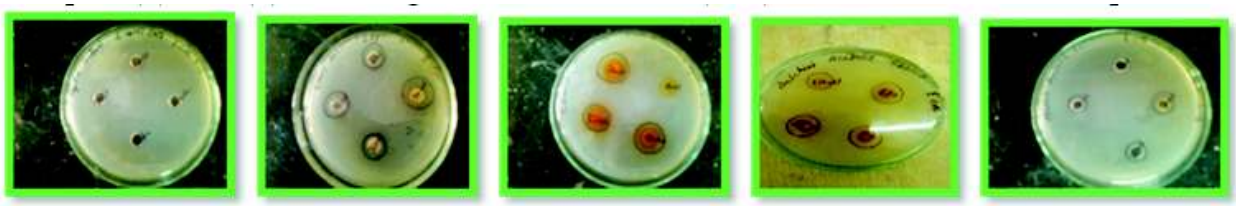
Plant Extract	Dilution of Plant Extract.(µl/Ml)								
	Acetone extract				Methanol extract				
Conc / Extract	5	20	50	100	5	20	50	100	Control
	Zone of Inhibition (mm)								
Clove	6	7	8	10	8	10	15	17	-
Ajwain	5	6	7	8	-	6	8	10	-
Turmeric	5	9	10	11	-	6	9	10	-

Table 1 (B): Zone of Inhibition of Spice Extracts Against *E.coli* in Well Diffusion Assay

Plant Extract	Dilution of Plant Extract.(µl/Ml)								
	Acetone extract				Methanol extract				
Conc / Extract	100	150	200	250	100	150	200	250	Control
	Zone of Inhibition (mm)								
Blackpepper	6	8	9	10	6	8	9	11	-
Dalchini	10	11	12	15	2	12	13	3	-



Graph 1(a) and (b): Showing Zone of Inhibition (mm) of Acetone Extract of Spices



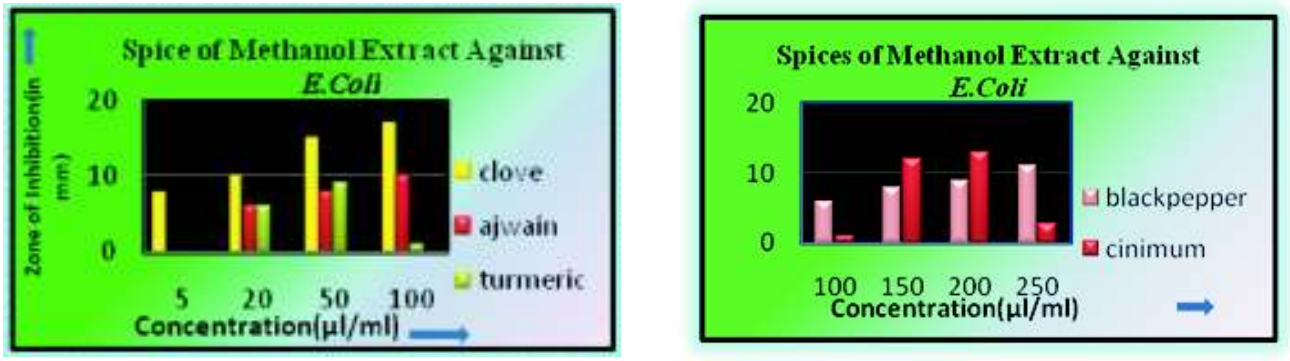
Negative control | **With Clove Extract** | **With Turmeric Extract** | **With Dalchini Extract** | **With Ajwain Extract**

Figure 1: Inhibition Zone Photographs of Gram-Negative Bacteria *Escherichia coli* Based on Agar Well Diffusion Assay for The Various Extracts of Spices

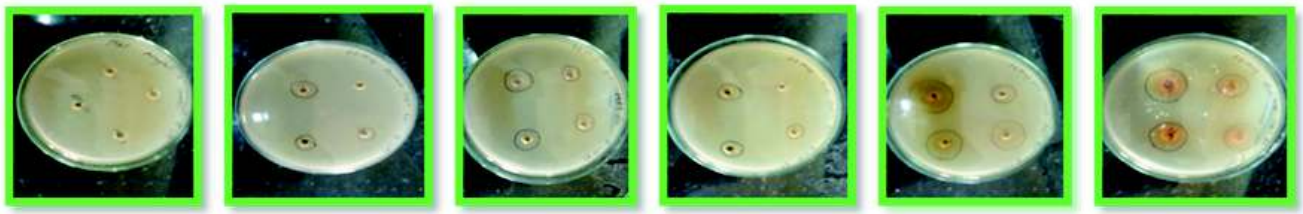
Minimum Inhibitory Concentration (MIC)

Well diffusion method

Determination of MICs of the spice extracts was done by well diffusion and agar dilution techniques and the concentrations of the extracts used were 5, 20, 50, 100 µl/ml.The lowest concentration that did not permit any visible growth when compared with the control was considered as the minimum inhibitory concentration.



Graph 2 (a) and (b): Showing Zone of Inhibition (mm) of Methanol Extract of Spices at Different Concentration in µl/ml on the *E.coli*



Control | **With Ajwain Extract** | **With Turmeric Extract** | **With Clove Extract** | **With Black Paper Extract** | **With Dalchini Extract**

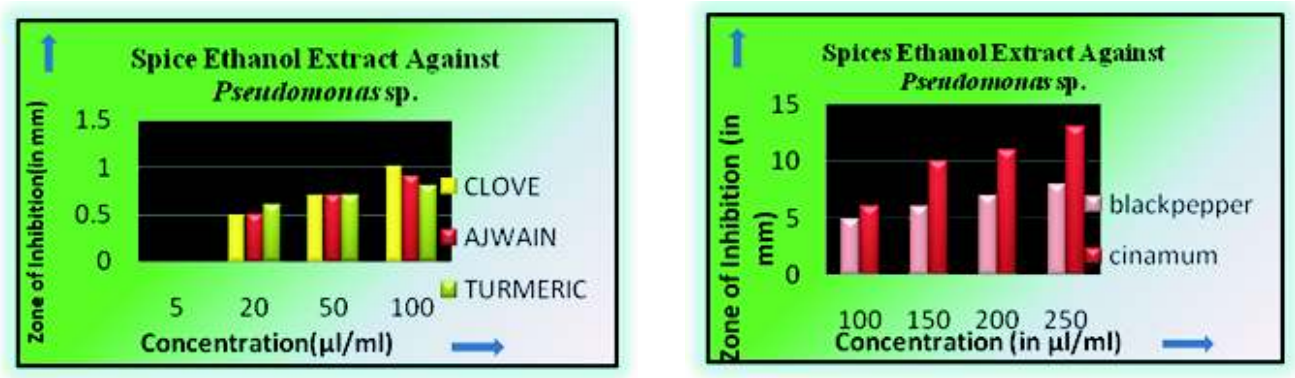
Figure 2: Inhibition Zone of *Escherichia coli* Based on Agar Well Diffusion Assay For The Methanol Extracts of Spices

Table 2 (A): Zone of Inhibition of Spice Extracts Against *Pseudomonas* sp.

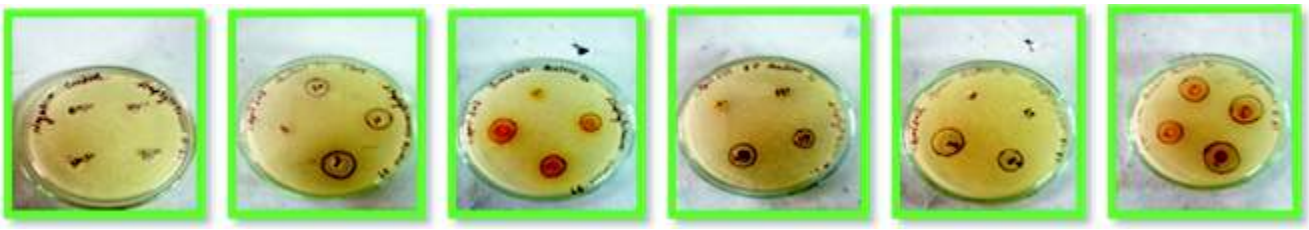
Plant Extract	Dilution of Plant Extract.(µl/Ml)								
	Acetone extract				Methanol extract				
Conc / Extract	5	20	50	100	5	20	50	100	Control
	Zone of Inhibition (mm)								
Clove	-	5	7	10	6	8	11	15	-
Ajwain	-	5	7	9	-	5	6	9	-
Turmeric	-.	6	7	8	-	5	6	8	-

Table 2(B): Zone of Inhibition of Spice Extracts Against *Pseudomonas* sp.

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

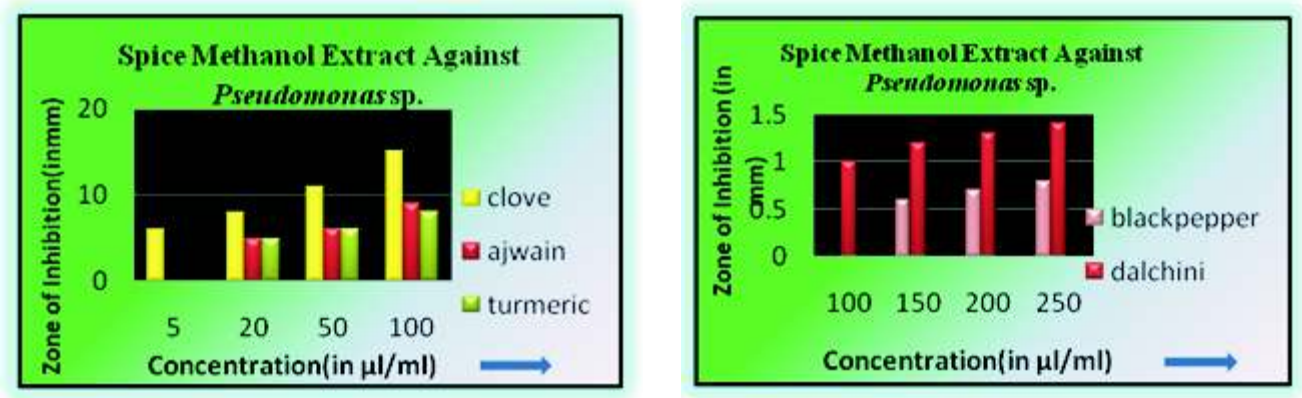


Graph 3(a) and (b): Showing Zone of Inhibition (mm) of Acetone Extract of Spices



Control | **With Clove Extract** | **With Turmeric Extract** | **With Black Paper Extract** | **With Ajwain Extract** | **With Dalchini Extract**

Figure 3: Inhibition Zone Photographs of Bacteria *Pseudomonas* 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

Figure 4: Inhibition Zone Photographs of Bacteria *Pseudomonas* sp. Based on Agar Well Diffusion Assay for The Methanol Extracts of Spices

RESULTS AND OBSERVATION

In present studies antimicrobial 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 *E.coli.*, in turmeric extract maximum zone of inhibition is 11mm at 100(µl/ml) and cinnamon showed maximum zone of inhibition is 15mm at 250(µl/ml) concentration. Similarly the antimicrobial activity of spices extracted in methanol against *E.coli*, in clove extract, maximum zone of inhibition is 17mm in concentration 100(µl/ml) and cinnamon shown maximum zone of inhibition is 15mm at 250(µl/ml) concentration.

Table, 2a, 2b graph 3a, 3b, 4a and 4b and figure 3 and 4, shown the antimicrobial activity of spices extracted in acetone against *Pseudomonas* sp., in clove extract, maximum zone of inhibition is 10mm 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 *Pseudomonas* sp. in clove extract, maximum zone of inhibition is 15mm in concentration 100(µl/ml), and cinnamon maximum zone of inhibition is 14mm at 250(µl/ml) concentration.

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 , ajwain and dalchini powder, they produced significant antimicrobial effects. In the antimicrobial study of spices , antimicrobial activity against *E.coli* maximum activity was shown in methanol extract of dalchini, and minimum activity was shown in both acetone and methanol black pepper extract. Antimicrobial activity against Pseudomonas maximum activity was shown in methanol extract of dalchini, 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 antimicrobial activity against different bacteria (*E. coli* and *Pseudomonas*) shown similarity with the previous results.

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