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Characterization of Antimicrobial Activity of Some Indian Spices

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ABSTRACT A study is designed to compare the antibacterial activity of spices like cloves, cinnamon, dry ginger, pepper, turmeric and betel leaves. The samples were formulated in three combinations as follows- turmeric and betel leaves; cinnamon and clove; dry ginger and pepper. All the combinations were mixed in the ratios (1:1), (1:2); (1:1), (1:2) and (1:1), (1:2) respectively. It was followed by sequential extraction with solvents like hexane, ethyl acetate and ethanol. Antibacterial activity of the extracts was done against *E.Coli* which was isolated. Among all the extracts the sample with cinnamon and clove showed the highest antibacterial activity and the zone of inhibition was measured. It showed highest activity in hexane solvent and showed moderate activity with ethanol. The future developments of this study may progress with quantification of compounds responsible for this activity and its further application in medicine.

KEYWORDS: Antimicrobial activity, inhibition zone,

I. INTRODUCTION

Various spices serve as a good taste enhancer. But apart from this they also provide good antimicrobial activity. They exhibit this quality naturally and therefore are very much employed in preservation techniques. The various spices are betel leaves, pepper, turmeric, cinnamon, cloves. The existence of microorganisms causes spoilage and results in reduction of the quality and quantity of possessed food (Anwar et al., 2009). 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. 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. Since long time, cinnamon and ginger have been used to treat dyspepsia, gastritis, blood circulation disturbance and inflammatory diseases in many countries (Wang et al., 2009). 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. 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). Each spice has a unique aroma and flavour, which derive from compounds known as phytochemicals or secondary compounds. These chemicals evolved in plants to protect them against herbivorous insects, vertebrates, fungi, pathogens, and parasites (Walker, 1994).

II. MATERIALS AND METHODS

The spices namely cinnamon (*Cinnamomumzeylanicum*), black pepper (*Pepper nigrum*), clove (*Syzygiumaromaticum*), turmeric (*Curcuma domestica*), Ginger (*Zingiberofficinale*), were used for the present study collected from the local market.

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1. Preparation of spice extract:

The samples were powdered and were formulated in various ratios. The samples formulated are (F1) betel leaves and turmeric, (F2) cinnamon and clove and (F3) dry ginger and pepper. The mixed samples subjected to extraction. All the samples were sequentially extracted using solvents like hexane, ethyl acetate and ethanol in their increasing order of polarity.

Extract of each spice was prepared by 10g of dry spices in 30ml of hexane, ethyl acetate and ethanol separately for 48hrs at room temperature for spice extract preparation. They were taken in ratios (Table: I) and mixed.

2. The Gram Stain:

A heat fixed bacteria smear was covered completely with a few drops of crystal violet solution. After 30- 60 sec the smear was rinsed with water by squirting the slide above the smear and letting the water wash over it until the water runs clear. Several drops of iodine (mordant) was applied to cover the smear and left for 60 sec then rinsed again. A few drops of Isopropanol-acetone mixture was added at a time until it become colorless, then the slide was rinsed again. Aqueous safranin was applied for 30- 60 sec followed by a rinse. The smear was blotted to remove excess water, using absorbent paper. The slide was then air dried and observed under a microscope. The *E.coli* was taken for the study.

3. Minimum Inhibitory concentration (MIC) Agar 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 100 (μ l). The size of the well was about 5mm in depth. Zone of inhibition was determined using Himedia zone of inhibition scale and results were expressed in millimeters (mm). The plates were then incubated at 35-37°C for 24 hours. Dimethylsulfoxide (DMSO), was used as negative control and Chloramphenicol was used as the positive control (Table II).

III. PROPOSED METHODOLOGY AND DISCUSSION

The Gram staining test confirmed the pathogen to be *E.coli*. It was also confirmed with EMB agar which shows metallic green sheath colonies. Among the ratios comprising cinnamon and clove showed the highest Zone of inhibition that results the highest antimicrobial activity against the pathogen. The others like ginger, pepper and turmeric and betel leaf showed no activity.

DISCUSSION

In present studies antimicrobial activity of six spices clove, betel leaf, cinnamon, turmeric, dry ginger and black pepper were done. Various results were showed by based on their antibacterial and antimicrobial properties. The highest activity was showed by cinnamon and the lowest was turmeric. According to the tests, cinnamon was found to be, with varying degrees, the most effective spice against tested microorganism. Cinnamon was detected to exhibit a similar inhibitory effect against *P.aeruginosa* and *E. faecalis*, and its weakest activity was against *E. coli* and *M. luteus* reported by Mabrouk and Shayeb, (1980). Ramadan *et al.* (1972) investigated the eugenol shown to have a stronger bactericidal activity against *E. coli* and *K. pneumoniae*. Antimicrobial activity against *Staphylococcus aureus* 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. (Sheetal Singh *et al.*, 2013).

IV. EXPERIMENTAL RESULTS

The Gram staining test confirmed the pathogen to be *E.coli*. It was also confirmed with EMB agar which shows metallic green sheath colonies Fig - 1. Among the ratios comprising cinnamon and clove showed the highest Zone of inhibition that results the highest antimicrobial activity against the pathogen. The others like ginger, pepper and turmeric and betel leaf showed no activity.

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FIGURES: 1 Growth of *E.coli* in EMB agar

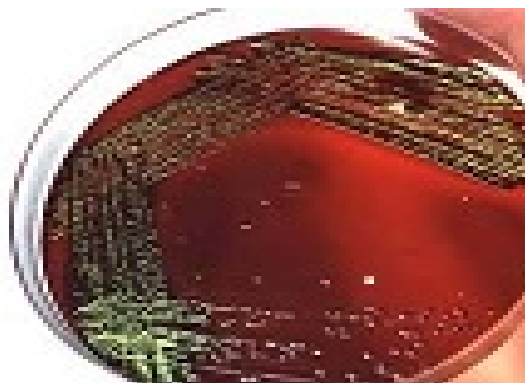


TABLE: I
Extraction preparation with different fractions

SAMPLES	RATIO: 1	RATIO:2	SOLVENT ADDED
Sample 1 Betel leaves and Turmeric	10 grams of betel leaves was mixed with 10 grams of turmeric.	10 gram of betel leaves was added with 20 gram of turmeric.	1)Hexane 2)Ethyl acetate 3)Ethanol
Sample 2 Cinnamon and Cloves F2	10 grams of cinnamon was mixed with 10 grams of clove.	10 grams of cinnamon was mixed with 20 grams of clove.	1)Hexane 2)Ethyl acetate 3)Ethanol
Sample 3 Dry ginger and pepper	10 grams of dry ginger was mixed with 10 grams of pepper.	10 grams of dry ginger was mixed with 20 grams of pepper.	1)Hexane 2)Ethyl acetate 3)Ethanol

The samples formulated are (F1) betel leaves and turmeric, (F2) cinnamon and clove and (F3) dry ginger and pepper. Different concentration also applied by differentiated their ratio by using the solvent Hexane, Ethyl acetate and Ethanol

Table ;II
In vitro antimicrobial activity of crude extractions and its different fractions
The Zone of Inhibition was measured in millimeter (mm)]

S.NO	RATIOS	HEXANE	ETHYL ACETATE	ETHANOL	POSITIVE	NEGATIVE
Sample1	F1(1:1)	10	0	0	20	0
	F1(1:2)	0	0	0	20	0
Sample2	F2(1:1)	17	11	14	20	0
	F2(1:2)	25	13	15	20	0
Sample3	F3(1:1)	0	0	0	20	0
	F3(1:2)	0	0	0	20	0

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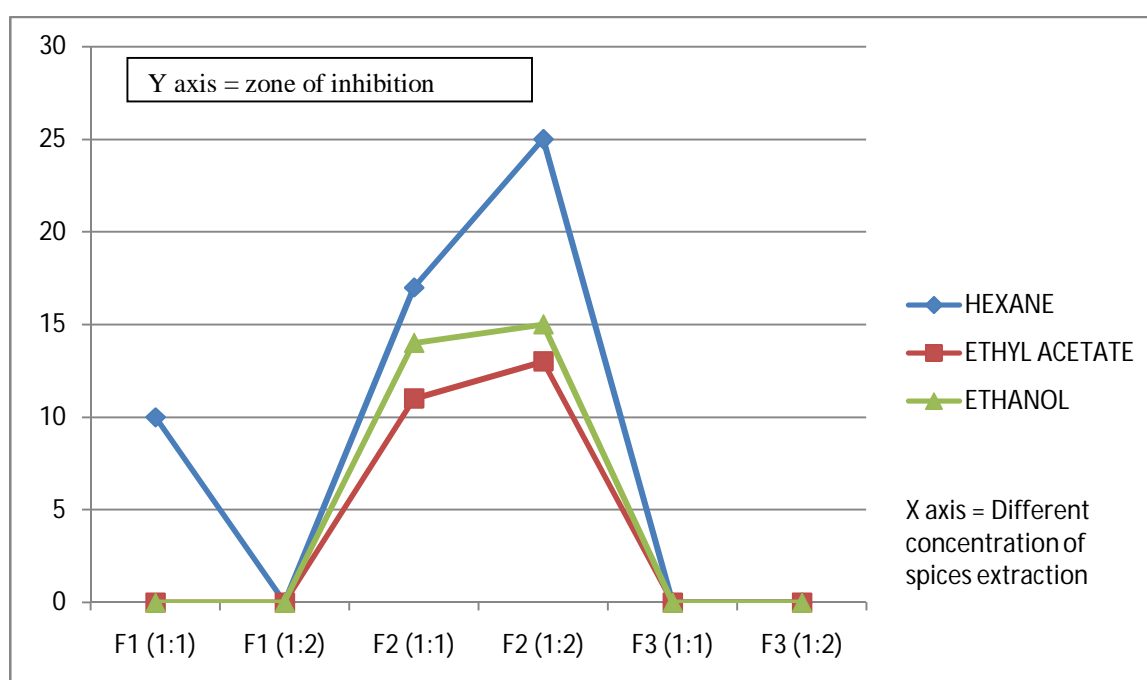
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Antimicrobial activity of different extraction against the pathogen E.coli with the negative control used as DMSO and positive control used as Chloramphenicol. The sampe 2 and F2 plant extraction has high antimicrobial activity compare to sample 1 and 2.

The graph were plot for *In vitro* antimicrobial activity of crude extractions and its different fractions



Graph represents Y axis as zone of inhibition and X axis as spices extraction with different solvent. On this sample 2 F2 (1:1) and F2 (1: 2) has high activity against E.coli.

DISCUSSION

In present studies antimicrobial activity of six spices clove, betel leaf, cinnamon, turmeric, dry ginger and black pepper were done. Various results were showed by based on their antimicrobial properties. The highest activity was showed by cinnamon and the lowest was turmeric. According to the tests, cinnamon was found to be, with varying degrees, the most effective spice against tested microorganism. Cinnamon was detected to exhibit a similar inhibitory effect against *P.aeruginosa* and *E. faecalis*, and its weakest activity was against *E. coli* and *M. luteus* reported by Mabrouk and Shayeb, (1980). Ramadan *et al.* (1972) investigated the eugenol shown to have a stronger bactericidal activity against *E. coli* and *K. pneumoniae*. Antimicrobial activity against *Staphylococcus aureus* 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. (Sheetalsinghet. *al.*, 2013).

V. CONCLUSION

The data supports the hypothesis that some common Indian spices have an inhibitory effect on the growth of certain food borne pathogen. The antimicrobial activity of six spices clove, betel leaf, cinnamon, turmeric, dry ginger and black pepper were done. According to the tests, cinnamon was found to be the most effective spice against tested microorganism. Cinnamon was detected to exhibit a similar inhibitory effect against *P. aeruginosa* and *E. faecalis*.

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Results concluded that these spices contain high amount of secondary metabolites and it can be used as good bio-preservative and it can also use for medicinal purpose.

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