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SCREENING AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF SOME COMMON INDIAN SPICES AGAINST CERTAIN HUMAN PATHOGENS

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ABSTRACT

In the present study, antimicrobial activity of six different common indian spices namely ajwain, cinnamon, clove, garlic, ginger and turmeric was evaluated against five human pathogens, *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The solvents used for preparation of spice extract were ethanol and distilled water. The *in vitro* evaluation of antimicrobial activity was carried out by agar well diffusion method. The results indicated that different extracts of spices had broad spectrum antibacterial activity. The aqueous extract of garlic and the alcoholic extract of clove showed highest antimicrobial activity. The aqueous extracts of ginger and turmeric were found to be least effective against pathogens**.** The bacterial pathogens showed variable degree of sensitivity toward these extracts. *Proteus mirabilis* and *Staphylococcus aureus* were found to be highly sensitive to aqueous and alcoholic extracts of the spices while *Pseudomonas aeruginosa and Klebsiella pneumoniae* were found to be least sensitive to aqueous and alcoholic extracts of the spices respectively. The results of present study scientifically prove importance of spices in therapeutic treatments.

Key Words: antimicrobial activity, spices, ajwain, cinnamon, cloves, garlic, ginger, turmeric, agar well diffusion method

INTRODUCTION

Infectious diseases are generally controlled by use of commercially available antimicrobial agents. Unfortunately, there is tremendous decrease in the susceptibility of pathogens towards various antibiotics in last few years (Fu et al., 2007). The indiscriminate use of commercially available antimicrobial agents has resulted in multiple drug resistance. Antibiotics may also cause adverse effects on the host. Therefore, there is a need to explore new antimicrobial agents for the treatment of infectious diseases.

Use of spices has been gradually increasing in developing as well as developed countries (Duman-Aydyn, 2008). Spices have been used for many centuries by various cultures to enhance flavour and aroma of foods. Many spices are reported to have antimicrobial activities. They are

proved to be effective against certain ailments (Nanasombat et al., 2002). In comparison to the formulated drugs, the herbs and spices have fewer side effects. They are also inexpensive, show better patient tolerance and are readily available (Adeshina et al., 2011).The inhibitory effects of spices are mostly due to specific phytochemicals or essential oils present in their composition (Avato et al., 2000).

In the present study, six important common indian spices namely ajwain, cinnamon, clove, garlic, ginger and turmeric were selected and their antimicrobial activity was evaluated against five human pathogens.

MATERIALS AND METHODS

Test Pathogens

Five different human pathogens used in the study namely *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* were collected from stock culture collections of Department of Microbiology, D. A. B. Naik Arts and Science College, Chikhali, Sangli (Shivaji University, Kolhapur), Maharashtra.. Pure cultures were preserved at 4°C on nutrient agar and MacConkeys agar slants.

Spices Sample Collection

Six different common indian spices Ajwain (*Trachypermum ammi*), Cinnamon (*Cinnamomum zeylanicum*), Cloves (*Syzygium aromaticum*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*) and Turmeric (*Curcuma longa*) used in the present study were purchased from the local markets of Shirala. Spices were washed, sliced and sun dried for seven days. After drying, they were ground to fine powder separately using electric blender.

PREPARATION OF SPICE EXTRACT

Preparation of ethanol extract of spices

5 gm of air-dried powder of spices was taken in 50 ml of ethanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 150 rpm for 72 hours. It was then centrifuged and filtered through Whatman No.1 filter paper. The filtrate was evaporated at 50^0C to near dryness and stored at 4^0C in airtight tubes. Afterwards, dimethyl sulphoxide was added to the evaporated extract before experiments.

Preparation of aqueous extract of spices

5 gm of air-dried powder of spices was added to 100 ml distilled water and boiled on slow heat for 30 minutes. It was then centrifuged and filtered through Whatman No.1 filter paper. The filtrate was collected. This procedure was repeated twice. The filtrate was concentrated at 80^0C to make the final volume one-fourth of the original volume with the help of water bath and stored at 4^0C in airtight tubes.

Screening of antimicrobial activity

Antimicrobial activity of different spice extracts was determined by agar well diffusion method. 0.1 ml of freshly grown culture of test organism (10^6cfu/ml) was aseptically introduced and spread on the surface of sterile nutrient agar plates. Wells of 6 mm diameter were made in

agar plate with the help of sterile cork-borer. 200 µl of different spice extracts and same volume of positive and negative controls were filled in the wells with the help of micro pipette. Sterile distilled water or 10% DMSO was used as negative control and streptomycin was used as positive control for the test organisms. Plates were left for some time in refrigerator at 4^0 C till the extract diffuses in the medium with the lid closed and incubated at 37˚C for 24 hours. The plates were observed for zone of inhibition. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition against the tested pathogen. Each assay in this experiment was replicated three times and mean values were represented.

RESULTS AND DISCUSSION

In present study, five bacteria which are known to cause diseases in human beings such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis* and *Staphylococcus aureus* were selected and antimicrobial activity of aqueous and alcoholic extracts of six different common indian spices namely ajwain, cinnamon, cloves, garlic, ginger and turmeric was evaluated against them by agar well diffusion method.

The results indicated that different extracts of spices had broad spectrum antimicrobial activity. The bacterial pathogens showed variable degree of sensitivity toward these extracts.

For the comparison, positive and negative controls were used. Positive control significantly inhibited growth of all test organisms, while negative controls did not show inhibitory action on any of the test organisms.

Aqueous extract of garlic (*Allium sativum*) exhibited highest antimicrobial activity against all tested pathogens. Aqueous extracts of clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum*) and ajwain (*Trachypermum ammi*) also showed significant antibacterial activity against pathogens. Aqueous extracts of ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) were found to be least effective against tested pathogens. The aqueous extract of garlic (*Allium sativum*) showed maximum inhibition zone of 20 mm against *Staphylococcus aureus.*

Staphylococcus aureus and *Proteus mirabilis* were found to be highly sensitive to aqueous extracts of all the spices used while *Pseudomonas aeruginosa* was found to be least sensitive to aqueous extracts of all the spices used.

Alcoholic extract of clove (*Syzygium aromaticum*) exhibited highest antibacterial activity against all tested pathogens. Alcoholic extracts of garlic (*Allium sativum*), cinnamon (*Cinnamomum zeylanicum*) and ajwain (*Trachypermum ammi*) also showed significant antibacterial activity against all tested bacteria. The alcoholic extract of clove (*Syzygium aromaticum*) showed maximum inhibition zone of 22 mm against *Proteus mirabilis.*

Proteus mirabilis and *Staphylococcus aureus* were found to be highly sensitive to alcoholic extracts of all the spices used while *Klebsiella pneumoniae* was found to be least sensitive to alcoholic extracts of all the spices used.

The aqueous extracts of garlic showed higher antibacterial activity as compared to alcoholic extracts. Both aqueous and alcoholic extracts of clove showed significant antimicrobial activity. The alcoholic extracts of turmeric and cinnamon showed better antimicrobial activity as compared to the aqueous extracts. Aqueous and alcoholic extracts of ajwain also showed

antimicrobial activity, while aqueous extracts of ginger and turmeric were found to be least effective against pathogen**.**

Joe et al. (2009) studied antimicrobial activity of some common spices against certain human pathogens and found that the garlic extracts showed excellent antimicrobial activity against almost of all pathogens tested while ginger extract showed only a moderate antimicrobial activity. Similar research on other bacteria studied by Bhak et al. (1990) showed cinnamon and clove to have strong inhibitory actions, while mustard and garlic had only slight antimicrobial activity. Allicin is the principal antimicrobial compound of freshly crushed garlic (Serge and David, 1999; Miron et al., 2000). The potent antimicrobial activity of clove and cinnamon can be predominantly attributed to eugenol and cinnamaldehyde. These are the phenolic components of clove and cinnamon, which render them effective against the tested micro-organisms. This was confirmed by Farag et al. (1989), where eugenol, a major component of clove was found to limit the growth of *B. cereus* by inhibiting the production of certain enzymes needed for its growth. The antimicrobial activity of ginger may be attributed to the fact that it contains antimicrobial substances such as zingiberol, zingiberine and bisabolene (Derrida M, 1999). The rhizome of ginger contains pungent vanillyl ketones including gingerol and paradole, etc. Gingerol is a mixture of crystal gingerone and it is the major cause of acidity of ginger and plays a role in inhibiting bacteria such as *S. aureus, Trichomonas vasmalis* and help to cure bacterial vaginosis and skin diseases (Derrida M, 1999). Chandara et al. (2005) reported that turmeric is effective against *E.coli, B.subtilus and S.aureus* due to the presence of a phenolic compound, curcuminoid.

Odhav et al. (2002) suggested that the mechanism of antibacterial action of spices involve the hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, membrane disruption, destruction of electron transport systems and cell wall disruption. The antimicrobial activity of aqueous extracts could be due to anionic components such as thiocyanate, nitrate, chlorides and sulphates in addition to many other compounds naturally present in plants (Darout, 2000). The ethanolic extracts showed better results as compared to aqueous as being organic dissolves more organic compounds resulting in the release of greater amount of active antimicrobial components (Cowan, 1999).

Table 1: Antimicrobial activity of aqueous extract of some common indian spices against certain human pathogens

Name of the Spices	**Diameter of zone of inhibition in mm of test pathogens against different Spice extracts**				
	Escherichia coli	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Ajwain	10	10	12	08	12
Cinnamon	12	08	12	08	10
Cloves	15	10	15	11	18
Garlic	15	16	16	12	20
Ginger	-	10	11	-	09
Turmeric	08	-	09	-	12
Streptomycin	20	19	23	19	18

(Positive Control)					
Distilled water (Negative Control)	-	-	-	-	-

(**Note**- Figures indicate mean diameter of inhibition zone in mm and '-' indicates no antimicrobial activity)

Figure 1: Antimicrobial activity of aqueous extract of some common indian spices against certain human pathogens

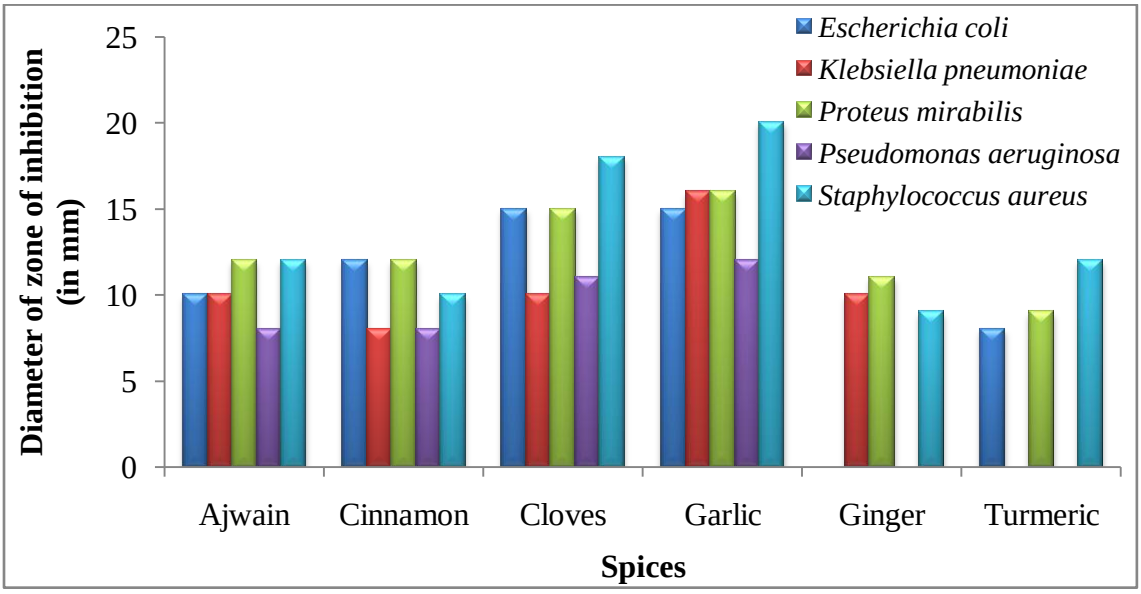


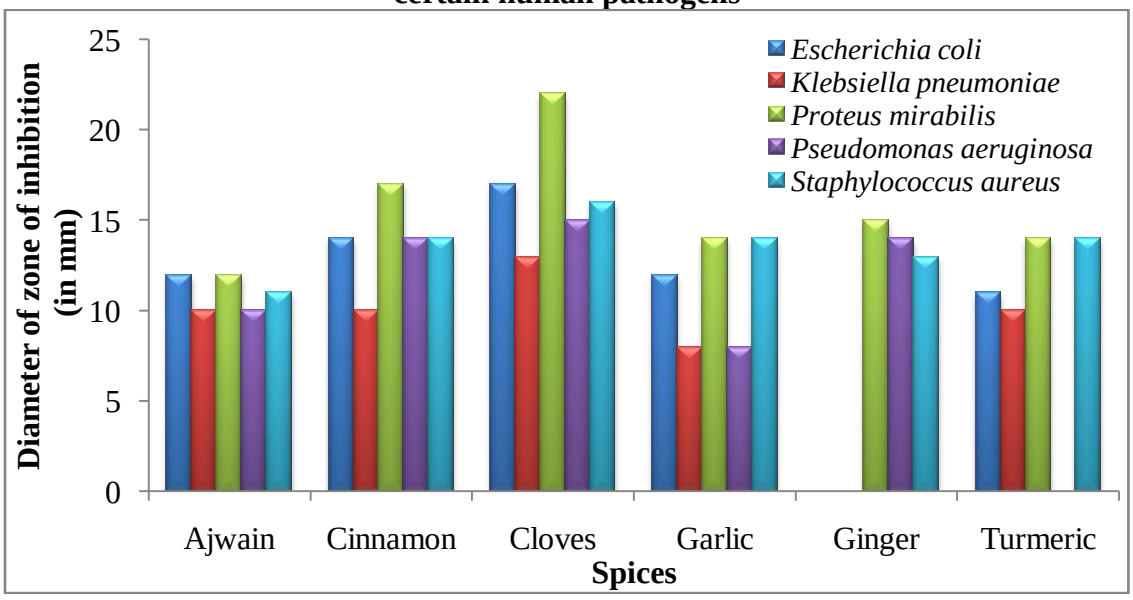
Table 2: Antimicrobial activity of alcoholic extract of some common indian spices against certain human pathogens

Name of the Spices	**Diameter of zone of inhibition in mm of test pathogens against different Spice extracts**				
	Escherichia coli	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Ajwain	12	10	12	10	11
Cinnamon	14	10	17	14	14
Cloves	17	13	22	15	16
Garlic	12	08	14	08	14
Ginger	-	-	15	14	13
Turmeric	11	10	14	-	14
Streptomycin (Positive Control)	20	19	23	19	18
Ethanol	-	-	-	-	-

(Negative Control)					
DMSO (Negative Control)	-	-	-	-	-

(**Note**- Figures indicate mean diameter of inhibition zone in mm and '-' indicates no antimicrobial activity)

Figure 2: Antimicrobial activity of alcoholic extract of some common Indian spices against certain human pathogens



CONCLUSIONS

The results of present study scientifically prove importance of spices in therapeutic treatments. Among six spices used, garlic and clove were found to be most effective against human pathogens. Further investigations can be carried out to find out bioactive molecules present in the spices like garlic and clove which can be used in development of new antimicrobial agents for the treatment of infectious diseases.

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