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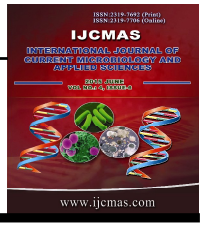

Original Research Article

Efficacy of Spice Extracts against UTI Isolates

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A B S T R A C T

Urinary tract infections are a serious health problem affecting millions of people each year. Infections of the urinary tract are the second most common type of infection in the body.In this study out of the 80 samples collected from 10 hospitals, 26 samples were positive urine samples; they were *Escherichia coli, Klebsiella pneumoniae, Citrobacter diverses, Pseudomonas aeruginosa and Proteus vulgaris.* Among these organisms *E.coli* was the most frequent causative agent in the urinary tract infections up to (46%). Out of the six different antibiotics Methicillin, Ampicillin, Streptomycin, Penicillin, ciprofloxacin and tetracyclin used the ciprofloxacin and tetracycline were found to be the most effective drugs against the urinary tract pathogens. The spice extract sensitivity patterns of urinary tract pathogens were determined. Among the five spice extracts *Eugenia caryophyllus* extract showed maximum antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa.* From these Findings it is observed that *Eugenia caryophyllus, Cinnamomum zeylanicum and Myristica fragrans* have the maximum antibacterial activity against all the five urinary pathogens.

Keywords

UTI , urinary tract pathogens, spice extracts, *Eugenia caryophyllus, Cinnamomum zeylanicum* and *Myristica fragrans*, Bioactive compounds.

Introduction

Urinary tract infection is second most common infection next to Respiratory tract in our human body. Different group of microorganisms involved in UTI. Different sex and age group of people affected by urinary tract infection. Sometimes, the UTI is symptomatic or asymptomatic and complicated or uncomplicated in nature. Usually UTI infection confirmed by significant bacteriuria. i.e., 10^5 organisms /ml considered to be suggestive infection. Isolation, characterization of Urinary tract infection, early detection and antibiotic therapy also very important for Urinary tract infection. Thomas *et al*., (1996) suggested that the plant extract disc showing an inhibitory zone greater that (8mm) were considered as sensitive to the particular extract. (Lai & Roy, 2004) Spices contain products of secondary metabolism such as phenolics, phenolic acids, quinines, flavonoides, tannins. (Pappachan *et al, 2007)* evaluated the antimicrobial activity of six Indian spices extracts namely clove,

cinnamon, mustard, garlic, ginger and mint against *E. coli, Staphylococcus aureus and Bacillus cereus.* The result showed that the extract of clove, cinnamon and mustard had good inhibitory action at 100 % while garlic showed medium ginger and mint showed negligible anti bacterial activity against these pathogens at the same concentration.

In this work comparison of the inhibitory characteristics of five spices extracts (Garlic, Clove, Cinnamon, Nutmeg and Fennel) on UTI isolates (*Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Citrobacter diversus*) were carried out. The active compounds present in essential oil of spices were determined through Gas chromatography.Hence The present study was undertaken with the following objectives. To isolate and characterize the bacteria involved in the urinary tract infection. To assess the antibacterial activity of spices extract in comparison with a known drug of antibacterial activity such as a few antibiotics.

Materials and Methods

Collection of urine sample

"Clean - catch", midstream urine samples were collected in sterile wide mouthed, screw-capped bottle after very thorough preliminary cleaning of external genitalia with soap and water. If there is any chance of delay, to examine the urine sample, they were refrigerated at 4^0 C. Early morning samples were mandatory. Enumeration and identification of bacteria were done in the laboratory.

Smear examination

For routine specimens, placed a loopful (ordinary bacteriological loop of 4mm diameter) of a small drop of the well-mixed urine sample without centrifugation process, on a clean side. Then it was air-dried. Heat fixed and stained by Gram's method. Then stained slide looked for pus cells and organisms in every field.

Bacteriological analysis

To evaluate the clinical significant of "positive" urine culture, estimation of the number of organisms present is essential. Results of Smear examination were used as a guide for choice of media

Calibrated loop method

A 4mm platinum loop which delivers 0.01 ml was used. One loopful of the well-mixed, uncentrifuged urine specimen, (undiluted) quickly transferred and inoculated by touching the loop to 3 or 4 places on the MacConkey Agar Medium. Immediate and complete transfer, and even distribution of the inoculum accomplished by cross streaking the entire plate for six times. Blood agar plate was inoculated by 4 areas streaking (Monica Cheesbrough, 1984).

Pour plate method

1.0 ml of undiluted urine pipetted out and poured into petri dish. 1.0 ml of a 1: 100 dilution, made by mixing 0.1 ml of with 9.9 ml of sterile normal saline, into another and 0.1 ml of the 1:100 dilution into the third petri dish. Approximately 10.0ml of melted and cooled Nutrient agar poured into each plate and mixed well by carefully rotating the plates. Then the plates were allowed to harden and then for incubation **(**Monica cheesbrough, 1984).

Colony counts

After overnight incubation of all plates at 37^oC, the number of colonies counted and

calculated the number of viable bacteria present in 1.0ml undiluted urine. Calibrated loop plates -The number of colonies multiplied with 100 because undiluted urine was used for inoculation.

Pour plates

In plate I, number of colonies counted directly; plate II, number of colonies multiplied with 100 and III, number of colonies multiplied with 1000. Then average of the counts taken on three plates.The significance of a positive urine culture is most reliably assessed in terms of the number of viable bacteria present in urine (Kass, 1997).

Patients Urine sample showing 10^5 organisms per ml was considered to be significant bacteriuria. After confirmation of quantitative evaluation, the urine sample was inculcated on MacConkey agar plate and Blood agar plate respectively. Lactose fermenting (LF) and non-lactose fermenting colonies (NLF) are identificated by Blood agar medium. Then the causative organisms of UTI identified and characterized by various biochemical test, Voges- Proskauer test, Citrate utilization test, Catalase test, Coagulase test, Urease test, Gelatinase test, Motility test and Oxidase test.

The antibiotic sensitivity pattern of urinary pathogens

The isolated Urinary pathogens were used for antibiotic sensitivity test. Kirby-Bauer method was used for present investigation. Before doing antibiotic sensitivity test. The test organisms were inoculated in to nutrient broth and incubated at 35 – 37^{o}C for overnight incubation. Muller - Hinton Agar plates were used for sensitivity test. Then the test organism was inoculated into the prepared plates. Sterile swabs were used for inoculation purpose simultaneously antibiotics which are common antibiotics relevant to urinary tract infection, they were placed in the plates. Himedia antibiotics discs were used for antibiotics sensitivity test.

The plates were kept in the refrigerator for one hour to arrest the growth of the test organism and make the diffusion of the antibiotics. These plates were then incubated at 37^{o}C for 24 hours. The zones of inhibitions were observed and recorded.

Concentration of antibiotics discs

Methicillin	-	5 mcg/disc
Ampicillin	-	10 mcg/disc
Streptomycin	-	10 mcg/disc
Penicillin	-	10 mcg/disc
Ciprofloxacin -	-	5 mcg/disc
Tetracycline	-	30 mcg/disc

Extraction of spices

The Powdered spice samples namely Garlic (*Allium sativum),* Cinnamon *(Cinnamomum zeylanicum),* Clove (*Eugenia caryophyllus),* Fennel (*Foeniculum vulgare),* Nutmeg (*Myristica fragrans)* were extracted with the solvent methanol in the soxhlet apparatus (Tanira, *et al*, 1995).The extract collected was concentrated by exposing them in a laminar air flow and stored 4^{o}C until further use (Caceres *et al*, 1995). These extracts were further used to study the antibacterial activity against the urinary tract pathogens.

Antibacterial assay of spices extract

In this present work four different concentrations of five extracts antibiotic discs were prepared. Sterile discs were used for the preparation. 100 microgram antibiotic or spice extracts dissolved in 100

microlitre of common solvent. We got 100 % concentration.

i.e.

100 µg spice extracts + 100 µl DMSO-100%
50 µg spice extracts + 100 µl DMSO-50%
25 µg spice extracts + 100 µl DMSO-25%
10µg spice extracts + 100 µl DMSO-10%

On the basis of this calculation for different concentrations were prepared and used for antibacterial activity. DMSO (Di Methyl Sulfoxide) is the common solvent and the spice extracts were dissolved in this solvent. There is no antibacterial activity for DMSO. With the help of sterile forceps, the sterile discs dipped in the prepared concentrations of spice extracts. The prepared discs were allowed to dry in an incubator at 37^{o}C for One hour. Inhibition zones with diameter less than 12 mm were considered having non antibacterial activity. Diameters between 12 and 16 mm considered moderately active and these with more than 16mm were considered as highly active (Indu, *et al*, 2006)

Results and Discussion

In this present work, Of the 80 samples of urine were cultured, 26 samples yield pathogenic bacteria. 54 Samples were yielded only saprophytic contaminants (Table .1) shows the incidence of urinary tract infection in different cases.

In this study nearly five different urinary pathogens were isolated from positive urine samples. According to percentage of incidents, they were *Escherichia coli, Klebsiella pneumoniae, Citrobacter diversus, Pseudomonas aeruginosa and Proteus vulgaris* (Table 2).The organisms were identified through different biochemical reactions (Table 3).

Antibiotic sensitivity pattern of urinary tract pathogens

The antibiotic sensitivity pattern of urinary tract pathogens were determined by disc diffusion technique**.** For this technique different antibiotics with various concentrations were used.

Ciprofloxacin showed zone of inhibition of 28 mm and Tetracycline showed 13 mm against *Escherchia coli*. Ciprofloxacin showed zone of inhibition of 10 mm and Tetracycline showed 18 mm against *Klebsiella pneumoniae.* Tetracycline showed zone of inhibition of 10 mm against *Citroobacter diversus.* Ciprofloxacin showed zone of inhibition of 25 mm against *Pseudomonas aeruginosa*. Ciprofloxacin showed zone of inhibition of 42 mm against *Proteus vulgaris*. (Table. 4)

Antibacterial activity of the spice extracts

The spice extract sensitivity patterns of urinary tract pathogens were determined. Among the five spice extracts *Eugenia caryophyllus* extract showed maximum antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. The antibacterial activity of 100% extract of *Eugenia caryophyllus* was on par with the antibiotic ciprofloxacin (Table.5).

Table.1 Incidence of urinary tract infection in 80 cases studied

Total number of Cultures.	Total number of positive Cultures.	Percentage of positive cultures
80	**26**	**32.5**

Table.2 Incidence and nature of organism observed

Sl. No	Name of the Bacteria	Single pure Isolates	Mixed Isolates	Total Number of Positive	Percentage of Incidence
1	*Escherichia coli*	8	4	12	46.15
2.	*Klebsiella pneumoniae*	4	2	6	23.07
3.	*Citrobacter diversus*	2	1	3	11.53
4.	*Pseudomonas aeruginosa*	2	1	3	11.53
5.	*Proteus vulgaris*	1	1	2	7.69
	Total	17	9	26	100

Table.3 Biochemical reactions

Tests	*Escherichia coli*	*Klebsiella pneumoniae*	*Citrobacter diversus*	*Pseudomonas aeruginosa*	*Proteus vulgaris*
Motility	Positive	Negative	Positive	Positive	Positive
Indole	Positive	Negative	Positive	Negative	Positive
Methyl Red	Positive	Negative	Positive	Negative	Positive
Vogus-Proskauer	Negative	Positive	Negative	Negative	Negative
Citrate Utilization Test	Negative	Positive	Positive	Positive	Negative
Hydrogen Sulphide Production	Negative	Negative	Negative	Negative	Positive
Lactose fermentation	Positive	Positive	Positive	Negative	Negative
Sucrose Fermentation	Positive	Positive	Positive	Negative	Positive
Gas from glucose	Positive	Positive	Positive	Positive	Positive
Gelatin Hydrolysis	Negative	Negative	Negative	Positive	Positive
Urea Hydrolysis	Negative	Positive	Positive	Positive	Positive
Catalase	Positive	Positive	Positive	Positive	Positive
Oxidase	Negative	Negative	Negative	Positive	Negative
Nitrate reduction	Positive	Positive	Negative	Positive	Positive

Table.4 Antibiotic sensitivity pattern of urinay tract pathogens

S. No.	Name of the Bacteria	Antibiotic sensitivity of Different Antibiotics (zone of inhibition in mm)					
		Cf 5mcg	T 30mcg	M 5mcg	S 10mcg	A 10mcg	P 10mcg
1	*Escherichia coli*	28	13	R	R	R	R
2	*Klebsiella Pneumoniae*	10	18	R	R	R	R
3	*Citrobacter diversus*	R	10	R	R	R	R
4	*Pseudomonas aeruginosa*	25	6	R	12	R	R
5	*Proteus vulgaris*	42	15	R	17	10	R

Cf - Ciprofloxacin ,M – Methicillin , T- Tetracycline , S - Streptomycin
A –Ampicillin , P - Penicillin., R - Resistant

Table.5 The spices sensitivity pattern of urinary pathogens

Name of the spice extracts	Concentrations	Zone of Inhibition in (mm)				
		E.coli	*Klebsiella pneumoniae*	*Citrobacter diversus*	*Pseudomons aeruginosa*	*Proteus vulgaris*
Allium sativum	10 %	7	7	7	7	8
	25 %	9	7	9	7	10
	50 %	13	10	12	8	12
	100 %	16	12	16	10	15
Cinnamomum Zeylanicum	10 %	10	10	8	11	8
	25 %	12	10	10	13	8
	50 %	14	16	10	16	10
	100 %	16	16	12	18	12
Eugenia caryophyllus	10 %	12	8	8	12	8
	25 %	15	8	10	15	10
	50 %	17	10	10	18	13
	100 %	20	12	12	20	15
Foeniculum vulgare	10 %	8	8	8	8	10
	25 %	8	10	80	8	10
	50 %	12	14	10	10	12
	100 %	14	14	10	14	14
Myristica Fragrans	10 %	8	8	10	10	8
	25 %	10	8	12	10	10
	50 %	14	12	14	12	10
	100 %	16	14	16	16	14

In this study , Among the five spice extracts *Eugenia caryophyllus* extract showed maximum antibacterial activity against urinary pathogens *Escherichia coli* and *Pseudomonas aeruginosa* . This methanolic extract showed maximum zone of inhibition against *E coli* and *Pseudomonas aeruginosa* .This result is in agreement with the findings of Zhan et al, (2009) found that ethanol extracts of clove and rosemary displayed

significant inhibitory properties against *Escherichia coli* and *Listeria monocytogenes* . As studied and listed in the results, it is clearly understood that spices play a vital role against the control of certain pathogenic microorganisms causing urinary tract infections which also shows no undesirable side effects.

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