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## STUDY OF SYNERGISTIC INTERACTIONS BETWEEN INDIAN SPICES AND DRUGS AGAINST UROPATHOGENS

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### ABSTRACT

The present study investigated the prevalence of uropathogens in urinary tract infection and the synergistic effect between antimicrobial potential of four Indian spices viz., *Cinnamomum zeylanicum* (cinnamon), *Piper nigrum* (black pepper), *Syzygium aromaticum* (clove) and *Trachyspermum ammi* (carom seeds) and drugs against uropathogens. *E. coli* was the most prevalent uropathogens (45%). Spices exhibit effective antimicrobial potential. The ethanolic extract of cinnamon showed highest potential against *E. coli* (21.4±0.30 mm) while that of black pepper showed highest potential against *E. coli* (22.3±0.56 mm). The ethanolic extract of clove was most effective against *E. coli* (25.0±0.41 mm) while that of carom seeds was most effective against *Proteus* (13.7±0.12 mm). The methanolic extract of cinnamon showed highest potential against *E. coli* (12.6±0.32 mm) while that of black pepper showed highest potential against *Staphylococcus* (14.6±0.32 mm). The methanolic extract of clove was most effective against *E. coli* (15.6±0.27 mm) while that of carom seeds was most effective against *Proteus* (10.7±0.17 mm). Aqueous extract of cinnamon showed highest potential against *E. coli* (14.6±0.12 mm) while that of black pepper showed highest potential against *E. coli* (16.5±0.20 mm). Aqueous extract of clove was most effective against *Staphylococcus* (15.8±0.17 mm) while that of carom seeds was most effective against *Klebsiella* (12.3±0.12 mm) and least against *Pseudomonas* (9.7±0.12 mm). A synergistic effect on the antimicrobial was observed when crude extracts of spices was used in combination with antibiotics.

**Keywords:** Uropathogens, Antimicrobial potential, Clove, Cinnamon, Black pepper, Carom seeds.

### INTRODUCTION

The increasing resistance against antibiotics amongst the bacterial pathogens due to widespread use of antibiotics have made treatment difficult. The various mechanisms for drug resistance has evolved in pathogens. The resistance can be natural due to some mutation in gene or acquired from plasmid or transposon [1]. The change in the physiological state of bacterial cell may also contribute in developing resistance against antibiotics. The bacteria may counteract the effect of antibiotics either by breaking down the antibiotics like ampicillin, or chemical modification of antibiotics like chloramphenicol [2-7]. The antibiotic resistance gene is usually plasmid mediated and therefore bacteria may lose this property over a period of time if selection pressure is not there. Moreover, certain bacterial variants have evolved mechanisms to resist multiple drugs, making such variants obstinate to chemotherapy against such bacterial strains that are the causative agents of infection in patients. The formation of biofilm by certain bacterial species may also contribute in resistance against the antibiotics [8]. The researchers are now searching medicinal plants whose extract may have

antimicrobial potential [9,10]. The active constituent of medicinal plants can be developed as drug and can be made more effective using combinatorial synthesis approach.

The present study was aimed at determining the antimicrobial activity of cinnamon (*Cinnamomum zeylanicum*), black pepper (*Piper nigrum*), clove (*Syzygium aromaticum*) and carom seeds (*Trachyspermum ammi*) and to evaluate whether these extracts exhibit any synergistic effect when used along with antibiotics.

### MATERIALS AND METHODS

#### Isolation of uropathogens

A total of 50 urine samples were collected aseptically from different patients in the hospitals in Dehradun, Uttarakhand, India. The samples were plated by T-streaking method on CLED agar and blood agar using calibrated loops. The samples in which bacterial count was >10<sup>5</sup> cfu/ml were taken for isolation of uropathogens. All samples were plated in triplicates.

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Isolates were purified by streaking on nutrient agar and pure cultures were maintained.

### Characterization of uropathogens

The morphological and biochemical characterization of recovered uropathogens was carried out. Cell morphology (Gram's reaction, cell shape and arrangement) of isolates were studied. The various biochemical tests viz., Oxidase test, Indole-Methyl Red-Voges-Proskauer-Citrate Utilization test (IMViC), Triple Sugar Iron (TSI) test, Urease test and Nitrate reduction tests were carried out according to [11].

### Acquisitions of spices & preparation of extracts

The spices viz., cinnamon, black pepper, clove and carom seeds were procured from the local market. The spices were sorted for separation of dirt and unwanted materials and grounded into fine powder. Three extractants i.e., water, ethanol and methanol were used. The extracts were prepared by dissolving spices in solvents in a concentration of 1:4 and keeping at room temperature for 24hrs in a sterile beaker covered with aluminium foil to avoid evaporation and then subjected to filtration through sterilized Whatman no. 1 filter paper. The solvent was dried and concentrated using orbital shaker at 40°C. The stock solutions of the extracts thus obtained were prepared by diluting the dried extracts with 50% of respective solvents.

### Evaluation of antimicrobial activity of extracts

The antimicrobial activity of crude extracts against uropathogens was evaluated by using agar well diffusion method. The isolates were inoculated into 10ml of sterile nutrient broth, and incubated at  $37\pm 1^{\circ}\text{C}$  overnight. The turbidity of culture was compared with Mac Farland standard number II. The cultures were swabbed on the surface of sterile Mueller-Hinton agar plates using a sterile cotton swab and allowed to dry for 3-5 minutes. Agar wells were prepared with the help sterilized borer with 10mm diameter. The extract of spices was diluted to give the final concentration 1000ppm, 2000ppm, 3000ppm and 4000ppm. 100  $\mu\text{l}$  of different dilutions of the extracts was added to the wells of the inoculated plates. 50% ethanol and 50% methanol was used as control which was introduced into the well instead of the extract. The plates were incubated in an upright position at  $37\pm 1^{\circ}\text{C}$  for 24hrs. The zone of inhibition was measured and expressed in millimetres (mm).

### Antibiotic sensitivity assay

All isolates were tested for antibiotic sensitivity by Kirby-Bauer disc diffusion method [12] on Mueller-Hinton agar (MHA). The cultures were enriched in sterile nutrient Broth overnight at  $37^{\circ}\text{C}$ . Using a sterile cotton swabs, the cultures were aseptically swabbed on the surface of surface MHA plates and allowed to dry for 3-5 minutes before applying the antibiotic discs. Using a sterile forcep, 4 antibiotic discs were aseptically placed over the inoculated plates sufficiently separated from each other

to avoid overlapping of inhibition zones. The plates were incubated in an upright position for 24hrs at  $37^{\circ}\text{C}$  and diameter of zone of inhibition was measured in mm.

### Evaluation of synergistic effect of crude extracts on the antimicrobial activity of drugs

The antibiotic and dilution of the crude extracts exhibiting maximum antimicrobial potential against uropathogens was chosen for further study. This test was carried out in the similar as described under antibiotic sensitivity assay with an addition that 100  $\mu\text{l}$  of the dilutions of the extracts exhibiting maximum antimicrobial potential was added to the antibiotic discs. The plates were incubated in an upright position at  $37\pm 1^{\circ}\text{C}$  for 24hrs. The zone of inhibition was measured and expressed in millimetres (mm).

## RESULTS

### Prevalence of uropathogens

A total of 70 uropathogens were obtained from positive urine samples which were identified based on morphological and biochemical characteristics (Fig. 1). *E. coli* was the most prevalent uropathogen (45%) followed by *Pseudomonas* (25%), *Proteus* (10%), *Staphylococcus* (10%), *Klebsiella* (6%) and *Serratia* (4%).

### Antimicrobial activity of Indian spices against uropathogens

All extracts of spices showed good antibacterial property (Table 1 to 4). The ethanolic extract of cinnamon showed highest potential against *E. coli* ( $21.4\pm 0.30$  mm) and least against *Staphylococcus* ( $9.7\pm 0.21$  mm). The methanolic extract of cinnamon showed highest potential against *E. coli* ( $12.6\pm 0.32$  mm) and least against *Staphylococcus* ( $7.6\pm 0.16$  mm). The aqueous extract of cinnamon showed highest potential against *E. coli* ( $14.6\pm 0.12$  mm) and least against *Staphylococcus* ( $8.3\pm 0.17$  mm). The ethanolic extract of black pepper showed highest potential against *E. coli* ( $22.3\pm 0.56$  mm) and least against *Serratia* ( $10.0\pm 0.47$  mm). The methanolic extract showed highest potential against *Staphylococcus* ( $14.6\pm 0.32$  mm) and least against *Serratia* ( $5.3\pm 0.22$  mm). The aqueous extract showed highest potential against *E. coli* ( $16.5\pm 0.20$  mm) and least against *Serratia* ( $8.6\pm 0.27$  mm). The ethanolic extract of clove was most effective against *E. coli* ( $25.0\pm 0.41$  mm) and least against *Klebsiella* ( $13.6\pm 0.25$  mm). The methanolic extract most effective against *E. coli* ( $15.6\pm 0.27$  mm) and least against *Serratia* ( $11.4\pm 0.24$  mm). The aqueous extract was most effective against *Staphylococcus* ( $15.8\pm 0.17$  mm) and least against *Serratia* ( $12.6\pm 0.18$  mm). The ethanolic extract of carom seeds was most effective against *Proteus* ( $13.7\pm 0.12$  mm) and least against *Pseudomonas* ( $10.0\pm 0.12$  mm). The methanolic extract most effective against *Proteus* ( $10.7\pm 0.17$  mm) and least against *Pseudomonas* ( $8.3\pm 0.15$  mm). The aqueous extract was most effective against *Klebsiella* ( $12.3\pm 0.12$  mm) and least against *Pseudomonas* ( $9.7\pm 0.12$  mm).

### Antimicrobial activity of antibiotics against uropathogens

Maximum isolates were observed to be resistant to clindamycin, oxacillin and ampicillin (Fig. 1). *E. coli* was found to be resistant to ciprofloxacin. *Staphylococcus* was observed to be resistant to clindamycin, erythromycin, oxacillin, vancomycin, ampicillin and ciprofloxacin. *Pseudomonas* was resistant to erythromycin while *Klebsiella* was resistant to clindamycin, chloramphenicol, oxacillin, vancomycin, ampicillin, ciprofloxacin and cephalothin. *Proteus* was resistant to clindamycin, erythromycin, oxacillin, ampicillin and cephalothin. *Serratia* was resistant to clindamycin, erythromycin,

oxacillin, vancomycin, ampicillin, ciprofloxacin and cephalothin.

### Synergistic effect of crude extracts on the antimicrobial activity of drugs

The extracts of different spices showed synergistic effect with antibiotics in exhibiting antimicrobial potential against uropathogens (Table 5 to 8). The zone diameters were found to increase when extracts were used in combination with antibiotics. The combination was found to be more potent than either of the two.

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**Table 1a. Antimicrobial activity of ethanolic extract of cinnamon against uropathogens**

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
<i>E. coli</i>	11.6±0.23	15.3±0.20	18.5±0.15	21.4±0.30
<i>Staphylococcus</i>	4.5±0.20	5.6±0.12	7.6±0.16	9.7±0.21
<i>Pseudomonas</i>	5.7±0.14	7.6±0.20	10.5±0.12	13.5±0.27
<i>Klebsiella</i>	10.6±0.35	12.3±0.47	15.6±0.34	17.3±0.32
<i>Proteus</i>	4.6±0.32	6.4±0.24	8.7±0.20	10.6±0.32
<i>Serratia</i>	7.6±0.14	8.2±0.25	9.6±0.37	10.5±0.27

Values are mean ± SD of three replicates

**Table 1b. Antimicrobial activity of methanolic extract of cinnamon against uropathogens**

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
<i>E. coli</i>	4.6±0.20	7.5±0.15	9.8±0.13	12.6±0.32
<i>Staphylococcus</i>	3.6±0.16	5.0±0.17	6.2±0.21	7.6±0.16
<i>Pseudomonas</i>	2.3±0.12	4.5±0.16	6.7±0.32	8.6±0.25
<i>Klebsiella</i>	3.6±0.7	6.3±0.17	8.3±0.27	10.6±0.24
<i>Proteus</i>	3.8±0.17	5.6±0.23	7.2±0.24	8.4±0.27
<i>Serratia</i>	6.5±0.10	7.3±0.12	8.4±0.15	9.3±0.12

Values are mean ± SD of three replicates

**Table 1c. Antimicrobial activity of aqueous extract of cinnamon against uropathogens**

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
<i>E. coli</i>	6.4±0.15	9.6±0.27	11.7±0.30	14.6±0.12
<i>Staphylococcus</i>	4.0±0.12	5.2±0.27	6.7±0.25	8.3±0.17
<i>Pseudomonas</i>	3.6±0.20	5.6±0.12	7.8±0.14	10.6±0.27
<i>Klebsiella</i>	5.6±0.31	7.6±0.32	9.3±0.33	11.7±0.17
<i>Proteus</i>	4.2±0.27	5.8±0.23	7.0±0.24	8.9±0.15
<i>Serratia</i>	6.9±0.17	7.8±0.12	8.9±0.34	9.8±0.47

Values are mean ± SD of three replicates

**Table 2a. Antimicrobial activity of ethanolic extract of black pepper against uropathogens**

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
<i>E. coli</i>	10.3±0.47	14.3±0.36	18.3±0.46	22.3±0.56
<i>Staphylococcus</i>	9.6±0.22	11.6±0.47	13.3±0.47	16.6±0.47
<i>Pseudomonas</i>	7.3±0.47	9.5±0.22	11.6±0.45	13.3±0.56
<i>Klebsiella</i>	7.6±0.94	8.3±0.45	9.6±0.35	10.3±0.47
<i>Proteus</i>	8.3±0.47	10.3±0.16	12.6±0.22	13.3±0.47
<i>Serratia</i>	5.3±0.42	7.3±0.33	9.6±0.47	10.0±0.47

Values are mean ± SD of three replicates

**Table 2b. Antimicrobial activity of methanolic extract of black pepper against uropathogens**

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
<i>E. coli</i>	8.5±0.34	10.5±0.32	12.3±0.32	14.3±0.24
<i>Staphylococcus</i>	7.3±0.22	9.6±0.25	11.3±0.24	14.6±0.32
<i>Pseudomonas</i>	4.5±0.14	5.3±0.24	6.5±0.33	7.3±0.47
<i>Klebsiella</i>	6.5±0.14	7.6±0.24	8.7±0.24	9.6±0.20
<i>Proteus</i>	5.3±0.24	7.3±0.25	9.6±0.32	11.3±0.34
<i>Serratia</i>	1.6±0.10	2.5±0.27	4.3±0.17	5.3±0.22

Values are mean ± SD of three replicates

**Table 2c. Antimicrobial activity of aqueous extract of black pepper against uropathogens**

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
<i>E. coli</i>	9.3±0.21	11.4±0.14	13.4±0.27	16.5±0.20
<i>Staphylococcus</i>	8.6±0.22	9.8±0.17	12.3±0.22	15.6±0.17
<i>Pseudomonas</i>	6.9±0.24	8.6±0.21	10.3±0.33	12.6±0.41
<i>Klebsiella</i>	7.0±0.24	8.6±0.26	10.3±0.25	12.4±0.21
<i>Proteus</i>	7.4±0.23	8.6±0.12	11.6±0.16	12.4±0.14
<i>Serratia</i>	3.6±0.17	5.3±0.22	7.3±0.21	8.6±0.27

Values are mean ± SD of three replicates

**Table 3a. Antimicrobial activity of ethanolic extract of clove against uropathogens**

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
<i>E. coli</i>	10.0±1.4	16.6±0.45	21.5±0.54	25.0±0.41
<i>Staphylococcus</i>	9.6±0.24	11.4±0.31	14.3±0.24	17.6±0.17
<i>Pseudomonas</i>	8.6±0.23	10.3±0.14	13.3±0.15	15.3±0.25
<i>Klebsiella</i>	8.4±0.24	10.2±0.20	12.6±0.14	13.6±0.25
<i>Proteus</i>	7.6±0.25	9.5±0.14	11.4±0.17	14.3±0.20
<i>Serratia</i>	6.3±0.17	8.3±0.15	11.5±0.25	15.2±0.16

Values are mean ± SD of three replicates

**Table 3b. Antimicrobial activity of methanolic extract of clove against uropathogens**

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
<i>E. coli</i>	6.6±0.16	9.3±0.12	11.5±0.25	15.6±0.27
<i>Staphylococcus</i>	7.6±0.15	9.3±0.36	11.0±0.21	14.3±0.17
<i>Pseudomonas</i>	6.7±0.18	8.6±0.22	11.6±0.17	13.3±0.17
<i>Klebsiella</i>	7.2±0.15	8.6±0.23	10.4±0.24	11.7±0.27
<i>Proteus</i>	6.2±0.15	8.4±0.12	10.6±0.20	12.3±0.23
<i>Serratia</i>	4.6±0.25	6.4±0.22	8.3±0.16	11.4±0.24

Values are mean ± SD of three replicates

**Table 3c. Antimicrobial activity of aqueous extract of clove against uropathogens**

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
<i>E. coli</i>	7.6±0.17	9.8±0.27	12.8±0.22	15.0±0.12
<i>Staphylococcus</i>	8.8±0.13	10.2±0.12	12.8±0.15	15.8±0.17
<i>Pseudomonas</i>	7.5±0.24	9.4±0.17	12.3±0.14	14.2±0.10
<i>Klebsiella</i>	7.8±0.12	9.6±0.15	11.6±0.17	12.8±0.14
<i>Proteus</i>	6.6±0.16	8.7±0.17	10.9±0.14	13.6±0.23
<i>Serratia</i>	5.8±0.13	7.6±0.17	9.7±0.17	12.6±0.18

Values are mean ± SD of three replicates

**Table 4a. Antimicrobial activity of ethanolic extract of carom seeds against uropathogens**

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
<i>E. coli</i>	7.5±0.20	8.3±0.14	9.7±0.15	10.7±0.12
<i>Staphylococcus</i>	6.5±0.14	7.6±0.17	8.6±0.14	10.3±0.14
<i>Pseudomonas</i>	4.5±0.11	6.7±0.10	8.7±0.10	10.0±0.12
<i>Klebsiella</i>	7.5±0.11	9.3±0.17	11.3±0.15	13.3±0.16
<i>Proteus</i>	6.3±0.14	8.7±0.12	10.5±0.13	13.7±0.12
<i>Serratia</i>	5.7±0.10	7.7±0.15	9.7±0.13	11.7±0.10

Values are mean ± SD of three replicates

**Table 4b. Antimicrobial activity of methanolic extract of carom seeds against uropathogens**

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
<i>E. coli</i>	5.7±0.15	7.3±0.12	8.4±0.10	9.5±0.14
<i>Staphylococcus</i>	4.6±0.12	5.7±0.14	6.9±0.10	8.5±0.10
<i>Pseudomonas</i>	3.7±0.14	5.2±0.14	6.9±0.15	8.3±0.15
<i>Klebsiella</i>	5.3±0.10	6.7±0.12	8.3±0.17	10.4±0.15
<i>Proteus</i>	4.9±0.18	6.7±0.14	8.6±0.14	10.7±0.17
<i>Serratia</i>	3.7±0.11	5.0±0.13	6.8±0.17	8.7±0.14

Values are mean ± SD of three replicates

**Table 4c. Antimicrobial activity of aqueous extract of carom seeds against uropathogens**

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
<i>E. coli</i>	6.3±0.23	7.8±0.21	9.0±0.23	10.2±0.21
<i>Staphylococcus</i>	5.7±0.14	6.9±0.14	8.0±0.10	9.8±0.11
<i>Pseudomonas</i>	4.0±0.14	5.8±0.13	7.6±0.11	9.7±0.12
<i>Klebsiella</i>	7.0±0.14	8.7±0.12	10.2±0.11	12.3±0.12
<i>Proteus</i>	5.6±0.13	7.3±0.12	9.4±0.13	11.6±0.17
<i>Serratia</i>	4.3±0.17	6.2±0.15	8.6±0.17	10.4±0.17

Values are mean ± SD of three replicates

**Table 5. Antimicrobial activity of cinnamon extracts in combination with antibiotics against uropathogens**

Name of organism	Antibiotic	Zone of inhibition (mm)	Antibiotic + ethanolic extract (mm)	Antibiotic + methanolic extract (mm)	Antibiotic + aqueous extract (mm)
<i>E. coli</i>	AMP	29.0±0.67	44±0.89	34±0.34	37±0.35
<i>Staphylococcus</i>	CHL	24.0 ±0.35	30±0.43	26±0.24	28±0.32
<i>Pseudomonas</i>	CIP	19.0 ±0.48	30±0.45	25±0.35	28±0.24
<i>Klebsiella</i>	AMK	11.0±0.62	25±0.34	20±0.24	22±0.27
<i>Proteus</i>	CHL	23.0±0.56	30±0.23	25±0.32	27±0.21
<i>Serratia</i>	CHL	13.0±0.45	20±0.37	14±0.25	16±0.26

AMP- Ampicillin; CHL- Chlorafloxacin; CIP- Ciprofloxacin; AMK- Amikacin

Conc. of extracts used: 4000 ppm

**Table 6. Antimicrobial activity of black pepper extracts in combination with antibiotics against uropathogens**

Name of organism	Antibiotic	Zone of inhibition (mm)	Antibiotic + ethanolic extract (mm)	Antibiotic + methanolic extract (mm)	Antibiotic + aqueous extract (mm)
<i>E. coli</i>	AMP	29±0.67	45±0.89	36±0.34	39±0.35
<i>Staphylococcus</i>	CHL	24 ±0.35	35±0.56	29±0.24	31±0.32
<i>Pseudomonas</i>	CIP	19 ±0.48	29±0.45	20±0.35	36±0.24
<i>Klebsiella</i>	AMK	11±0.62	19±0.34	14±0.24	16±0.27
<i>Proteus</i>	CHL	23±0.56	30±0.23	24±0.32	26±0.21
<i>Serratia</i>	CHL	13±0.45	20±0.37	15±0.25	17±0.26

Amp- Ampicillin; Chl- Chlorafloxacin; Cip- Ciprofloxacin; Amk- Amikacin

Conc. of extracts used: 4000 ppm



**Table 7. Antimicrobial activity of clove extracts in combination with antibiotics against uropathogens**

Name of organism	Antibiotic	Zone of inhibition (mm)	Antibiotic + ethanolic extract (mm)	Antibiotic + methanolic extract (mm)	Antibiotic + aqueous extract (mm)
<i>E. coli</i>	AMP	29±0.67	47±1.50	32±0.67	38±0.35
<i>Staphylococcus</i>	CHL	24 ±0.35	36±0.90	28±0.65	30±0.32
<i>Pseudomonas</i>	CIP	19 ±0.48	29±0.56	22±0.48	25±0.24
<i>Klebsiella</i>	AMK	11±0.62	21±0.85	13±0.78	17±0.27
<i>Proteus</i>	CHL	23±0.56	34±0.75	26±0.87	29±0.21
<i>Serratia</i>	CHL	13±0.45	25±0.65	18±0.56	20±0.26

*Amp- Ampicillin; Chl- Chlorafloxacin; Cip- Ciprofloxacin; Amk- Amikacin*

Conc. of extracts used: 4000 ppm

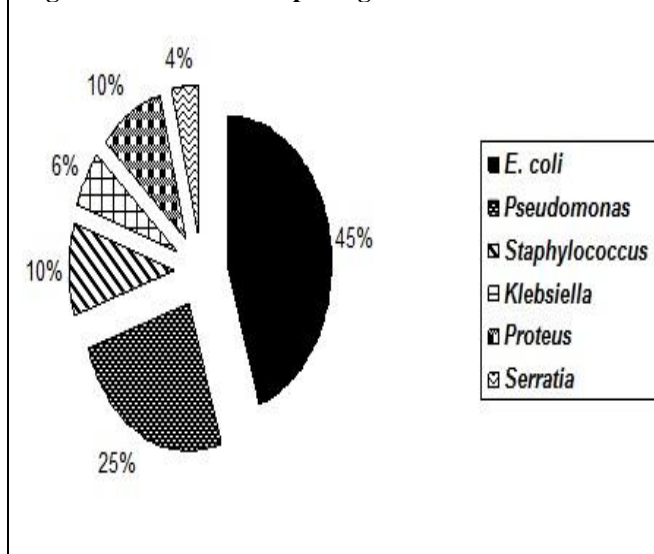
**Table 8. Antimicrobial activity of carom seeds extracts in combination with antibiotics against uropathogens**

Name of organism	Antibiotic	Zone of inhibition (mm)	Antibiotic + ethanolic extract (mm)	Antibiotic + methanolic extract (mm)	Antibiotic + aqueous extract (mm)
<i>E. coli</i>	AMP	29±0.67	36±1.30	30±0.67	32±0.35
<i>Staphylococcus</i>	CHL	24 ±0.35	30±0.50	24±0.45	21±0.32
<i>Pseudomonas</i>	CIP	19 ±0.48	26±0.36	22±0.28	24±0.24
<i>Klebsiella</i>	AMK	11±0.62	21±0.47	16±0.48	18±0.27
<i>Proteus</i>	CHL	23±0.56	30±0.75	24±0.47	26±0.21
<i>Serratia</i>	CHL	13±0.45	20±0.34	14±0.26	17±0.26

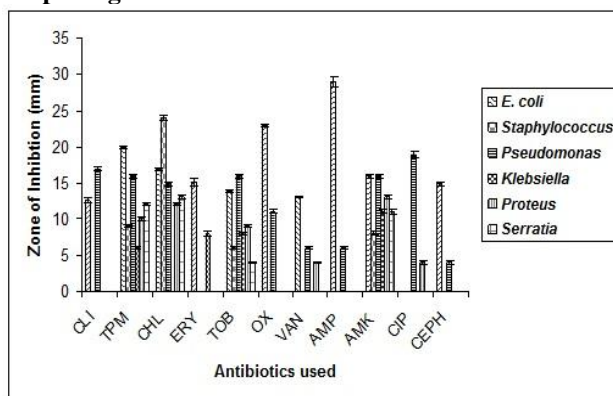
*Amp- Ampicillin; Chl- Chlorafloxacin; Cip- Ciprofloxacin; Amk- Amikacin*

Conc. of extracts used: 4000 ppm

**Fig 1. Prevalence of uropathogens**



**Fig 1. Effect of antibiotics on growth inhibition of uropathogens**



*CLI- Clindamycin; TPM- Trimethoprim; CHL- Chloramphenicol; ERY- Erythromycin; TOB- Tobramycin; OX- Oxacillin; VAN- Vancomycin; AMP- Ampicillin; AMK- Amikacin; CIP- Ciprofloxacin, CEPH- Cephalothin*

**DISCUSSION AND CONCLUSION**

The indiscriminate use of antibiotics for treatment of infectious diseases has led to increasing resistance amongst pathogens [13, 14]. Resistance to multiple drugs has become a common feature in most of the organisms associated with diarrhoea and other enteric diseases. The emergence of multidrug resistance among bacteria causing several life threatening infections, the increasing failure and spiralling cost of antibiotic therapy has led to screening of several medicinal plants for potential antimicrobial activity.

Spices are an integral part of human diet all across the world. They have been looked upon as flavor and aroma enhancement. Though our traditional health practices have written lots on the beneficial effect of these spices in the treatment of various ailments however it is in recent years they have become a feature of attraction for researchers working on phytomedicines [15, 16].

A lot of research is going on searching various medicinal plants for their antimicrobial property but people are not looking upon whether these plant extracts can work synergistically together and also with commonly

prescribed antibiotics. The present research work was focused on determining the prevalence of uropathogens in urinary tract infection and to study the antimicrobial activity of Indian spices alone and in combination with antibiotics against the uropathogens.

*E. coli* was observed to be dominant uropathogens as has already been reported in various previous studies [17, 18]. All extracts of spices showed good antibacterial property. All extracts of cinnamon showed highest potential against *E. coli*. The ethanolic and aqueous extract of black pepper was most effective against *E. coli* while the methanolic extract exhibited highest potential against *Staphylococcus*. The ethanolic and methanolic extract of clove was most effective against *E. coli* while aqueous extract was most effective against *Staphylococcus*. The ethanolic and methanolic extract of carom seeds was most

effective against *Proteus* while aqueous extract was most effective against *Klebsiella*.

The active constituent of spices may exhibit their antimicrobial effect either by degradation of cell wall, disruption of cytoplasmic membrane, leakage of cellular components, damage protein, interfere with the enzymatic activities inside cell, affect synthesis of DNA and RNA, affect electron transport and nutrient uptake, leakage of cellular components, impair the energy production inside cell, change fatty acid and phospholipid constituent. The extracts showed synergistic effect with antibiotics in exhibiting antimicrobial potential against uropathogens. The combination was found to be more potent than either of the two. Thus it may be concluded that the combination of antibiotics along with spices can be effectively used to combat urinary tract infections.

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