Evaluation of antimicrobial activity of cuminum cyminum & piper nigrum seed extract against isolated urinary pathogens

[1]Sharmila Donepudi, [2]U. Mahalakshmi, [3]M. Chandana, [4]R. Soniya, Mounika Sai, [5]T. Saileela
[1]associate professor, [2]Student, [3]Student, [4]Student, [5]Student
V. V. Institute of Pharmaceutical Sciences

***Abstract* - Urinary tract infection (UTI) is one of the most widely recognized bacterial disease in everybody all through the world. with growing concern about the greater occurrence of antibiotic resistance among bacteria and particularly uropathogens, many efforts were done to find effective agents. Cuminum cyminum L. (cumin) and Piper nigrum L. (black pepper) were reported with good antimicrobial activity. This study evaluated the activity of selected herb extract in combination against isolated uropathogens from 50 urine sample of patients suffering from Urinary tract infection. After isolation and screening of organism, susceptibility testing was carried out by disc diffusion method and minimum inhibitory concentration values by broth dilution testing. The activity was assessed by comparing alcoholic extract of selected herbs with standard drugs like Ciprofloxacin, Gentamicin and ampicillin common drugs used in UTI. Based on the results obtained it was made clear that both Cumin and Pepper extract exhibited suitable inhibition against urinary pathogens and in combination they exhibited improved activity. The MIC value of cumin extract against isolated urinary pathogens was determined by broth dilution method.**

***keywords* - Urinary tract infection, Cuminum cyminum L., Piper nigrum L., Disc diffusion method, Mininmum Inhibitory concentration**

Introduction:

Urinary tract infection (UTI) is one of the most widely recognized bacterial disease in everybody all through the world, influencing 150 million individuals consistently around the world. UTIs are a significant reason for morbidity among male newborn children, elderly people and in women of all ages (1). Serious complications of UTIs include repeated recurrences, pyelonephritis and subsequent sepsis and premature birth can be developed. The prolonged use of antibiotics may lead to high-level antibiotic resistance and Clostridium difficile colitis, are also very probable. In recent years, there has been growing concern about the greater occurrence of antibiotic resistance among bacteria and particularly uropathogens. The uncontrolled over use of antimicrobial drugs could lead to increased amounts of antibiotic-resistant pathogens, and could eventually compromise treatments of bacterial infections in humans (2-4).

Many herbs such as, cinnamon, myrrh, aloe, ginseng, basil, fennel, chives, cilantro, thyme, etc., are some important medicinal herbs and can be planted in kitchen garden. Many herbs are used as blood purifiers to alter or change a long-standing condition by eliminating the metabolic toxins. Certain herbs improve the immunity of the person, thereby reducing conditions such as fever. Some herbs are also having antibiotic properties such as Turmeric (5-7). In similar manner Cumin has reported to have various activities like carminative, antispasmodic (used in dyspepsia and diarrhoea), stimulant, diuretic, antibacterial, emmenagogue, galactagogue (8-13). Pepper has reported antioxidant, antitumor, antifungal, antibacterial, hepatoprotective, anti-asthmatic, GI stimulant, lipid metabolism accelerator, diuretic, etc (15-16). The present work aims to study the synergetic activity of cumin and pepper towards isolated urinary pathogens.

Material and Methods:

Chemicals: Cumin and Pepper seeds were purchased from local market. Urine samples of people who are proven to be having Urinary Tract Infection (UTI) were collected from local diagnostic centres. Solvents used for extraction purpose was procured from Merck chemicals and the media used was prepared in house.

Preparation of cumin and pepper extracts by using Soxhlet extraction:

Seeds of the "cumin" were coarsely powdered and subjected for "Soxhlet extraction" method. In this way, 200g of the powder was percolated in 1600ml of absolute methanol. At the end, samples were placed at 50°C in oven to remove the solvent. The extractions were stored in sterile dark vials at 4°C until used.

Isolation of microbes:

Primary isolation of uropathogens was performed by spread plate technique on Mac Conkey agar and Cysteine Lactose and Electrolyte Deficit agar (CLED). The plates were incubated at 37°C for 24 hrs. The plates with ≥105 CFU/ml were selected and colonies were further identified and isolated by conventional methods. The identification includes morphological and biochemical characterization. Which include gram staining, motility test, catalase test, Oxidase test, Nitrate reductase test, gelatin hydrolysis test, IMVic's tests and Urease test.

Antimicrobial activity:

The antimicrobial activity of alcoholic extract of selected herb in comparison with standard antibiotics was carried out by using disc diffusion method. Urine sample dilution equivalent to 110 CFU/ml was spread over the plates containing Nutrient agar using a sterile glass spreader in three directions in order to get a uniform microbial growth. In aseptic conditions, blank sterile discs (5 mm in diameter) were impregnated in methanolic extract of cumin and drug solution of 1mg/ml concentration. Discs were left for 5 min at room temperature for better absorption and were then placed on the inoculated agar surface. The Petri dishes were then placed in an incubator at 37°C for 24 hrs. After an incubation period, diameters of inhibition zones around the discs were measured.

Minimum Inhibitory Concentration (MIC):

The minimum inhibitory concentration was carried by broth dilution method. The MIC was carried on each isolated colonies individually. Nutrient broth (double Strength) test tubes were prepared, sterilized and label as UT (Uninoculated), CT (Control),1,2,3,4,5,6,7,8,9,10. In the first tube (UT) inoculums is not added which is used for checking the sterility of the medium and as a negative control and to other all test tubes inoculums 0.5ml is added. In all the test tubes, test anti-microbial compound was added ranging from 0.5 – 5ml with 0.5ml increment except uninoculated (negative control) & control (positive) tube. The positive control tube was used to check the suitability of the medium for growth of test micro organism and viability of the inoculums. The final volume (10ml) in all the test tubes was adjusted to 10ml by using sterile water. All test tubes were properly shaken and then incubated at 37◦C for 24hr.

RESULTS AND DISCUSSION

Morphological Characterization

MacConkey and CLED agar plates were inoculated by urine samples by spread plate and were observed for colony formation. The plates were subjected to total viable count. Plates with 112-186 CFU/ml were selected and morphological characteristics were observed. The observations were recorded in table -1.

Table -1: Morphological Characterization

Name of Colony	COLONY FEATURES		CELL FEATURES		Motility
	Colour of Colony	Nature of Colony	Gram Staining	Shape	
Mc(1)	Pink	Round, smooth & bulged	Gram negative	Rod	Non motile
Mc(2)	Pink	Round & smooth	Gram negative	Rod	Motile
Mc(3)	White	Smooth, bulged & convex	Gram negative	Rod	Motile
C(1)	Yellow	Small, Round & smooth	Gram negative	Rod	Motile
C(2)	Yellow	Round, smooth & bulged	Gram negative	Rod	Non motile
C(3)	White	Rough, bulged & convex	Gram negative	Rod	Motile
C(4)	Deep yellow	Round smooth	Gram positive	Cocci	Non motile

About three different colonies were observed on MacConkey agar and four different colonies, on CLED agar respectively. MacConkey is a very important medium as it supports the growth of gram negative bacteria and little gram positive bacteria. It also differentiates lactose fermented from non(-)lactose fermented. CLED medium supports many urinary pathogens growth. In isolated colonies four colonies were of gram negative motile organism, two were gram negative non motile and one colony gram positive non motile.

5.2 Biochemical tests

The isolated colonies were further subjected to biochemical testing. Biochemical tests are the tests used for the identification of bacteria species based on the differences in the biochemical activities of different bacteria. These differences in carbohydrate metabolism, protein metabolism, fat metabolism, production of certain enzymes, ability to utilize a particular compound, etc., help them to be identified by the biochemical tests. The observation of biochemical characterization was compiled in table-2.

Table-2: Biochemical Characterization

TESTS	Mc(1)	Mc(2)	Mc(3)	C(1)	C(2)	C(3)	C(4)
Catalase	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Oxidase	(-)	(-)	(+)	(-)	(-)	(+)	(-)
Nitrate reduction	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Gelatin hydrolysis	(-)	(+)	(+)	(+)	(-)	(+)	(+)
Indole	(-)	(+)	(-)	(+)	(-)	(-)	(-)
Methyl Red	(-)	(+)	(-)	(+)	(-)	(-)	(+)
Voges proskauer	(+)	(-)	(-)	(-)	(+)	(-)	(+)
Citrate utilisation	(+)	(-)	(+)	(-)	(+)	(+)	(+)
Urease	(+)	(-)	(-)	(-)	(+)	(-)	(+)

(+) = positive, (-) = negative

The above results gave an idea of the morphology, colony characteristics and biochemical nature of the isolated strains which helped in deriving an idea about the genus of the strains. The biochemical nature of the strains suggested that the genus of the isolated strains may be Escherichia, Kelbisella, Pseudomonas and Staphylococcus.

5.3 Antimicrobial activity by disc plate method

The antimicrobial activity was assessed by comparing alcoholic extract of *Cuminum Cyminum*, *piper niger* with standard drugs like Ciprofloxacin, Gentamicin and ampicillin common drugs used in UTI. The antibiotics were used at a concentration of 1mg/ml. the observation was presented in table-3. The graphical representation of comparative assessment was represented in fig. -1.

Table – 3: Antimicrobial activity

S. No.	Test compound	Zone of Inhibition (mm)					Average diameter (mm)
		1	2	3	4	5	
1	Solvent (methanol)	5	4.5	4.5	4.5	5	4.7
2	Ciprofloxacin (1mg/ml)	20	19	20	18	19	19.2
3	Gentamicin (1mg/ml)	10	9	9.5	10	9.9	9.7
4	Ampicillin (1mg/ml)	10	8	8.5	8.5	8.5	8.7
5	*Cuminum Cyminum*	10.5	12	10.5	11.5	10	10.9
6	*Piper Nigrum*	11.0	11.0	10.5	10.5	11.5	10.9
7	*Cuminum Cyminum*: *Piper Nigrum* (50:50)	15.0	14.5	14.5	15.0	15.2	14.84

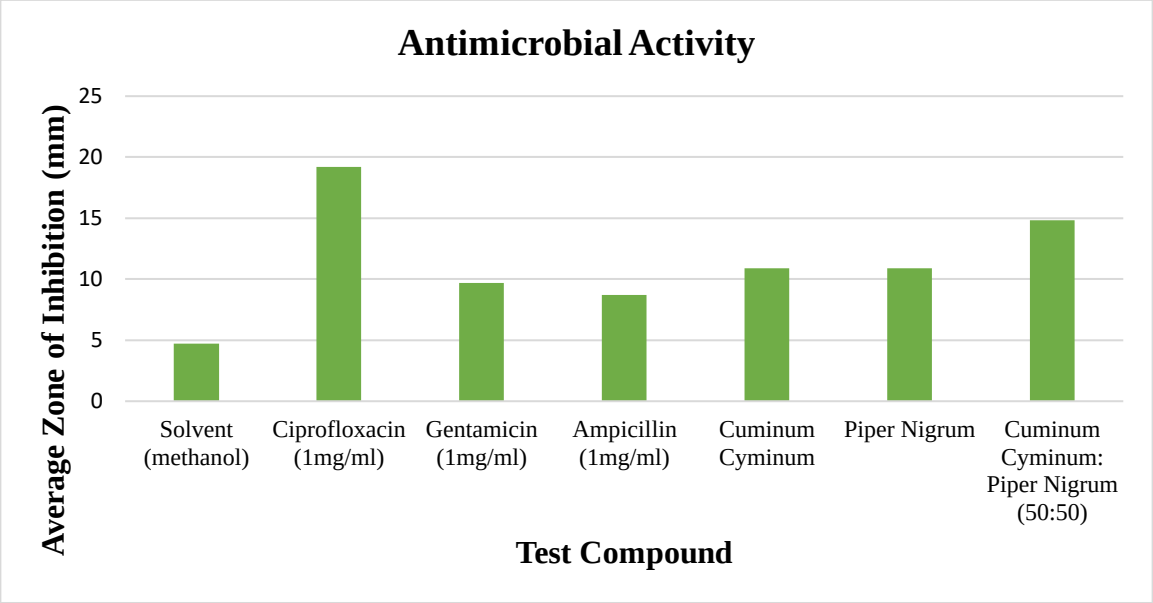

Figure – 1: Antimicrobial activity of *Cuminum Cyminum*

Ciprofloxacin showed higher inhibitory activity among all test compounds. It is followed by Gentamicin, Cumin and Ampilcillin. Based on the results obtained it was made clear that Cumin extract exhibited suitable inhibition against urinary pathogens.

5.4 Minimum inhibitory concentration

The MIC value of cumin extract against isolated urinary pathogens was determined by broth dilution method. The amount of Cumin Extract added varied from 0.5ml to5ml with 0.5ml increment. The results were obtained after 24 hr incubation. The data obtained through the determination of MIC are revealed in table-4.

Table – 4: MIC of selected herbs against isolated pathogens

Micro Organism	*Cuminum Cyminum* Extract	*Piper Nigrum* Extract	*Cuminum Cyminum*: *Piper Nigrum* (50:50)
Mc(1)	3.0ml	2.5ml	1.5ml
Mc(2)	3.0ml	3.5ml	2.0ml
Mc(3)	4.0ml	4.0ml	2.0ml
C(1)	3.0ml	2.5ml	1.5ml
C(2)	3.0ml	2.5ml	1.0ml
C(3)	4.0ml	3.0ml	1.5ml
C(4)	3.5ml	3.0ml	1.5ml

The minimum inhibitory activity of cumin varied from 30 to 40%. This indicated cumin had good inhibitory activity against urinary pathogens.

Summary and Conclusion:

From the infected sample seven different colonies were isolated. By the morphological and biochemical characterization helped in deriving an idea about the genus of the strains. The biochemical nature of the strains suggested that the genus of the isolated

strains may be *Escherichia, Kelbisella, Pseudomonas and Staphylococcus*. The antimicrobial activity was assessed by comparing alcoholic extract of Cuminum Cyminum and *Pepper Nigrum* with standard drugs like Ciprofloxacin, Gentamicin and ampicillin common drugs used in UTI. Based on the results obtained it was made clear that both Cumin and Pepper extract exhibited suitable inhibition against urinary pathogens and in combination they exhibited improved activity. The MIC value of cumin extract against isolated urinary pathogens was determined by broth dilution method. Activity of cumin varied from 30 to 40%, pepper varied from 25 to 40% whereas the combination herbs showed 10 to 20%. This indicated cumin and pepper in combination had good inhibitory activity against urinary pathogens.

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