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Threapeutic Analysis of Terminalia Chebula Against Uropathogenic *Escherichia Coli* (UPEC)

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Abstract: Urinary tract infection was caused by uropathogenic *Escherichia coli.* The most of strains which showed the resistance against the broad spectrum antibiotics were tested against fruit extracts of *Terminalia chebula*. The various fruits extracts of *Terminalia chebula* showed good antibacterial activity and killed the resistant uropathogenic *Escherichia coli* strains. The acetone and ethanol extracts of fruit *Terminalia chebula* showed the superior antibacterial activity than the cold and hot water extracts of *Terminalia chebula*. The minimal inhibitory concentration of acetone and ethanol extracts of *Terminalia chebula* was so effective to inhibit the uropathogenic *Escherichia coli* bacteria at low concentration. Thus Terminalia chebula fruit can be used as medicinal important fruit to over come the urinary tract diseases.

Key words: Uropathogenic *E. coli* · Fruit Extracts · *Terminalia chebula* · Antibacterial Activity · Minimal Inhibitory Concentration

INTRODUCTION

Urinary tract infection is a common cause of fever and one of the most common community acquired infections. In females 75- 90 % of infections are caused by *E. coli* followed by *Klebsiella sp* and *Proteus sp* [1, 2]. Identification of diarrhaegenic *E. coli* strains requires that these organisms be differentiated from nonpathogenic members of the normal flora [3]. Bacterial resistance to antimicrobial agents has been emerging and rapidly disseminating among many nosocomial and community acquired pathogens [4]. The development of resistance to older agents such as Ampicillin and Trimethoprim-sulfamethoxazone, as well as the emerging problem of fluoroquinolone resistance, may substantially limit our antibiotic choice [5]. One of the most important medicinal plants, which are widely used in the traditional system of medicine, is *Terminalia chebula*, which is also known as black myroblan a king of medicine [6, 7]. Eighty percent of nosocomial UTIs are related to urethral catheterization, while 5-10% is related to genitourinary manipulation [8]. Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals intermediates bioactive principles and lead compounds in synthetic drugs [9, 10]. *Terminalia chebula* dried fruit powders are used for treatment of various diseases [11, 12].

MATERIALS AND METHODS

Urine Sample Collection: A total of 40 Infected mid stream urine sample were screened for significant symptomatic bacteriuria [colony count > 1, 00, 000 CFU/ml]. Urine samples were collected from District Govt Hospital Erode during December 2011- February 2012. The samples were brought out into the microbiological laboratory and stored at 4°C.

Complete Urine Analysis

Physical Examination of Urine Sample: The physical examinations such as color, odor, appearance, volume and reaction of urine sample were observed and recorded.

Wet Mount Preparation: Five ml of well-mixed Urine samples was centrifuged at 3000rpm for 10 minutes. The supernatant was discarded and the sediment was examined microscopically under the high power objective (45X) for the presence of pus cells, epithelial cells,

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red blood cells, cast, crystals and bacteria motility. Based on the findings of bacteria in the urine sample, they were subjected for further cultivation process.

Processing of Urine Sample: Urine samples were examined microscopically [1] for gram staining, flagella staining then cultured on Eosin Methylene blue agar, Mac-Conkey agar and incubated for 24 hrs at 37°C. Following incubation the bacterial colonies were observed and result was recorded.

Identification of Pathogens: Isolated organisms were identified [13] and characterized on the basis of staining method and biochemical characteristics and results were identified with the help of Bergey's Manual of systematic Bacteriology.

Collection of Plant: Herbal Plant *Terminalia chebula* was collected from the dense forest at Bengali, Karnataka state, India, based on its ethno medical importance.

Preparation of Seed Powder: The seed powder was prepared by Nube *et al.* [10] washing with distilled water, surface sterilized with 10% sodium hypochlorite solution, rinsed with sterile distilled water and air dried at room temperature. The *Terminalia* fruits were dried in shadow and then milled to fine powder.

Extraction of Plant Material: The various fruit extractions of *Terminalia chebula* were obtained [14].

Cold Aqueous Extract: Ten grams 10g of powder was dissolved in 60ml of distilled water and kept in water bath at 80°C 80 degree Celsius for 2 hours. The extraction was filtered using a sterile muslin cloth. Then the filtrate was evaporated and concentrated to 4.25g.

Hot Aqueous Extract: Ten grams of powder was dissolved in 60ml of boiled distilled water and kept in water bath at 80°C80 degree Celsius for 2 hours. The extraction was filtered using a sterile muslin cloth. Then the filtrate was evaporated and concentrated to 4.03g.

Ethanol Extract: 10g of powdered sample was soaked in 50ml of ethanol and it was kept in Soxhlet apparatus at 80°C 80 degree Celsius for 48 hours. This extraction was taken, allowed for evaporation and then concentrated with Dimethyl Sulfoxide to 3.37g.

Acetone Extract: Ten grams of powdered sample was soaked in 50ml of acetone and it was kept in Soxhlet apparatus at 80°C degree Celsius for 48 hours. This extraction was taken and allowed for evaporation. It was concentrated with Dimethyl Sulfoxide to 3.95g.

Antibacterial Activity of Terminalia Chebula: The antibacterial activity of *Terminalia chebula* fruit powder was carried by Kim *et al*.[15] taking sterile paper discs (6mm, Hi-media, Mumbai) loaded with 30µl (500mg/ml) of the extracts dissolved in 10% Dimethyl sulfoxide and were left to dry for 12 hrs at 37°C in a sterile room. Bacterial suspensions were diluted to match the 0.5 McFarland standard scale 8 (approximately 1.5x10 CFU/ml) and they were further diluted to obtain a final inoculum. The Mueller Hinton agar was poured into petri dishes and allowed for solidification, then inoculated with 100µl of suspension containing 1x10 CFU/ml of bacteria. The discs treated with extracts were applied on the medium. Paper discs treated with DMSO were used as negative control. The plates were incubated at 37°C for 24 hrs. After incubation the inhibition zone diameters around each of the disc were measured and recorded.

Minimum Inhibitory Concentration: The minimal inhibitory concentration was carried by Kumarasamy *et al.* [16] using microtitre plate. Wells from each column in row 1 were marked and 100µl of stock acetone, ethanol, cold water, hot water (500mg/ml) and blank dimethyl sulfoxide solution was added. The 50µl of saline was added to rows 2-11. Two fold serial dilutions were performed by transferring 50µl of solution from row 1 to row 2, using a multichannel pipette. This was repeated down the row 2 to row12. The 40µl of double strength nutrient broth and 10µl of different bacterial solution was added to all the wells in separate column, so the final concentrations of the inoculum in all the wells were 5x10 CFU/ml. To prevent dehydration, the plates were covered with a plastic cover and then incubated at 37°C overnight. The bacterial growth was determined after addition of 40µl of detrazolium red (0.2mg/ml). The minimum inhibitory concentrations of the isolates were taken as the lowest concentration of the antibiotic of which the bacterial tested did no t show visible growth.

RESULTS

Urine Analysis: The urine samples were having bad odour with deep yellowish appearance appreance and showed acidic reactions. A few hyaline cast, sulpher

Table 1: Morphological and biochemical characteristics of uropathogenic Escherichia coli (UPEC)

S.No	Tests	Uropathogenic Escherichia coli	S.No	Tests	Uropathogenic Escherichia coli
1.	Gram's Reaction	Negative	11.	Nitrate Reduction	+
2.	Shape	Rod	12.	Carbohydrate Fermentation	A/A, Gas +
3.	Motility	+		Lactose	AG
4.	Indole	+		Glucose	AG
5.	Methyl Red	+		Xylose	AG
6.	Voges Proskauer	+		Galactose	AG
7.	Citrate Utilization	+		Maltose	AG
8.	Oxidase	-	14.	Media:	
9.	Catalase	-		Eosine Methylene Blue Mac Conkey	Metallic Green Sheen Pink colour colonies
10.	Urease	-			

(A) indicates Acid production, (AG) indicates Acid and Gas production

Table 2: Antibiotic resistant profile of uropathogenic Escherichia coli strains against various types of antibiotis Antibiotics Name Concentration (µg)

		Antibiotics Name Concentration(µg)									
S.No	Urinary Isolates	A (30)	Ak (30)	Ca (30)	C (30)	Co (25)	G (50)	Na (30)	Nx (30)	Of (30)	T (30)
1	E.coli 1	S	R	R	R	S	S	R	S	R	S
2	E.coli 2	S	S	R	R	S	S	R	S	R	S
3	E.coli 3	R	R	R	S	R	S	R	R	R	R
4	E.coli 4	R	R	R	R	R	R	R	R	R	R
5	E.coli 5	S	R	R	S	R	R	R	R	R	R

Note: A(30µg) - Ampicillin, Ak(30µg) - Amikacin, Ca(30µg) - Ceftazidime, C(30µg) -Chloramphenicol, C(25µg) - Cotrimaxazole, G(50µg)- Gentamycin, Na(30µg)-Nalidixic acid, Nx (30µg) -Norfloxacin, Of (30µg) -Ofloxacin, T(30µg) -Tetracycline. where R- indicates the resistance, S- Indicates sensitive

Table 3: The Minimum Inhibitory Concentration of acetone treated Terminalia chebula

		Minimum Inhibitory Concentration (mg/ml)											
S. No	Urinary isolates	500	250	125	62.5	31.25	15.63	7.81	3.91	1.953	0.977	0.488	0.244
1	E.coli 1	-	-	-	-	-	-	-	-	-	-	-	-
2	E.coli 2	-	-	-	-	-	-	-	-	-	+	+	+
3	E.coli 3	-	-	-	-	-	-	-	-	-	+	+	+
4	E.coli 4	-	-	-	-	-	-	-	-	-	+	+	+
5	E.coli 5	-	-	-	-	-	-	-	-	-		+	+

Where (-) Indicates the no bacterial growth thus showed susceptibility of minimal inhibitory concentration of acetone extracts of Terminalia chebula while as (+) indicates the bacterial growth thus showed maximum resistance of bacterial strains.

Table 4: The Minimum Inhibitory Concentration of ethanol extracts of Terminalia chebula

		Minimum Inhibitory Concentration (mg/ml)											
S.No	Urinary isolates	500	250	125	62.5	31.25	15.63	7.81	3.91	1.953	0.977	0.488	0.244
1	E.coli 1	-	-	-	-	-	-	-	-	-	-	-	-
2	E.coli 2	-	-	-	-	-	-	-	-	-	-	+	+
3	E.coli 3	-	-	-	-	-	-	-	-	-	-	-	+
4	E.coli 4	-	-	-	-	-	-	-	-	-	-	+	+
5	E.coli 5	-	-	-	-	-	-	-	-	-	-	-	+

Where (-) Indicates the no bacterial growth thus showed susceptibility of minimal inhibitory concentration of ethanol extracts of Terminalia chebula while as (+) indicates the bacterial growth thus showed maximum resistance of bacterial strains

Table 5: The Minimum Inhibitory Concentration of cold water extracts of Terminalia chebula

		Minimum Inhibitory Concentration (mg/ml)											
S.No	Urinary isolates	500	250	125	62.5	31.25	15.63	7.81	3.91	1.953	0.977	0.488	0.244
1	E.coli 1	-	-	-	-	-	-	-	-	-	+	+	+
2	E.coli 2	-	-	-	-	-	-	-	-	-	+	+	+
3	E.coli 3	-	-	-	-	-	-	-	+	+	+	+	+
4	E.coli 4	-	-	-	-	-	-	-	-	+	+	+	+
5	E.coli 5	-	-	-	-	-	-	-	-	+	+	+	+

Where (-) Indicates the no bacterial growth thus showed susceptibility of minimal inhibitory concentration of cold water extracts of Terminalia chebula while as (+) indicates the bacterial growth thus showed maximum resistance of bacterial strains

Table 6:The Minimum Inhibitory Concentration of hot water extracts of Terminalia chebula

		Minimum Inhibitory Concentration (mg/ml)											
S.No	Urinary isolates	500	250	125	62.5	31.25	15.63	7.81	3.91	1.953	0.977	0.488	0.244
1	E.coli 1	-	-	-	-	-	-	-	-	-	-	-	-
2	E.coli 2	-	-	-	-	-	-	-	-	+	+	+	+
3	E.coli 3	-	-	-	-	-	-	-	-	+	+	+	+
4	E.coli 4	-	-	-	-	-	-	-	+	+	+	+	+
5	E.coli 5	-	-	-	-	-	-	-	+	+	+	+	+

Where (-) Indicates the no bacterial growth thus showed susceptibility of minimal inhibitory concentration of hot water extracts of Terminalia chebula while as (+) indicates the bacterial growth thus showed maximum resistance of bacterial strains

crystals, pus cells, epithelial cells and red blood cells were found. The bacteria showed active motility under hanging drop method.

Identification of Uropathogenic Escherichia Coli: The morphology and biochemical characteristics of the uropathogenic isolates were identified and tabulated (Table 1).

Antibiotic Sensitivity Test: In this study, ten antibiotic discs were used for treatment of urinary tract infections. The isolates showed 100% resistance to Ceftazidime (50µg) followed by 96% to Ofloxacin (30µg) 92% of Norfloxacin (30µg), 84% of Nalidixic acid (30µg) and 80% of Tetracycline (30µg). 51-70% isolates were resistant to Ampicillin (30µg), Amikacin (30µg), Chloramphenicol (25µg),Co-trimoxazole (50µg) and Gentamycin (20µg) are presented in presented in Table 2.

Terminalia Chebula: Kingdom-Plantae, Subkingdom-Tracheobionta, Superdivision-Spermatophyta, Division-Magnoliophyta, Class-Magnoliopsida, Subclass -Rosidae, Order- Myrtales, Family- Combretaceae, Genus-Terminalia, Species- *Terminalia chebula*

Antibacterial Activity of T. Chebula: Antibacterial activity of the dried fruit extract from *T. chebula* against urinary tract pathogens using disc method. The acetone and ethanol extracts showed good antibacterial activity against *E.coli* strains than the cold and hot water extract.

Minimum Inhibitory Concentration: The Minimum inhibitory concentration of various *T.chebula* extracts against uropathogenic *E. coli* isolates showed that acetone extract (Table 3) and Ethanol extracts (Table 4) exhibit superior activity against uropathogenic *E. coli* strains. While as aqueous extracts of cold water (Table 5) and hot water extracts (Table 6) exhibit the least activity against uropathogenic *E. coli* strains.

DISCUSSION

Most infection arises from one type of bacterium, *E. coli* which normally lives in the colon. The urinary system is structured in such a way that prevents the entry of infection [17]. Pathogenic isolates of *E. coli* have relatively high potentials for developing resistance [5]. High resistance of *E. coli* to various antimicrobial agents tested was observed in this study. This is similar to what was observed by who reported 100% resistance of their *E. coli* isolates to ampicillin and amoxicillin [18]. Their finding is in harmony with the report of this study, showing 69% and 88% resistance to cotrimoxazole and tetracycline respectively [19]. Multiple drug resistance among urinary tract isolates in USA was reported to be 7.1% in 2000 [20]. Such multi drug resistance has serious implications for the empiric therapy of infections caused by *E. coli* and for the possible co-selection of antimicrobial resistance mediated by multi drug resistance plasmids [21].The extracts of *T. chebula* exhibited growth inhibitory activity against two dental carries causing bacterial strain [6]. The susceptibility of the positive control for bacteria i.e Ciprofloxacin towards the acetonic extract of *S. aureus* was more or less the same [22]. It is also observed that the acetone extract *T. chebula* was more potent against *E.coli* compared to other tested extracts. The acetone and ethanol extracts of fruits of the *T. chebula* showed greater antimicrobial activity than the corresponding cold and hot water extracts [23]. The extract of *T. chebula* at the concentration of 100% has anti-bacterial activity on the tested microorganisms from high to low respectively [24]. On other prospects extracts of *T. chebula* showed activity on *B. subtilus, S. aureus, S.epidermis, E. coli*, *S. flexineria, P. aeruginosa* (25]. It was observed that *S. aureus* and *P. aeruginosa* have shown minimum inhibitory concentration value at 1% concentration of plant extract [15]. Other bacteria have shown very small zone at 1% concentration of the extract. On comparing the inhibition zone of the extract to that of standard

antibiotics such as Tobramycin, Cephlaxin and Amoxicillin, *Terminalia chebula* extract showed better activity is not potent than erythromycin and amoxicillin in these condition [26]. In the end of study we have found *T. chebula* extract exhibit anti-bacterial activities against uropathogenic *Escherichia coli* [12].

REFERENCES

1. Kunin, C.M., 1994. Urinary tract infection in fermale. Clinical Infect Diseases, 18: 1-12.
2. Thomas, M. and M.D. Hooton, 2012. Uncomplicated Urinary Tract Infection. N. Engl. J. Med., 366: 1028-1037.
3. Toma, C., Y. Lux, N. Higa, N. Nakasone and C. Isbel, A. Baschkier, M. Rivas and M. Iwanaga, 2003. Multiplex PCR Assay for Identification of Human Diarrheagenic *Escherichia coli*. Journal of Clinical Microbiology. 41(6): 2669-2671.
4. Manikandan, S., S. Ganesapandian, S. Manoj and A.K. Kumaraguru, 2011. Emerging of Multidrug Resistance Human Pathogens from Urinary Tract Infections. Current Research in Bacteriology. 4(1): 9-15.
5. Karlowsky, J.A., L.J. Kelly, C. Thornsberry, M.A. Jones and D.F. Sahm, 2002. Trends in Antimicrobial Resistance among Urinary Tract Infection Isolates of Escherichia coli from Female Outpatients in the United States. Antimicrobial Agents Chemother. 46(8): 2540-2545.
6. Anitha, M., B.N. Satish Kumar, B.M. Vrushabendra Swamy and S. Archana, 2010. A Review on Natural Diuretics. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 1(4): 615-634.
7. Kannan, P., S.A. Ramadevi and H. Waheeta, 2009. Antibacterial activity of *Terminalia chebula* fruit extract. Journal of Microbiology, 3(4): 180-184.
8. Tunn, R. and E. Petri, 2003. Introital and transvaginal ultrasound as the main tool in the assessment of urogenital and pelvic floor dysfunction: an imaging panel and practical approach. Ultrasound Obstet. Gynecol., 22: 205-213.
9. Ebi, G.C. and S.I. Ofoefule, 2000. Antimicrobial activity of Pterocarpus osun stems. Fitoterapia, 71(4): 433-435.
10. Ncube, N.S., A.J. Afolayan and A.I. Okah, 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology. 7(12): 1797-1806.
11. Ashish, A., G. Anil, N.K. Naveen and D. Kapilesh, 2010. Antibacterial Activity of Hydroalcoholic Extract of *Terminalia chebula* Retz on Different Gram-positive and Gram-negative Bacteria. International Journal of Pharmaceutical & Biological Archives. 1(4): 485-488.
12. Ghosh, A., B.K. Das, A. Roy, B. Mandal and G. Chanda, 2008. Antimicrobial activity of some medicinal plants extracts. Journal of Nat. Med., 62(2): 259-262.
13. Holt, J., N. Krieg, P. Stanely and S. William, 1994. Enterobacteriaceae. In: Bergey's manual of Determinative Bacteriology, 9th edn. Baltmore: Williams and Walkin Co., pp: 87-175.
14. Chessbrough, M. ,2000. Medicinal laboratory manual for tropical countries, Lianacre Houses. Volume II: Microbiology. Chap. 44: 289-311. Butterworth-Heinemann Ltd. Linacre House, Jordan. Hill, Oxford.
15. Kim, H.G., J.H. Cho, E.Y. Jeong, J.H. Lim, S.H. Lee and H.S. Lee, 2006. Growth inhibitory activity of active compound isolated from *Terminalia chebula* fruits against intestinal bacteria. Journal of Food Production. 69(9): 2205-2209.
16. Kumarasamy, Y., P.J. Cox, M. Jaspars, L. Nahar and S.D. Sarker, 2002. Screening seed of scotish plants antibacterial activity. Journal of Ethnopharmacon. 83(1-2): 13-77.
17. Goossenen, H., P. Mol, M. Hall and J.B. Butzler, 1985. Prevalence of asymptomatic bacteria and comparison between different screening for its detection in infants methods European Journal of Epidermology. 1(4): 301-304
18. Taiz, Q. and E. Zeiger, 2006. Plant physiology 4th Edn., 13: 315- 344.
19. Susan, S., J. Huang Brian, C. Labus Michael, T. Samuel Dairian and L.R. Arthur, 2002. Antibiotic Resistance Patterns of Bacterial Isolates from Blood in San Francisco County, California, 1996-1999. Emerging Infectious Diseases. 8(2): 195-201.
20. David, M.B. and J.P. Antoni, 2011. Does clinical examination aid in the diagnosis of urinary tract infections in women? A systematic review and meta-analysis. BMC Fam Pract. 12: 111-119.
21. Ahmad, I., Z. Mehmood and F. Mohammad, 1989. Screening of some Indian medical plant for their antimicrobial properties. Journal of Ethnopharmacon. 62(2): 183-193.

22. Kiyota, H., 2006. Synthesis of naturally derived bioactive compounds of agricultural interest. Biosci Biotechnol Biochem., 70(2): 317-24.
23. Nirmala, N.R., M.T. Urmila and A.D. Sharadini, 1999. Adaptogenic properties of six rasayana herbs used in Ayurvedic medicine. Phytotherapy Research. 13(4): 275-291.
24. Ates, D.A. and O.T. Erdoúrul, 2003. Antimicrobial Activities of Various Medicinal and Commercial Plant Extracts. Truk. Journal of Biol., 27:157-162.
25. Archana, S., C. Abhishek, S. Madhuliki, J. Farrukh, P. Preethi, M.R. Siron, W.B. Falgun and V. Lakshmi, 2010. Inhibition of Hyaluronidase activity of human and rat spermatozoa *in vitro* by Terminalia, Flavanoid richplant. Reproductive Toxicology. 29(2): 214-224.
26. Chattopadhyay, R.R., S.K. Bhattacharyya, B. Anwesa, B. Premananda and P. Nitish Kumar, 2009. Evaluation of antibacterial properties of *Chebulic myrobalan* (fruit of *Terminalia chebula* Retz.) extracts against methicillin resistant *Staphylococcus aureus* and trimethoprim-sulphamethoxazole resistant uropathogenic *Escherichia coli*. African Journal of Plant.