Research Article

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TERMINALIA CHEBULA: A TREATMENT AGAINST PATHOGENIC *PROTEUS VULGARIS* STRAINS ASSOCIATED WITH URINARY TRACT INFECTION

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Received on: 02/01/13 Revised on: 20/02/13 Accepted on: 10/03/13

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DOI: 10.7897/2277-4343.04422

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ABSTRACT

Terminalia chebula was used to find out the new sort of treatment for the urinary tract infections caused by *Proteus vulgaris.* The causative agent was identified as *Proteus vulgaris* by staining and biochemical methods. It is responsible to cause urinary tract infection and most of strains show the resistance against the broad spectrum antibiotics: Ceftazidime (30µg), Ofloxacin (50µg), Norfloxacin (30µg), Tetracycline (30µg), Ampicillin (30µg), Chloramphenicol (25µg) and Gentamycin (20µg). The preparation of seed powders and antibacterial activity of *Terminalia chebula* was carried out. The acetone and ethanol fruits extracts of *Terminalia chebula* showed good antibacterial activities and killed the resistant urinary tract associated pathogens of *Proteus vulgaris* strains. The ethanol extracts of fruit *Terminalia chebula* showed the superior antibacterial activity than the acetone extracts of *Terminalia chebula*. The minimal inhibitory concentrations of acetone and ethanol extracts of *Terminalia chebula* were effective to inhibit the urinary tract pathogenic *Proteus vulgaris* strains at low concentration. Thus *Terminalia chebula* fruit can be used as medicinal important fruit to overcome the urinary tract diseases.

Key words: Antibiotic resistance, *Proteus vulgaris, Terminalia chebula*, urinary tract infection

INTRODUCTION

Bacterial resistance to antimicrobial agents has been emerging and rapidly disseminating among many nosocomial and community acquired pathogens.[1] 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.[2] 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.[3] Eighty percent of nosocomial UTIs are related to urethral catheterization while 5-10% is related to genitourinary manipulation.[4] Identification of diarrhaegenic *Escherichia coli* strains requires that these organisms be differentiated from nonpathogenic members of the normal flora.[5] Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceuticals intermediates bioactive principles and lead compounds in synthetic drugs.[6] 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 myrobalan a king of medicine.[7] *Terminalia chebula* dried fruit powders are used for treatment of various diseases[8, 9].

MATERIALS AND METHODS

Urine Sample Collection

A total of 40 infected mid-stream urine samples were screened for significant symptomatic bacteriuria [colony count > 1, 00, 000 CFU/ml]. Urine samples were collected from District Govt Hospital Erode, India during December 2011- February 2012. The samples were brought out into the microbiological laboratory and stored at 4^0C.

Analysis of Urine Samples

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 3000 rpm for 10 minutes. After centrifugation the supernatant was discarded and sediment was examined microscopically under high power objective (45x) for the presence of pus cells, epithelial cells, 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 for gram staining and flagella staining. Then cultured on Heart infusion agar, Mac-Conkey agar and incubated for 24 hours at 37^0C. Following incubation the bacterial colonies were observed and results were recorded.

Identification of Pathogens

Isolated organisms were identified[10] and characterized on the basis of staining methods as well as biochemical characteristics. The results were identified with the help of Bergey's Manual of systematic Bacteriology.

Collection of Fruit Plants
Herbal fruit Plants of *Terminalia chebula* were collected from the dense forest at Bengali, Karnataka State, India, based on its ethnomedical importance.

Preparation of Seed Powder
The seed powder was prepared[6] by washing with distilled water and surface sterilized with 10% sodium hypochlorite solution then rinsed with sterile distilled water and air dried at room temperature. The *Terminalia chebula* fruits were further dried under shadow and milled to fine powder.

Extraction of Plant Material
The various extracts of *Terminalia chebula* fruits were obtained[11].

Ethanol Extract
10 g of powdered *Terminalia chebula* sample was soaked in 50 ml of ethanol and it was kept in Soxhlet apparatus at 80 ^{0}C for 48 hours. This extraction was taken and allowed for evaporation then concentrated with Dimethyl Sulfoxide to 3.37g.

Acetone Extract
10 g of powdered *Terminalia chebula* sample was soaked in 50ml of acetone and it was kept in Soxhlet apparatus at 80 ^{0}C for 48 hours. This extraction was taken and allowed for evaporation. It was concentrated with Dimethyl Sulfoxide to 3.95 g.

Antibacterial Activity of *Terminalia chebula*
The antibacterial activity of *Terminalia chebula* fruit powder was carried out[12] by taking sterile paper discs (6mm, Hi-media, Mumbai) loaded with 30 µl (500 mg/ml) of the extracts dissolved in 10% Dimethyl sulfoxide and were left to dry for 12 hours at 37 ^{0}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 100 µl 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 ^{0}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[13] using microtitre plate. Wells from each column in row 1 were marked and 100µl of stock acetone and ethanol (500mg/ml) and blank Dimethyl sulfoxide solution was added. The 50µl of saline was added to rows 2-10. 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 row 11. 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^{0}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 on which the test bacteria did not show visible growth.

RESULT

Urine Analysis
The urine samples were having bad odour with deep yellowish appearance and showed acidic reactions. A few hyaline cast, sulpher crystals, pus cells, epithelial cells and red blood cells were found. The bacteria showed active motility under hanging drop method.

Identification of Urinary Tract Associated *Proteus vulgaris* Strains
The morphology and biochemical characteristics of the urinary tract associated pathogenic isolates were identified and tabulated (Table 1).

Antibiotic Sensitivity Test
In this study, seven antibiotic discs were used against the bacteria isolated from urinary tract infected urine sample. The isolates showed 100% resistance to Ceftazidime (30 µg), Ofloxacin (50 µg) followed by 96% to 92% of Norfloxacin (30 µg), 84% to Tetracycline (30 µg), 51-70% isolates were resistant to Ampicillin (30 µg), Chloramphenicol (25 µg) and Gentamycin (20 µg) (Table 2).

Identification of Fruits
Kingdom- Plantae, Sub-kingdom- Tracheobionta, Super-division- Spermatophyta, Division- Magnoliophyta, Class Magnoliopsida, Subclass- Rosidae, Order- Myrtales, Family- Combretaceae, Genus- Terminalia, Species- *Terminalia chebula*

Antibacterial activity of *Terminalia chebula*
Antibacterial activity of acetone and ethanol extracts from *Terminalia chebula* against urinary tract infection associated with *Proteus vulgaris* strains by using well method showed good antibacterial activity against *Proteus vulgaris* strains.

Minimum Inhibitory Concentration
The Minimum inhibitory concentration of various *Terminalia chebula* extracts against urinary tract infection associated with *Proteus vulgaris* isolates showed that in acetone extract (Table 3) and ethanol extracts (Table 4) ethanol extracts exhibit superior activity against urinary tract associated *Proteus vulgaris* strains .

DISCUSSION

Multiple drug resistance among urinary tract isolates in United States of America was reported to be 7.1% in 2000.[14] Such multi drug resistance had serious implications for the empiric therapy of infections caused by urinary tract associated *Proteus vulgaris* for the possible co-selection of antimicrobial resistance mediated by multi drug resistance plasmids.[15]

Table 1: Morphological and biochemical characteristics of urinary tract associated isolates of *Proteus vulgaris* strains

Tests	Urinary tract associated *Proteus vulgaris* strains
Gram's Reaction	Negative Rod shaped
Motility	Active motile with peritrichious flagella
Indole	Positive
Methyl Red	Positive
Voges Proskauer	Negative
Citrate Utilization	Negative
Oxidase	Negative
Catalase	Positive
Urease	Positive
Nitrate Reduction	Positive without gas
Carbohydrate Fermentation	Ferment Glucose, Sucrose and Maltose Gas +

Table 2: Antibiotic resistant profile of urinary tract associated *Proteus vulgaris* strains against various types of antibiotics

Urinary Tract Associated Isolates	A (30)	Ca (30)	C (30)	G (50)	Nx (30)	Of (30)	T (30)
Proteus vulgaris strain TP1	S	R	S	S	R	R	S
Proteus vulgaris strain TP2	R	R	R	R	S	R	S
Proteus vulgaris strainTP3	S	R	R	S	R	R	R
Proteus vulgaris strain TP4	R	R	R	R	S	R	R
Proteus vulgaris strainTP5	S	R	S	S	R	R	R

Note: A (30µg) - Ampicillin, Ca (30µg) - Ceftazidime, C (30µg) -Chloramphenicol, G (50µg)- Gentamycin, Nx (30µg) -Norfloxacin, Of (30µg) -Ofloxacin, T (30µg) -Tetracycline. Where R- indicates the resistance and S- Indicates sensitive

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

Urinary tract associated isolates	250	125	62.5	31.25	15.63	7.81	3.91	1.953	0.977	0.488	0.244
Proteus vulgaris strainTP1	-	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris strainTP2	-	-	-	-	-	-	-	-	+	+	+
Proteus vulgaris strainTP3	-	-	-	-	-	-	-	-	+	+	+
Proteus vulgaris strain TP4	-	-	-	-	-	-	-	-	+	+	+
Proteus vulgaris strain TP5	-	-	-	-	-	-	-	-		+	+

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*

Urinary Tract Associated isolates	250	125	62.5	31.25	15.63	7.81	3.91	1.953	0.977	0.488	0.244
Proteus vulgaris strainTP1	-	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris strainTP2	-	-	-	-	-	-	-	-	-	+	+
Proteus vulgaris strainTP3	-	-	-	-	-	-	-	-	-	-	+
Proteus vulgaris strain TP4	-	-	-	-	-	-	-	-	-	+	+
Proteus vulgaris strain TP5	-	-	-	-	-	-	-	-	-	-	+

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

Pathogenic isolates of *Proteus vulgaris* have relatively high potentials for developing resistance.[2] This is similar to what was observed by WHO report where 100% resistance of *Proteus vulgaris* isolates to ampicillin and amoxicillin were found that *Proteus vulgaris* strains showed 100% resistance against Ceftazidime and Ofloxacin. Their finding is in harmony with the report of this study showing 51% and 70% resistance to ampicillin, chloramphenicol and tetracycline respectively.[16] The extracts of *Terminalia chebula* exhibited growth inhibitory activity against two dental carries causing bacterial strain.[17] The acetone and ethanol extracts of fruits of the *Terminalia chebula* showed good antimicrobial activity.[18] It is also observed that the ethanol extract of *Terminalia chebula* was more potent against urinary tract associated pathogenic *Proteus vulgaris* strains when compared to acetone extracts. The susceptibility of the positive control Ciprofloxacin and acetone extract towards *Staphylococcus aureus* was comparable.[19] The extract of *Terminalia chebula* at the concentration of 100% has anti-bacterial activity on the tested microorganisms from high to low *Escherichia coli*, *Shigella flexineria* and *Pseudomonas aeruginosa* respectively.[20] On comparing the inhibition zone of the extract to that of standard antibiotics such as Ampicillin, Ceftazidime, Ofloxacin, Chloramphenicol, Gentamycin, Norfloxacin and Tetracycline the *Terminalia chebula* extract showed better activity against urinary tract associated *Proteus vulgaris* strains.[21]

CONCLUSION

This study revealed that *Terminalia chebula* fruit plays dual benefits as medicinal values and food values. Our study found that *Terminalia chebula* fruit were found to be potential antibacterial agents against urinary tract pathogens of *Proteus vulgaris* strains. The acetone and ethanol extract showed better activities against urinary tract associated *Proteus vulgaris* strains and also shown the maximum zone of inhibition when compared with standard antibiotics Ampicillin, Ceftazidime, Ofloxacin, Chloramphenicol, Gentamycin, Norfloxacin and Tetracycline. Thus this research suggested that *Terminalia chebula* fruit can be used an alternative medicine against the bacterial infection which can overcome the problem of drug resistant strains of *Proteus vulgaris*.

REFERENCES

1. Manikandan S, Ganesapandian S, Manoj S, Kumaraguru AK. Emerging of Multidrug Resistance Human Pathogens from Urinary Tract Infections. Current Research in Bacteriology 2011; 4(1):9-15. http://dx.doi.org/10.3923/crb.2011.9.15
2. Karlowsky JA, Kelly LJ, Thornsberry C, Jones MA, Sahm DF. Trends in Antimicrobial Resistance among Urinary Tract Infection Isolates of Escherichia coli from Female Outpatients in the United States. Antimicrobial Agents Chemother, 2002;46(8): 2540–2545. http://dx.doi.org/10.1128/AAC.46.8.2540-2545.2002 PMCid:127340
3. Thomas M, Hooton MD. Uncomplicated Urinary Tract Infection.N. Engl. J. Med. 2012;366:1028-1037 http://dx.doi.org/10.1056/NEJMcp1104429 PMid:22417256
4. Tunn R, Petri E. 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. 2003;22:205–213.http://dx.doi.org/10.1002/uog.189PMid:12905521
5. Toma C, Lux Y, Higa N, Nakasone N, Isbel C, Baschkier A, Rivas M, Iwanaga M, Multiplex PCR. Assay for Identification of Human DiarrhaegenicEscherichia coli. Journal of Clinical Microbiology, 2003; 41(6): 2669–2671. http://dx.doi.org/10.1128/JCM.41.6.2669-2671.2003 PMid:12791900 PMCid:156568
6. Ncube NS, Afolayan AJ, Okah AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology, 2008; 7(12):1797-1806.
7. Kannan P, Ramadevi, SA, Waheeta H. Antibacterial activity of Terminalia chebula fruit extract.Journal of Microbiology, 2009; 3(4):180-184.
8. Ashish A, Anil G, Naveen KC, Kapilesh D. Antibacterial Activity of Hydroalcoholic Extract of Terminalia chebula Retz on Different Gram-positive and Gram-negative Bacteria. International journal of pharmaceutical & Biological Archives, 2010; 1(4): 485-488.
9. Ghosh A, Das BK, Roy A, Mandal B, Chanda G. Antimicrobial activity of some medicinal plants extracts. Journal of Nat. Med, 2008; 62(2):259-262. http://dx.doi.org/10.1007/s11418-007-0216-x PMid:18404337
10. Holt J, Krieg N, Stanely P, William S. Enterobacteriaceae. In: Bergey's manual of Determinative Bacteriology, 9th edn. Baltmore: Williams and Walkin Co., 1994; pp. 87-175.
11. Chessbrough M. Medicinal laboratory manual for tropical countries, Lianacre Houses. Volume II: Microbiology.2000;Chap. 44:289-311. Butterworth-Heinemann Ltd., Linacre House, Jordan. Hill, Oxford.
12. Tariq AL and Reyaz AL. Extraction of cannabinoids from Cannabis sativa L plant and its potential antimicrobial activity. Universal Journal of Medicine and Dentistry, 2012; 1(4):051-055.
13. Kumarasamy Y, Cox PJ, Jaspars M, Nahar L, Sarker SD. Screening seed of Scottish plants antibacterial activity. Journal of Ethnopharmacon, 2002; 83(1-2): 13-77. http://dx.doi.org/10.1016/S0378-8741(02)00214-3
14. David MB, Antoni JP. Does clinical examination aid in the diagnosis of urinary tract infections in women? A systematic review andmeta-analysis.BMCFamPract,2011;12:111-119. http://dx.doi.org/10.1186/1471-2296-12-111 PMid:21985418 PMCid:3207883
15. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medical plant for their antimicrobial properties. J. Ethnopharmacon. 1989;62(2):183-193.http://dx.doi.org/10.1016/S0378-8741(98)00055-5
16. Susan S, Huang Brian J, Labus Michael C, Samuel Dairian T, Arthur LR. Antibiotic Resistance Patterns of Bacterial Isolates from Blood in San Francisco County, California, 1996–1999. Emerging Infectious Diseases, 2002; 8(2):195-201. http://dx.doi.org/10.3201/eid0802.010102 PMid:11897073 PMCid:2732439
17. Anitha M, Satish Kumar BN, Vrushabendra Swamy BM, Archana S. A Review on Natural Diuretics. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2012; 1(4):615-634.
18. Nirmala NR, Urmila MT, Sharadini AD. Adaptogenic properties of sixrasayana herbs used in Ayurvedic medicine. Phytotherapy Research, 1999; 13(4):275-291. http://dx.doi.org/10.1002/(SICI)1099-1573(199906)13:4<275::AID-PTR510>3.3.CO;2-J
19. Kiyota H. Synthesis of naturally derived bioactive compounds of agricultural interest. Biosci Biotechnol Biochem, 2006; 0(2):317-24. http://dx.doi.org/10.1271/bbb.70.317
20. Archana S. Abhishek C, Madhuliki S, Farrukh J, Preethi P, Siron MR, Falgun WB, Lakshmi V. Inhibition of Hyaluronidase activity of human and rat spermatozoa invitro by Terminalia, Flavanoid richplant. Reproductive Toxicology, 2010; 29(2):214-224. http://dx.doi.org/10.1016/j.reprotox.2009.11.001 PMid:19903524
21. Chattopadhyay RR, Bhattacharyya SK, Anwesa B, Premananda B, Nitish Kumar P. Evaluation of antibacterial properties of Chebulicmyrobalan (fruit of Terminalia chebula Retz.) extracts against methicillin resistant Staphylococcus aureus and trimethoprim-sulphamethoxazole resistant uropathogenic Escherichia coli. African Journal of Plant Science, 2009; 3(2): 025-029.

Cite this article as:
Tariq A.L. and Reyaz A.L. Terminalia chebula: A treatment against pathogenic Proteus vulgaris strains associated with urinary tract infection. Int. J. Res. Ayurveda Pharm. 2013;4(4):560-563 http://dx.doi.org/10.7897/2277-4343.04422

Source of support: Nil, Conflict of interest: None Declared