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

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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: Cefazidime (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- sulfamethoxazole 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 diarrheagenic 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 diseases8,9.

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°C.

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°C. Following incubation the bacterial colonies were observed and results were recorded.

Identification of Pathogens
Isolated organisms were identified10 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 Bengal, Karnataka State, India, based on its ethnomedical importance.

Preparation of Seed Powder
The seed powder was prepared 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.

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 °C for 48 hours. This extraction was taken and allowed for evaporation then concentrated with Dimethyl Sulfoxide to 3.37 g.

Acetone Extract
10 g of powdered *Terminalia chebula* sample was soaked in 50 ml of acetone and it was kept in Soxhlet apparatus at 80 °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 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 °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 °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 using microtitre plate. Wells from each column in row 1 were marked and 100 μl of stock acetone and ethanol (500 mg/ml) and blank Dimethyl sulfoxide solution was added. The 50 μl of saline was added to rows 2-10. Two fold serial dilutions were performed by transferring 50 μl of solution from row 1 to row 2, using a multichannel pipette. This was repeated down the row 2 to row 11. The 40 μl of double strength nutrient broth and 10 μl 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 40 μl of detrazonium 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, sulphur 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), Oloxacim (50 μg) followed by 96% to 92% of Norflaxcin (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, Superdivision- 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. 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.
Pathogenic isolates of *Proteus vulgaris* have relatively high potentials for developing resistance. 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. The extracts of *Terminalia chebula* exhibited growth inhibitory activity against two dental carriers causing bacterial strain. The acetone and ethanol extracts of fruits of the *Terminalia chebula* showed good antimicrobial activity. 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. 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 flexneria* and *Pseudomonas aeruginosa* respectively. On comparing the inhibition zone of the tested microorganisms from high to low *Escherichia coli*, *Shigella flexneria* and *Pseudomonas aeruginosa* respectively. On comparing the inhibition zone of the tested microorganisms from high to low *Escherichia coli*, *Shigella flexneria* and *Pseudomonas aeruginosa* respectively.

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

<table>
<thead>
<tr>
<th>Tests</th>
<th>Urinary tract associated <em>Proteus vulgaris</em> strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram’s Reaction</td>
<td>Negative Rod shaped</td>
</tr>
<tr>
<td>Motility</td>
<td>Active motile with peritrichous flagella</td>
</tr>
<tr>
<td>Indole</td>
<td>Positive</td>
</tr>
<tr>
<td>Methyl Red</td>
<td>Positive</td>
</tr>
<tr>
<td>Voges Proskauer</td>
<td>Negative</td>
</tr>
<tr>
<td>Citrate Utilization</td>
<td>Negative</td>
</tr>
<tr>
<td>Oxidase</td>
<td>Negative</td>
</tr>
<tr>
<td>Catalase</td>
<td>Positive</td>
</tr>
<tr>
<td>Urease</td>
<td>Positive</td>
</tr>
<tr>
<td>Nitrate Reduction</td>
<td>Positive without gas</td>
</tr>
<tr>
<td>Carbohydrate Fermentation</td>
<td>Ferment Glucose, Sucrose and Maltose Gas +</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus vulgaris</em> strain TP1</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
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<tr>
<td><em>Proteus vulgaris</em> strain TP2</td>
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<td>R</td>
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<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> strain TP3</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> strain TP4</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> strain TP5</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Urinary Tract associated isolates</th>
<th>250</th>
<th>125</th>
<th>62.5</th>
<th>31.25</th>
<th>15.63</th>
<th>7.81</th>
<th>3.91</th>
<th>1.953</th>
<th>0.977</th>
<th>0.488</th>
<th>0.244</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus vulgaris</em> strain TP1</td>
<td>-</td>
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<tr>
<td><em>Proteus vulgaris</em> strain TP2</td>
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<tr>
<td><em>Proteus vulgaris</em> strain TP3</td>
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<td>+</td>
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<tr>
<td><em>Proteus vulgaris</em> strain TP4</td>
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<td>+</td>
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</tr>
<tr>
<td><em>Proteus vulgaris</em> strain TP5</td>
<td>-</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>

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*

<table>
<thead>
<tr>
<th>Urinary Tract Associated isolates</th>
<th>250</th>
<th>125</th>
<th>62.5</th>
<th>31.25</th>
<th>15.63</th>
<th>7.81</th>
<th>3.91</th>
<th>1.953</th>
<th>0.977</th>
<th>0.488</th>
<th>0.244</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus vulgaris</em> strain TP1</td>
<td>-</td>
<td>-</td>
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<tr>
<td><em>Proteus vulgaris</em> strain TP2</td>
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<td>+</td>
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</tr>
<tr>
<td><em>Proteus vulgaris</em> strain TP3</td>
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<td>-</td>
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<td>+</td>
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<tr>
<td><em>Proteus vulgaris</em> strain TP4</td>
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</tr>
<tr>
<td><em>Proteus vulgaris</em> strain TP5</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>

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.
CONCLUSION

This study revealed that Terminalia chebula fruit plays dual benefits as medicinal values and food values. Our study found that Terminalia chebula fruit was 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 showed the maximum zone of inhibition when compared with standard antibiotics Ampicillin, Cefazidime, 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.

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