Antibacterial Activity of Medicinal Plants used against UTI (Urinary Tract Infection) causing Pathogens

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ABSTRACT

The study was to investigate the Antibacterial Activity of Some Medicinal Plants used against UTI Causing Pathogens. Bacteria were isolated from the UTI infected patients and characterized by using microscopic, staining, morphological and biochemical methods. The Ethanolic Extracts from plants were prepared and then used to check their antibacterial activity against the bacteria isolated from UTI infected patients and the zone of inhibition were compared with the zone of inhibition of standard antibiotics. Results from the present study showed that Punica granatum and Cinnamom verum had more antibacterial activity compared to other plant extracts that were used and Tribulus terrestris had lowest antibacterial activity against the bacteria isolated from UTI.

Keywords: Antibacterial Activity, Urinary Tract Infection, Antibiotics, Punica Granatum

INTRODUCTION

Urinary tract infections are the second most common type of infection in the body, accounting for about 8.1 million visits to health care providers each year. Women are especially prone to UTIs for anatomical reasons. One factor is that a woman’s urethra is shorter, allowing bacteria quicker access to the bladder. Also, a woman’s urethral opening is near sources of bacteria from the anus and vagina[14]. For women, the lifetime risk of having a UTI is greater than 50 percent [2]. UTIs in men are not as common as in women but can be serious when they occur. More than 95% of UTI are caused by single bacterial species E. coli which is the most frequently infecting organisms [10]. However, many other bacteria can also because an infection for example Klebsiella, Pseudomonas, Enterobacter, Proteus, Staphylococcus, Mycoplasma, Chlamydia, Serratia and Neisseria spp. It is reported that about 35% of healthy women suffer symptoms of Urinary tract infection and about 5% of women each year suffer with the problem of painful urination (dysuria) and frequency [7]. The incidence of UTI is greater in women as compared to men. Several potent antibiotics are available for the treatment of UTI, but increasing drug resistance among bacteria has made therapy of UTI difficult. Bacteria have the genetic ability to transmit and acquire resistance to drugs [15]. Essential extracts of certain plants have been shown to have antimicrobial effects, as well as imparting flavor to foods [6]. The synergistic effect of the mixture of phytochemicals plays an important role to use plant extracts as an antimicrobial agents [12]. It has been suggested that volatile oils, either inhaled or applied to the skin, act by means of their lipophilic fraction reacting with the lipid parts of the cell membranes, and as a result, modify the activity of the calcium ion channels [5]. The antimicrobial and other biological activities of the plant extracts varied depending upon the origins and cultivars [8]. In this study antimicrobial activity of the medicinal plants Tribulus terrestris, Cinnamom verum and Punica granatum were checked against the bacterial isolates from patients (Pregnant Women) with UTI.
MATERIALS AND METHODS

Isolation and identification of bacteria from UTI infected patients: First the microorganism present in urine samples of UTI infected patients were cultured in the nutrient broth and the morphology of organisms were studied with the help of light microscope and shape, size, odour, margin and surface characteristics of bacteria. Gram staining procedure was adopted to differentiate between gram positive and gram negative organisms. Selective agar medium was used for further identification. Different media for different organism were used as given below:

- Mac Conkey: *E. coli* and *Staphylococcus*
- EMB: *E. coli*
- MSA: *Staphylococcus*
- PABM: *Pseudomonas*
- XLDA: *Shigella*
- Blood agar: *Proteus*

After this step final identification of bacteria was done on the bases of biochemical testing.

Biochemical Tests

Seven biochemical tests were performed for each organism as given below:

- Catalase test
- Indole production test
- Citrate utilization test
- TSI (Triple sugar iron) agar test
- Urease activity test
- MR and VP test

Ethanolic Extraction of Medicinal Plants

The three Medicinal plants namely *Tribulusterrestris* - Nerungi, *Punica granatum* - Madhulai and *Cinnamom verum* - Ilavangam were selected and their antimicrobial activities were tested against bacteria affecting Urinary Track. Dried plant material (10g) was finely ground and extracted with Distilled Water and 99.9% Ethanol using Soxhlet’s apparatus. The extract was kept in oven at 50°C for 1hrs to evaporate excess ethanol and water. The residues (both ethanolic and aqueous extract) were suspended in DMSO to give 100 mg residue/ml by following procedure described [15].

TESTING OF ANTIMICROBIAL ACTIVITY

Testing the Antimicrobial Activity by Disc Diffusion Method

The paper disc diffusion method [4] was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (HA). Sterilized filter paper discs (Whatman, 6 mm in diameter) were placed on the surface of the solidified media soaked with 20 µl of a solution of each plant extracts. The standard antibiotic strips were also used for comparison. DMSO was used as negative control. The inoculated plates were stored at 4°C for 2hrs and then incubated at 37°C for 24hrs in the inverted position. The diameters (mm) of the inhibition zones were measured (diameter of paper disc, 6 mm is included). Studies were performed in triplicate and the results are expressed as means along with the Standard Deviation (SD) of three parallel measurements.
RESULTS & DISCUSSION

25 urine samples were obtained from urinary tract infected pregnant women respectively who were admitted at Namakkal Government Hospital, Namakkal. Table - 1 shows percentage contribution of bacterial isolates isolated from urinary tract infected patients.

Table-1. Etiology in 25 cases of Urinary Tract Infection were studied during the study period

<table>
<thead>
<tr>
<th>S.No</th>
<th>Pathogens</th>
<th>No. of Positive cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia coli</em></td>
<td>9</td>
<td>36%</td>
</tr>
<tr>
<td>2</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>6</td>
<td>24%</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>5</td>
<td>16%</td>
</tr>
<tr>
<td>4</td>
<td><em>Proteus vulgaris</em></td>
<td>3</td>
<td>14%</td>
</tr>
<tr>
<td>5</td>
<td><em>Staphylococcus aureus</em></td>
<td>2</td>
<td>10%</td>
</tr>
</tbody>
</table>

After studying the colony morphology on nutrient agar medium, colony morphology was also studied on the selective media. After the secondary identification on selective media, all samples were examined microscopically here the shape, size arrangement (pair, cluster and chain) and motility was checked and the gram staining techniques were followed Table: 2. The final identification were done on the basis of biochemical analysis Table:3 and Plate-1.

Table-2: Morphology and Staining of Test Isolates

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the Bacterial species</th>
<th>Gram staining</th>
<th>Morphology</th>
<th>Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia coli</em></td>
<td>-</td>
<td>GNR</td>
<td>Motile</td>
</tr>
<tr>
<td>2</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>-</td>
<td>GNR</td>
<td>Non motile</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
<td>GNR</td>
<td>Motile</td>
</tr>
<tr>
<td>4</td>
<td><em>Proteus vulgaris</em></td>
<td>-</td>
<td>GNR</td>
<td>Motile</td>
</tr>
<tr>
<td>5</td>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
<td>GPC</td>
<td>Non motile</td>
</tr>
</tbody>
</table>

(-) – Negative;  (+) – Positive; GNR – Gram negative rod; GPC – Gram positive cocci.

Table - 3 Biochemical tests of Recovered Isolates of Urine Samples

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the Bacterial species confirmed</th>
<th>Indole</th>
<th>M R</th>
<th>VP</th>
<th>Citrate</th>
<th>TSI</th>
<th>H₂S</th>
<th>Urease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia coli</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>A/A</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>A/A</td>
<td>G+ve</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>A/A</td>
<td>G+ve</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><em>Proteus vulgaris</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AK/H₂S</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td><em>Staphylococcus aureus</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>A/A</td>
<td>G-ve</td>
<td>-</td>
</tr>
</tbody>
</table>

(-) – Negative; (+) - Positive, A - Acid, K - Alkaline , G – Gas production
Antimicrobial Activity

Three plant extracts of the *Tribulus terrestris*, *Cinnamomum verum presl* and *Punica granatum* were extracted to test the antimicrobial activity on the five different bacteria isolated from urine samples of UTI infected patients with respect to standard antibiotic by the “Disc diffusion diffusion method” and the diameter zone of inhibition was measured in mm. Antimicrobial activity of different plant extracts on the different organisms is given as in Table 4.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Organism</th>
<th><em>Tribulus terrestris</em> (Inhibition zone in mm)</th>
<th><em>Cinnamomum verum presl</em> (Inhibition zone in mm)</th>
<th><em>Punica granatum</em> (Inhibition zone in mm)</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia coli</em></td>
<td>11</td>
<td>26</td>
<td>29</td>
<td>Norfloxacin-22</td>
</tr>
<tr>
<td>2</td>
<td><em>Klesiella pneumoniae</em></td>
<td>15</td>
<td>22</td>
<td>25</td>
<td>Norfloxacin-22</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>10</td>
<td>24</td>
<td>18</td>
<td>Norfloxacin-23</td>
</tr>
<tr>
<td>4</td>
<td><em>Proteus vulgaris</em></td>
<td>12</td>
<td>25</td>
<td>26</td>
<td>Ciprofloxacin - 25</td>
</tr>
<tr>
<td>5</td>
<td><em>Staphylococcus aureus</em></td>
<td>10</td>
<td>25</td>
<td>30</td>
<td>Gentamycin-19</td>
</tr>
</tbody>
</table>
Tribulus Terrestris extract against E.coli

Cinnamomum verum extract against E.coli

Punica grannatum extract against E.coli

Cinnamomum verum against K. pneumonia

Punica grannatum extract against K. pneumonia

Punica grannatum extract against S. aureus

Tribulus Terrestris K. pneumonia

Cinnamomum verum presl S. aureus
Results showed that the ethnolic extracts of *Punica grannatum* has greater anti bacterial activity among all the other extracts its maximum value of the zone of the inhibition is noted against *E.coli* 29mm than Norfloxacin 22mm. Then *Punica grannatum* and *Cinnamomum verum* extracts has showed almost equal activity, *Punica grannatum* has maximum activity against *Staphylococcus* with diameter 30mm in comparison to Gentamycin 19mm. *Cinnamon* extract had showed maximum results of antimicrobial activity against the organisms *Proteus vulgaris* and *Pseudomonas* 26mm in comparison to Norfloxacin 22mm and 20mm. After that *Cinnamomum verum* has showed almost equal results to the antibiotics with maximum activity 26mm against the bacteria *E. coli* in comparison to Norfloxacin 22mm and the *Tribulus Terristris* has show least activity of all the plant extracts and also less than the standard antibiotic maximum zone of inhibition 15mm in comparison to standard antibiotic ciprofloxacin 25mm.

*Cinnamomum verum* bark possess potent antimicrobial properties against *Staphylococcus aureus* and dermatophytic fungi. It is useful in the tropical treatment of superficial skin infections [9]. *Punica granatum* used to treat urinary astrigent, abdominal ulcers, glycosuria and skin diseases [11]. Prior study also reported that presence of inhibitory effect in *Punica granatum* against *Salmonella, E. coli,* and *Staphylococcus aureus*[13].

**CONCLUSION**

From the above results we can conclude that extracts of plants origin has remarkable antimicrobial activity as compare to antibiotic activity. We know that organisms are gaining resistance day by day towards the antibiotics, so that some natural products should be tried to overcome these antibiotic resistant organisms. The plants extracts or oils have no side effect; medicinal products may be one of our choices because it contains hydrophobic liquid which can be easily extracted by the process of distillation. Plant products or oils contain volatile aroma and phytochemicals which show the antimicrobial activity. More over plants can be grown easily and the production of their products is sophisticated than antibiotics. Expense on these material is bearable than antibiotic. The present study has been undertaken to identify effective herbal medicines to control UTI caused by bacterial organisms because, for all human beings herbal medicines are available in our environment and safe also. It does not cause any side effects. From these properties of plants we can say that natural medicine can take place of antibiotics in future.

**REFERENCE**


