Original Research Article

Antimicrobial Potential of Aqueous, Methanolic and Ethanolic Extracts of *Azadirachta indica* against Bacterial Pathogens Isolated from Urinary Tract Infection Patients

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**ABSTRACT**

Medicinal plants offer a number of bioactive compounds which can lead to development of new antimicrobial agents. These agents will be cheaper with improved safety and efficacy than the synthetic drugs. In the present study we compared the aqueous, methanolic and ethanolic extracts of *Azadirachta indica* (Neem) leaves against human pathogenic bacteria causing urinary tract infections. Soxhlet method was used for extraction of the various extracts while agar well diffusion method was used to determine the antimicrobial activity against bacteria. Our results suggest that ethanolic extracts exhibit highest antimicrobial activity with more activity against Gram negative than Gram positive.

**Keywords**

Neem, Agar well diffusion method, Urinary tract infection

**Introduction**

Selman Waksman, the discoverer of Streptomycin, for the first time defined Antibiotic as “any class of organic molecule that inhibits or kills microbes by specific interactions with bacterial targets, without any consideration of the source of that particular compound or class”. Antibiotics are the mainstay of therapy for bacterial infections and have always been considered one of the wonder discoveries of the 20th century (Demain and Elander, 1999).

However, the successful use of any therapeutic agent is compromised by the potential development of tolerance or resistance to that compound from the time it is first employed. This has created immense problems in the treatment of diseases. Further these antibiotics have several side effects. Therefore, there is a need to develop antimicrobial agents which are safer, cheaper and more efficacious.

Medicinal plants offer a number of bioactive compounds which can lead to development of new antimicrobial agents (Sumathi and Parvathi, 2010). About 10% of all flowering plants on earth are used to treat various infections, although only 1% have gained recognition by modern scientists (Lewis and
Ausubel, 2006). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties (Subapriya and Nagini, 2005).

Amongst these medicinal plants *Azadirachta indica* (Neem) is a source of potential therapeutic agents. *Azadirachta indica* belongs to family Meliaceae. It exhibits antibacterial, antifungal and antiviral activities (Biswas, 2002). Also exhibit wide pharmacological activities including: antioxidant, antimalarial, anticarcinogenic, anti-inflammatory, antiulcer, antimutagenic, antihyperglycaemic, and anti-diabetic properties (Talwar et al., 1997; Subapriya and Nagini, 2005).

The present study was designed to compare the three extracts of neem leaves: aqueous, methanol and ethanol against human pathogens causing UTI.

**Materials and Methods**

Collection of raw materials: The neem leaves were collected from Forest Research Institute (F.R.I.), Dehradun and authenticated by a botanist.

Preparation of different leaf extracts: For this purpose, shade dried powder of leaves was used for extraction with different solvents. The preparation of the three extracts was carried out according to the Soxhlet method (Gabriela et al., 2010) (Fig. 1).

Study population: Non repetitive 100 patients with significant bacteuria attending Muzaffarnagar Medical College, Muzaffarnagar were included in the study. For isolation of bacterial strains, loop full of urine samples were streaked on cystiene lactose electrolyte deficient (CLED) medium (Hi Media, India). After overnight incubation at 37°C organism was identified on the basis of standard biochemical tests (Koneman et al., 1997).

Antibacterial Susceptibility assay: The antibacterial activity of the three extracts was carried out using the Agar well diffusion method (Perez et al., 1990). Lawn culture of the test organism adjusted to 0.5 McFarland turbidity Standard was made on the Mueller Hinton Agar (HiMedia) plates using sterile cotton swabs (11, 12). A sterile cork borer was then used to make wells (6mm diameter) for the three extracts. 100µl of the extracts were introduced into the wells with the help of micropipettes.

The culture plates were allowed to stand on the working bench for 30 min for pre-diffusion and were then incubated in upright position at 37°C for 24 h. After 24 hrs, antibacterial activity was determined by measurement of diameter of zones of inhibition (mm). Standard antibiotic discs of Ampicillin (10mcg/disc) and Nitrofurantoin (300mcg/disc) were used as positive control. All the tests were done in triplicate to minimize the test error.

**Results and Discussion**

Amongst 100 urine samples which were included in the study *E. coli* was the commonest organism isolated 55 times (55%) alone and 4 times (4%) with *E. faecalis*. Other organisms isolated were *K. pneumonia* (19%), *P. aeruginosa* (7%), *P. mirabilis* (3%), *E. faecalis* (6%) and *S. aureus* (6%) each (Table 1).

The three extracts aqueous, methanolic and ethanolic were examined for their antimicrobial activity against the above mentioned bacteria, showed good antimicrobial potential (Table 2). Ethanolic
extracts exhibited higher antimicrobial effect than aqueous and methanolic extracts. Similar results were also obtained in studies by Tabassum et al. (2003).

Table 1 Bacteria isolated from Urine culture

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Bacteria isolated</th>
<th>Number</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td>1.</td>
<td>Escherichia coli</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>2.</td>
<td>Klebsiella pneumoniae</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>3.</td>
<td>Pseudomonas aeruginosa</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>4.</td>
<td>Proteus mirabilis</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5.</td>
<td>Enterococcus faecalis</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>6.</td>
<td>Staphylococcus aureus</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>7.</td>
<td>Escherichia coli+ Enterococcus faecalis</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2 Antimicrobial activity of various extracts of A. indica leaf

<table>
<thead>
<tr>
<th>Extract/Drug</th>
<th>Diameter of inhibition zone (average) in mm.</th>
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<tbody>
<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>Aqueous</td>
<td>16</td>
</tr>
<tr>
<td>Methanol</td>
<td>19</td>
</tr>
<tr>
<td>Ethanol</td>
<td>23</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>15</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>25</td>
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</table>

Fig. 1 Soxhlet Apparatus

Further maximum activity of ethanol extracts was seen on E. coli and least on K. pneumoniae. These results are in compliance with the results obtained by Sohail et al.
Based on the results obtained in this study leaf extracts of Neem expressed broad spectrum antibacterial activity on bacterial urinary tract infection isolates with highest activity recorded for ethanolic extracts.

Prevalence of drug resistance to various antibiotics among pathogenic bacteria has paved way for the search of new compounds that could replace the synthetic antimicrobial agents. Medicinal plants offer immense antimicrobial potential; all that is needed is to evaluate their antimicrobial activity. It is clear from the results obtained in our study that the leaf extracts of Neem exhibits good antimicrobial activity against urinary tract pathogens.

References


