Comparative anti microbial studies of aqueous, methanolic and saponins extract of seeds of *Trigonella Foenum-Graecum* on human vaginal pathogens causing UTI infection

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**ABSTRACT**

The aqueous, methanolic and saponin extracts of *Trigonella foenum-Graecum* seeds were screened for antimicrobial activities against some human vaginal pathogens *Staphylococcus aureus, Pseudomonas aeruginosa, streptococcus faecalis, klebsiella pneumoniae, Escherichia coli, Enterobacter faecalis, Enterobacter faecium and Proteus mirabilis* isolated from patient samples. Extracts were found to produce significant inhibition against all the pathogens. Saponin extract were observed to be more active than methanolic and aqueous fraction. Extracts are found to be more active against klebsiella pneumoniae, Escherichia coli, *Pseudomonas aeruginosa* and *Enterobacter faecalis* strains.

**Key Words:** *Trigonella foenum- Graecum, Human Vaginal Pathogens, Saponin.*

**INTRODUCTION**

Medicinal plants have always been integral to the traditional healthcare system all over the world. In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants,
animal tissues, or microorganisms. The shortcomings of the drugs available today, propel the
discovery of new pharmacotherapeutic agents in medicinal plants. [1-3]

Fenugreek also known as Methi-dana is seeds of Trigonella foenum –graecum belongs to the
family Leguminosae. The seeds are hard, yellow to reddish brown in color, oblong, rhomboidal,
with deep furrow running obliquely from the side which divides the seed in unequal parts. The
seeds are 2- 5mm long and 1.5-3 mm wide have pleasant odor and bitter taste.[4-7]

Urinary tract infections (UTIs) are a leading cause of morbidity and health care expenditures in
persons of all ages. Sexually active young women are disproportionately affected, but several
other populations, including elderly persons and those undergoing genitourinary instrumentation
or catheterization, are also at risk. An estimated 40 percent of women report having had a UTI at
some point in their lives. Urine located within the urinary tract, excluding the distal region of the
urethra is considered sterile in healthy individuals, as indicated by the absence of cultivable
bacterial cells. A urinary tract infection (UTI) describes a condition in which there are micro
organisms established and multiplying within the urinary tract. It is most often due to bacteria
(95%), but may also include fungal and viral infection. [8-11]

In the present study methanolic, aqueous and saponin Extracts of seeds of Trigonella Foenum-
Graecum plants were screened for potential antibacterial activity toward vaginal pathogens
causimg urinary tract infections (UTIs).

MATERIALS AND METHODS

Plant materials
Seeds of Trigonella foenum –graecum were collected from Local Market of Indore, Madhya
Pradesh and were identified by the Botany Department, Janata PG College, A.P.S. University,
Rewa (M.P.). The seeds were stored in an air-tight container for further use.

Preparation of extracts
Seeds were shattered and screened with 40 mesh. It was soxhlet extracted three times with
petroleum benzene for 4hr at 60°C. After drying and levigation, the residues were inverse flow
extracted 10 times with 70% methanol for 4hr at 85°C, then were filtrated and the residue was
extracted with distilled water for 48hr under reflux condition. The alcohol solution (Filtrate) was
evaporated to dryness with reduced pressure at 60°C, and dissolved with water. After filtration
and discarding the extraneous components, the solution was extracted by adding water-saturated
n-butanol (1:1v/v), the n-butanol phase was then treated by 1M KOH, alkaline–water phase was
removed. The n-butanol phase evaporated to dryness under pressure and the raw saponin was
obtained. All extracts were screened for phytochemical analysis.

Preparation of microorganisms for experiment
All the microorganisms were isolated from in & outpatients samples from Chotiram hospital and
research centre Indore. For use in experiments, the organisms were sub-cultured in nutrient
broth, nutrient agar, Macconky agar and Blood agar media. Muller Hinton agar was used in
antibiotic sensitivity testing.
Preparation and application of disks for experiment [12-21]
Different concentration of the extracts (10-60 µg/ml) was prepared by reconstituting with DMSO. The test microorganisms were streak to Muller Hinton agar medium by streaking plate method. After streaking the autoclaved filter paper discs (5 mm in diameter) impregnated with the extracts were placed on plates using flame-sterilized forceps. The antibacterial assay plates were incubated at 37°C for 24hr. For positive control Amoxycillin/cefitaxime/Ampicillin (60µg/ml) and for negative control solvent DMSO was used.

Observation of results
Results were recorded as presence or absence of zone of inhibition. The inhibitory zone around test paper disks indicated absence of bacterial growth and it was reported as positive (growth inhibition observed) and absence of zone as negative. The test was repeated thrice in interday interval to insure reliability of the results. The diameters of the inhibition zones were measured in mm (after subtraction the diameter of disc i.e 5mm), shown in table 1. The concentration of extract showing inhibition were further diluted and experiment was repeated to identify the minimum inhibitory concentration (MIC), shown in table 2. The Percentage of relative inhibition zone diameter (% RIZD) as compare to inhibition obtained from standard drug at same concentration was calculated, shown in table 3.

Table 1. Zone of inhibition for extracts, Standard & Control

<table>
<thead>
<tr>
<th>Con in µg/ml</th>
<th>Zone of Inhibition (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC</td>
</tr>
<tr>
<td>ME</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>8.16±0.16</td>
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<tr>
<td>40</td>
<td>11.5±0.28</td>
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<tr>
<td>60</td>
<td>16.16±0.16</td>
</tr>
<tr>
<td>AE</td>
<td></td>
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<tr>
<td>10</td>
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</tr>
<tr>
<td>20</td>
<td>7.16±0.16</td>
</tr>
<tr>
<td>40</td>
<td>11.33±0.16</td>
</tr>
<tr>
<td>60</td>
<td>15.33±0.33</td>
</tr>
<tr>
<td>SE</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>7.16±0.16</td>
</tr>
<tr>
<td>40</td>
<td>13.5±0.28</td>
</tr>
<tr>
<td>60</td>
<td>17.16±0.16</td>
</tr>
<tr>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>22.5±0.763</td>
</tr>
<tr>
<td>Con</td>
<td></td>
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<td>-</td>
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</tbody>
</table>

* mm= Mean of three replicates±SEM
Met: Methanolic extract AE: Aqueous Extract SE: Saponin Extract Con: Control (DMSO) SD: Standard (a = ceftaxime, b = Amoxycillin)
EC= Escherichia coli, PA= Pseudomonas aeruginosa, EFa= Enterobacter faecalis, EFI= Enterobacter faecium, KP= klebsiella pneumoniae, SF= Streptococcus faecalis, SA= Staphylococcus aureus and PM= Proteus mirabilis
Table 2 Minimum Inhibitory Concentration (MIC) for extracts

<table>
<thead>
<tr>
<th>Organism</th>
<th>EC</th>
<th>PA</th>
<th>EFa</th>
<th>EFI</th>
<th>KP</th>
<th>SF</th>
<th>SA</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME</td>
<td>2.66±0.16 (8µg/ml)</td>
<td>3.16±0.33 (20µg/ml)</td>
<td>2.5±0.28 (36µg/ml)</td>
<td>2.66±0.66 (14µg/ml)</td>
<td>2.66±0.44 (30µg/ml)</td>
<td>3.16±0.16 (18µg/ml)</td>
<td>2.83±0.16 (48µg/ml)</td>
<td></td>
</tr>
<tr>
<td>AE</td>
<td>2.5±0.28 (8µg/ml)</td>
<td>3.16±0.16 (20µg/ml)</td>
<td>2.16±0.28 (38µg/ml)</td>
<td>2.66±0.33 (20µg/ml)</td>
<td>3.16±0.16 (48µg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>3.33±0.16 (8µg/ml)</td>
<td>3.83±0.16 (20µg/ml)</td>
<td>2.33±0.16 (34µg/ml)</td>
<td>2.83±0.16 (26µg/ml)</td>
<td>2.83±0.44 (14µg/ml)</td>
<td>3.0±0.28 (44µg/ml)</td>
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</tr>
</tbody>
</table>

Met: Methanolic extract AE: Aqueous Extract SE: Saponin Extract
EC= Escherichia coli, PA= Pseudomonas aeruginosa, EFa= Enterobacter faecalis, EFI= Enterobacter faecium, KP= klebsiella pneumoniae, SF= Streptococcus faecalis, SA= Staphylococcus aureus and PM= Proteus mirabilis

Table 3 Percentage of relative Inhibition Zone diameter (% RIZD) for extracts as compare to standard at 60µg/ml

<table>
<thead>
<tr>
<th>Organism</th>
<th>EC</th>
<th>PA</th>
<th>EFa</th>
<th>EFI</th>
<th>KP</th>
<th>SF</th>
<th>SA</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME</td>
<td>71.82%</td>
<td>66.88%</td>
<td>59.79%</td>
<td>55.10%</td>
<td>75.83%</td>
<td>45.44%</td>
<td>56.27%</td>
<td>51.73%</td>
</tr>
<tr>
<td>AE</td>
<td>68.13%</td>
<td>65.52%</td>
<td>62.35%</td>
<td>49.21%</td>
<td>67.78%</td>
<td>48.25%</td>
<td>41.73%</td>
<td>41.21%</td>
</tr>
<tr>
<td>SE</td>
<td>76.26%</td>
<td>71.02%</td>
<td>66.66%</td>
<td>59.07%</td>
<td>85.21%</td>
<td>63.61%</td>
<td>62.24%</td>
<td>58.73%</td>
</tr>
</tbody>
</table>

Met: Methanolic extract AE: Aqueous Extract SE: Saponin Extract
EC= Escherichia coli, PA= Pseudomonas aeruginosa, EFa= Enterobacter faecalis, EFI= Enterobacter faecium, KP= klebsiella pneumoniae, SF= Streptococcus faecalis, SA= Staphylococcus aureus and PM= Proteus mirabilis

RESULTS AND DISCUSSION

In this study the results of the investigations show that all the extracts from the bark possess antimicrobial activities against mentioned test organisms. The minimum inhibitory concentration lies in the range from 08µg/ml to 48µg/ml.

Saponin extract were observe to be more active than ethanol and aqueous extracts. As compare to the standard, extracts were observed to be less active at concentration 60µg/ml. The percentage of relative inhibition zone diameter (% RIZD) observed to be in the range 41.73%-85.21% shown in table 3. Results clearly indicate that further purification of this compounds can leads to isolation of potent antibacterial compound active against some urinary pathogens.

Acknowledgment
Sincerely thanks to Dr. S.N. Dwivedi, Principal Investigator, Department of Botany, Janata College, APS University (M.P.), Dr. Chitins, Head, Department of Microbiology, Choitram Hospital & Research Centre, Indore. Dr. (Mrs.) V. Kothari, Head, Pathology Department, CHL Apollo, Indore for their valuable support.

REFERENCES


