Antimicrobial activity of *Terminalia chebula*

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**Abstract**: *Terminalia chebula* is a popular medicinal plant according to Ayurveda for its broad spectrum medicinal value including in the treatment of enteric disorders. Leaf extracts in water as well as in various organic solvents (namely methanol, ethanol, ethyl acetate and chloroform) were analyzed to testify its antibacterial activities against four different bacteria causing enteric disorders, viz. *Escherichia coli*, *Salmonella* sp, *Shigella* sp and *Vibrio cholerae* in vitro along with *Saccharomyces cerevisiae*. The analysis was carried out by taking the extracts at a concentration of 10 mg/ml and their activities were recorded by estimating zones of inhibition as produced by disc-diffusion method on Mueller-Hinton agar media. While all the organisms were resistant to chloroform extract and some of them to that of ethyl acetate, the methanol as well as the aqueous extracts of the plant showed the potential bactericidal activity, however nothing was evident against the yeast candidate. When compared with the traditional antibiotics, this activity was especially competent against *Escherichia coli* and *Shigella* sp, followed by *Vibrio* sp. and *Salmonella* spp. The broth dilution assay revealed that the bactericidal values fall in the range of 5000 to 8000 µg/ml.

**Keywords**: *Terminalia chebula*, antibiotics, drug-resistance, antimicrobial activity.

**Introduction**

The discovery of antibiotics more than 70 years ago initiated a period of drug innovation and implementation in human and animal health and agriculture. These discoveries were tempered and questioned in all cases by the emergence of resistant microbes (Teuber 2001; Heuer et al. 2006). For which, we are now facing the threat of superbugs, i.e. pathogenic bacteria resistant to most or all available antibiotics. It was warned by the World Health Organization that those multiple antibiotic-resistant pathogens would very likely bring the world back to the pre-antibiotic era. This clearly highlights the need for new antibacterial agents with fundamentally different modes of action than that of traditional antibiotics. The enormous demand has triggered worldwide efforts in developing novel antibacterial alternatives, particularly the screening of several medicinal plants for their potential antimicrobial activity.

Many Bangladeshi plants have been used from time immemorial to treat various diseases and infections in traditional medicinal systems. *Terminalia chebula* (Family Combretaceae; local name, haritaki) is one of the most commonly used plants in traditional systems of medicine in Indian sub-continent. This study aims to find out the potential antimicrobial activity of the leaves of *Terminalia chebula* by extracting them on organic as well as aqueous solvents, and then compare its antimicrobial activity with traditionally used chemotherapeutic drugs. The activity of the extract was finally quantitatively estimated in terms of minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values.

**Materials and Methods**

**Test plant and its extraction**

The dried powder of *Terminalia chebula* was collected from Holy Drugs, a local pharmaceutical company for indigenous medicine. 10 g of the powder was mixed with 40 ml of chloroform and was kept at 25°C for 12 h, filtered through a Whatman no. 4 filter paper and the filtrate was evaporated by vacuum dryer at 40°C.
overnight to get the chloroform extract. After chloroform extraction, the solid residue was dried at 40°C overnight to remove residual chloroform. The solid powder was resuspended in 40 ml ethyl acetate and kept at 25°C for 12 h. Ethyl acetate extract was recovered following the same procedure as stated for chloroform extract. Similarly, methanol and ethanol extracts were prepared by applying the same procedure. In addition to the organic extracts, an aqueous extract was made by taking 10 g of herb in a sterile conical flask and distilled water was added up to 100 ml and kept at room temperature for 24 hrs. The mixture was filtered and the filtrate was centrifuged at 10,000 x g for 15 min to remove the dust of dried leaf. The supernatant containing water-soluble components was collected, dried in vacuum dryer at 40°C for 6 hours to obtain the aqueous extract. Finally, the respective solvents (i.e. chloroform, ethyl acetate, methanol, ethanol and distilled water) were added to each of the extracts respectively in order to make a final concentration of 10 mg/ml.

**Determination of antibacterial activity**

Bacterial susceptibility to antimicrobial agent was determined in vitro by using the standardized agar-disc diffusion method known as the Kirby Bauer method (Bauer et al. 1966). Four bacterial species, viz. *E. coli*, *Salmonella* sp, *Shigella* sp and *Vibrio cholerae*, collected from a local diagnostic centre were employed as test organisms together with *Saccharomyces cerevisiae*. Inocula were prepared by adding an overnight culture of the organism in Mueller-Hinton (MH) broth to obtain an OD₆₀₀ 0.1. The cells were allowed to grow until they obtain the McFarland standard 0.5 (approximately 10⁸ CFU/ml). For *S. cerevisiae*, sabaurouds dextrose broth (SDB) was used.

Sterile discs (Oxoid) were soaked separately with 30 µl of each of the organic extract prepared in chloroform, ethyl acetate, methanol and ethanol solvents and were placed on Mueller-Hinton agar plates, previously swabbed with the target bacterial isolate at a concentration of 10⁸ CFU/ml. In one disc, the respective organic solvent was added as negative control to determine possible inhibitory activity of the solvent. Plates were kept at 4°C for 1½ hour for better spreading of the extract material around the discs and then incubated for a period of 24 h at 37°C. For *S. cerevisiae* MYGP agar was used. Antibacterial activity was defined as the diameter (mm) of the clear inhibitory zone formed around the discs.

The MIC of the extract was determined by tube dilution techniques in Mueller-Hinton broth (Merck) according to NCCLS (1998). The range of concentration used was 2000-10000 µg/ml. Stock solution of Haritaki leaf dried powder water extract were prepared in distilled water at concentration of 10,000 µg/ml and 8000 µg/ml. The solutions were then serially diluted. 0.9 ml of Mueller Hinton Broth (MHB) was taken in each of sterile and dry glass vials and 1.0 ml of the respective extract concentration was dispensed into respective vials. 100 µl of bacterial suspension of interest that was previously grown in nutrient broth were added to vial and incubated at 37°C for 24 hrs. The highest concentration that exhibited no visible growth was recorded as the MIC. The last vials with no growth were streaked on nutrient agar plates and incubated at room temperature for 24 hrs. The lowest concentration that killed 100% of the inoculum bacteria (no growth on plate) was recorded as Minimum Bactericidal Concentrations (MBC).

**Results**

Ten standard antibiotic discs, viz ampicillin, streptomycin, chloramphenicol, ciprofloxacin, nalidixic acid, trimethoprim, rifampicin, polymyxin B, ceftriaxone and oxytetracycline, all purchased from Oxoid, UK were used to construct the antibiograms of the microorganisms, *Salmonella* sp, *Shigella* sp, *Escherichia coli* and *Vibrio cholerae* that are commonly regarded as enteric pathogens. The diameter of clear zone of inhibition was determined in mm scale and the finding was interpreted as ‘sensitive’, ‘intermediate sensitive’ or ‘resistant’ to the respective drug based on the standard (NCCLS 1998).
Table 1: Antibiogram of enteric pathogens based on the production of zone of inhibition (in mm diameter) around the antibiotic discs in Mueller-Hinton agar

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>AMP</th>
<th>STR</th>
<th>C</th>
<th>CIP</th>
<th>NA</th>
<th>TMP</th>
<th>RP</th>
<th>PB</th>
<th>CRO</th>
<th>OTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td>30</td>
<td>20</td>
<td>28</td>
<td>40</td>
<td>26</td>
<td>25</td>
<td>0</td>
<td>14</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>Shigella</td>
<td>0</td>
<td>23</td>
<td>0</td>
<td>32</td>
<td>0</td>
<td>48</td>
<td>20</td>
<td>10</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>E. coli</td>
<td>0</td>
<td>36</td>
<td>12</td>
<td>32</td>
<td>0</td>
<td>26</td>
<td>9</td>
<td>12</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>35</td>
<td>22</td>
<td>22</td>
<td>18</td>
<td>0</td>
<td>20</td>
<td>32</td>
<td>14</td>
<td>22</td>
<td>8</td>
</tr>
</tbody>
</table>

Antibiotics susceptibility pattern (NCCLS 1998) is coded by S = sensitive, I = intermediate, and R = resistant.

By comparing the antibacterial activity, it was observed that the chloroform extract failed to produce antibacterial activities to all the four organisms tested, the methanol and aqueous extracts were the dominant ones in producing greater zones of inhibition against the targets (Figure 1). Conversely, none of the extract was able to produce any biocidal effect on yeast (data not shown); hence the plant material can be better used as an antibacterial agent, rather than an antifungal agent.

Figure 1: Antibacterial activities of different organic and aqueous extracts of the leaves of T. chebula (10 mg/ ml) against enteric pathogens by employing disc-diffusion technique.
In order to analyze the relative efficacy of the plant extract compared to that of the standard chemotherapeutic drugs, following equation was exercised. This data will give an impression of the activity of the extract when compared to chemotherapeutic drugs (Table 2).

\[
\text{Relative effectiveness} = \frac{\text{diameter of zone of inhibition produced by extract}}{\text{diameter of zone of inhibition produced by antibiotics}} \times 100\%
\]

Table 2: Relative effectiveness of extract of *T. chebula* (in water and in methanol)

<table>
<thead>
<tr>
<th>RELATIVE EFFECTIVENESS (%)</th>
<th>in terms of methanol extract</th>
<th>in terms of aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salmonella</td>
<td>Shigella</td>
</tr>
<tr>
<td>Ampicillin (25 µg)</td>
<td>53</td>
<td>*</td>
</tr>
<tr>
<td>Ceftriaxone (30 µg)</td>
<td>61</td>
<td>66</td>
</tr>
<tr>
<td>Chloramphenicol (30 µg)</td>
<td>57</td>
<td>*</td>
</tr>
<tr>
<td>Ciprofloxacin (5 µg)</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td>Nalidixic acid (30 µg)</td>
<td>61</td>
<td>*</td>
</tr>
<tr>
<td>Oxytetracycline (30 µg)</td>
<td>72</td>
<td>60</td>
</tr>
<tr>
<td>Polymyxin B (300 unit)</td>
<td>114</td>
<td>120</td>
</tr>
<tr>
<td>Rifampicin (5 µg)</td>
<td>*</td>
<td>60</td>
</tr>
<tr>
<td>Streptomycin (10 µg)</td>
<td>80</td>
<td>52</td>
</tr>
<tr>
<td>Trimethoprim (5 µg)</td>
<td>64</td>
<td>25</td>
</tr>
</tbody>
</table>

*-filled boxes indicate that while the pathogens exhibited sensitive response towards the experimental extract, they were completely resistant to the corresponding drugs tested, hence no comparison could be calculated.

Table 2 reveals that both the methanolic and water extract, produced equal or even greater biocidal activities against two of the species, *E. coli* and *Shigella* when compared to that of ampicillin, chloramphenicol, nalidixic acid and polymyxin-B. Their activities were superior to Rifampicin but only against *E. coli*. This is particularly important given the fact that while these drugs are failed to produce any inhibitory effect against *E. coli* and *Shigella*, the plant’s extract is a solution. However, the activity of the extracts had more or less half of the efficacy against *Salmonella* and *V. cholerae* when compared to eight of the drugs except polymyxin B and Rifampicin. Their superior activities against *V. cholerae* over oxytetracycline are worth mentioning.

The MIC and MBC of the aqueous extract against the pathogens of interest were determined by using macro-dilution method and the results are summarized in Figure 2. While a relatively small dose (6-7 mg/ml) is required to have complete killing of *E. coli* and *Shigella* sp, 8 mg/ml was found sufficient to abolish both *Salmonella* and *V. cholerae*.

![Figure 2: MICs and MBCs of aqueous extract of *T. chebula* against enteric pathogens](http://www.openaccessscience.com)

**Discussion**

The extract of *T. chebula* showed broad spectrum antibacterial activity (Phadke and Kulkarni 1989). The ethanol extract at a concentration of 1 mg/disc showed maximum inhibition against *S. epidermidis*, followed by *B. subtilis* (Kannan et al. 2009; Gupta et al. 2002). It was reported that a *T. pallida* fruit methanolic extract showed maximum activity against gram-negative bacteria, while that of *T. bellerica* showed the highest inhibition zones against...
Pseudomonas aeruginosa and E. coli (Ghosh et al. 2008). Our results demonstrated that both the methanol and aqueous extracts of the leaves of T. chebula are well effective in producing antibacterial activities against gram-negative bacteria, particularly the agents causing gastroenteritis. Furthermore, in a few cases, these plant extracts were active against antibiotic resistant bacteria under very low concentration, thus minimizing the possible toxic effects. Such a potential of this medicinal plant, therefore demands further research to unfold its therapeutic values.

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References


