Antibacterial Activity of Mother Tinctures of Cholistan Desert Plants in Pakistan

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The mother tinctures of desert were screened for antibacterial activity against bacterial strains of Gram-positive and Gram-negative bacteria. Mother tinctures were prepared by maceration process and antibacterial activity of different plants was evaluated and compared by measuring their zones of inhibition. The results indicated that Boerhavia diffusa mother tincture had excellent activity only against Escherichia coli. Mother tincture of Chorozophora plicata showed highly effective results against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa whereas Echinops echinatus mother tincture showed highly effectiveness only against Salmonella typhi. Heliotropium europaeum mother tincture exhibited highly effective results against Bacillus subtilis in all concentrations. Tamrix aphylla presented maximum activity only against Bacillus subtilis in all three concentrations. Among the selected species Heliotropium europaeum, Chorozophora plicata and Tamrix aphylla were more effective plants against many microorganisms. However, Boerhavia diffusa and Echinops echinatus were less effective plants against tested pathogenic bacteria.

Key words: Antibacterial activity, Cholistan desert plants, homoeopathic mother tinctures

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Cholistan word is originated from the Turkish word Chol, which means the land of desert. Cholistan desert locally known as Rohi desert that surrounds near about 30 km from Bahawalpur, Punjab, Pakistan and covering an area of 26,000 km² which is full of resources of medicinal plants. Its length is 480 km and 32-192 km varying breadth[1]. Cholistan desert is uniquely located wild land of its own kind with scarcity of endemic flora, containing only 128 species belonging to 32 families. The specialized medicinal knowledge of plants is not common, but confined to the local specialist/herbalist called ‘hakims’. They offer human and animal disease treatments by providing herbal decoction, pellets, fresh, crushed or dried[2].

The collected plant samples were crushed and dried in open air under shade for 15 days and milled to fine powder in an electric grinder and stored in a well closed container. To prepare homoeopathic mother tinctures 100 g of each powdered plant material was soaked in 900 ml of 70% ethanol[9,10]. After 15 days, the soaked material of each plant was filtered and mother tincture was preserved. For the essay of antibacterial activity, two Gram-positive (Bacillus subtilis and Staphylococcus aureus) and four Gram-negative (Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Shigella sonnei) pathogenic bacterial strains were selected. To avoid the strain to go to the death phase, the inoculated broth media was shifted to shaking water bath adjusted at 37° for about 24 h. A 0.5 ml inoculated broth media was poured into petri plates and then 15 ml of nutrient agar were poured in each of them[11,12]. After solidifying, sterile sharp end cork borer (8 mm diameter) was used to bore three wells in petri plates labelled with the name of bacterial strains along with a positive standard ciprofloxacin as control[13,14]. With the help of micropipette the wells were filled with three different doses of plants mother tinctures (200, 400 and 800 µg/ml) and a standard dose of ciprofloxacin and finally incubated for 24 h at 37°. After all dishes were observed for zones of inhibition and the diameters of these zones were measured. The experiments were done in triplicate and the values obtained were statistically analysed by using Statistical Package for the Social Sciences (SPSS, IBM Corporation, USA) software[15].

B. diffusa mother tincture obtained by maceration method showed moderate activity against S. aureus, P. aeruginosa and S. sonnei and less activity was observed against E. coli, whereas B. subtilis and S. typhi were found resistant at 200 µg/ml concentration. When dose was increased to 400 µg/ml, it was highly effective against E. coli and still showed moderate activity against S. aureus, P. aeruginosa and S. sonnei and was ineffective against B. subtilis and S. typhi bacterial strains. However, at 800 µg/ml concentration, mother tincture was highly effective against E. coli; moderate activity was shown against B. subtilis, S. aureus, P. aeruginosa and S. sonnei; less activity was found in S. typhi microorganism (Table 1). The zone of inhibition in the range of 20-22 mm was considered moderate and more than 25 mm
was considered highly effective when compared to ciprofloxacin.

The data of antibacterial activity of C. plicata mother tincture are given in Table 1. Results showed its moderate activity against S. aureus, P. aeruginosa and E. coli; less activity was observed against B. subtilis, S. typhi and ineffective against S. sonnei bacterial strain at 200 μg/ml concentration. When dose of mother tincture was increased to 400 μg/ml, moderate activity was observed against S. aureus, P. aeruginosa, S. typhi and E. coli; less activity was found against B. subtilis and S. sonnei bacterial strains. However, at 800 μg/ml concentration, maximum antibacterial activity was observed against S. aureus, P. aeruginosa and E. coli and moderate activity was observed against B. subtilis, S. typhi and S. sonnei bacterial strains.

**E. echinatus** mother tincture was highly effective against S. typhi, moderate activity was observed in E. coli and less activity was found against P. aeruginosa microorganism; data revealed that E. echinatus mother tincture was ineffective against B. subtilis, S. aureus and S. sonnei at 200 μg/ml mother tincture concentration. When dose of mother tincture was increased to 400 μg/ml, highly effectiveness against S. typhi was obsered and moderate activity was again noted against E. coli and less activity was found against P. aeruginosa. However, at 800 μg/ml concentration mother tincture was highly effective against S. typhi; moderate activity was shown against P. aeruginosa and E. coli; less activity was found against B. subtilis and S. sonnei compared to standard used.

Results of antibacterial activity of H. europaeum mother tincture are given in Table 1. The data showed that it was highly effective against B. subtilis, moderately active against S. typhi and less active against S. aureus and S. sonnei microorganism and was ineffective against P. aeruginosa and E. coli at 200 μg/ml concentration. At 400 μg/ml concentration, it showed high activity only against B. subtilis; moderate activity was observed against S. typhi; less activity was found against S. aureus and S. sonnei as well as least activity was recorded against P. aeruginosa and E. coli bacterial strain. However, at 800 μg/ml concentration, mother tincture was highly effective against two microorganism i.e. B. subtilis and S. typhi; moderate activity was found against S. aureus; less activity was found against P. aeruginosa and S. sonnei microorganism as well as negligible activity against E. coli compared to standard.

Zone of inhibition of T. aphylla mother tincture are reported in Table 1. Results did not show high or moderate activity against any bacterial strain but less activity was found against S. aureus, S. typhi and E. coli. however, B. subtilis, P. aeruginosa and S. sonnei were found strongly resistant to T. aphylla mother tincture at 200 μg/ml mother tincture concentration. At higher doses of 400 μg/ml, similar results were observed. However, at 800 μg/ml concentration, mother tincture was highly effective against B. subtilis

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**TABLE 1: ZONE OF INHIBITION OF MEDICINAL PLANTS AGAINST DIFFERENT PATHOGENIC BACTERIAL SPECIES**

<table>
<thead>
<tr>
<th>Plants</th>
<th>Dose (μl)</th>
<th>Bacillus subtilis</th>
<th>Staphylococcus aureus</th>
<th>Pseudomonas aeruginosa</th>
<th>Salmonella typhi</th>
<th>Shigella sonnei</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boerrhavia diffusa</td>
<td>200</td>
<td>12.67±0.36</td>
<td>20.00±0.88</td>
<td>20.00±1.15</td>
<td>10.67±5.81</td>
<td>20.00±1.15</td>
<td>18.00±2.00</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>13.67±5.45</td>
<td>20.33±2.40</td>
<td>21.33±1.85</td>
<td>13.33±0.88</td>
<td>21.00±0.00</td>
<td>25.33±0.33</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>21.67±1.20</td>
<td>21.00±2.88</td>
<td>22.67±0.66</td>
<td>16.00±0.00</td>
<td>22.33±1.15</td>
<td>25.67±2.30</td>
</tr>
<tr>
<td>Chorozophora plicata</td>
<td>200</td>
<td>16.33±0.66</td>
<td>23.67±0.57</td>
<td>23.67±1.85</td>
<td>19.33±2.18</td>
<td>12.67±0.66</td>
<td>21.00±0.00</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>17.00±1.52</td>
<td>23.67±3.05</td>
<td>21.00±2.08</td>
<td>15.33±1.45</td>
<td>23.00±0.66</td>
<td>25.67±2.30</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>21.00±0.57</td>
<td>26.33±1.85</td>
<td>26.00±4.58</td>
<td>21.67±0.57</td>
<td>20.00±2.88</td>
<td>26.33±2.18</td>
</tr>
<tr>
<td>Echinops echinatus</td>
<td>200</td>
<td>Nil</td>
<td>10.67±4.00</td>
<td>18.67±2.84</td>
<td>26.00±5.57</td>
<td>13.00±1.52</td>
<td>23.00±5.85</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>13.00±1.00</td>
<td>12.33±0.33</td>
<td>19.67±1.20</td>
<td>26.33±0.66</td>
<td>13.67±0.66</td>
<td>23.33±1.85</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>14.67±2.66</td>
<td>12.67±0.33</td>
<td>20.67±6.00</td>
<td>27.33±2.02</td>
<td>17.33±0.33</td>
<td>24.00±2.08</td>
</tr>
<tr>
<td>Heliotropium europaeum</td>
<td>200</td>
<td>25.67±7.88</td>
<td>16.67±3.83</td>
<td>12.67±6.56</td>
<td>20.33±0.33</td>
<td>14.67±1.33</td>
<td>10.00±5.50</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>26.67±1.33</td>
<td>18.00±2.51</td>
<td>13.00±0.57</td>
<td>20.67±7.88</td>
<td>15.67±1.66</td>
<td>11.33±5.36</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>30.00±2.64</td>
<td>21.00±2.08</td>
<td>14.67±1.76</td>
<td>26.00±5.50</td>
<td>16.00±5.50</td>
<td>13.00±0.57</td>
</tr>
<tr>
<td>Tamarix aphylla</td>
<td>200</td>
<td>11.00±5.85</td>
<td>16.00±1.20</td>
<td>10.67±5.36</td>
<td>16.00±2.66</td>
<td>11.00±0.00</td>
<td>19.33±1.85</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>15.67±1.33</td>
<td>16.67±2.00</td>
<td>11.33±0.33</td>
<td>16.67±2.30</td>
<td>12.33±0.33</td>
<td>19.67±2.66</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>25.67±0.33</td>
<td>18.33±6.22</td>
<td>24.00±2.00</td>
<td>19.00±8.32</td>
<td>13.00±0.57</td>
<td>21.00±2.64</td>
</tr>
<tr>
<td>Ciprofloxacin (200 mg/100 ml)</td>
<td>200</td>
<td>33.67±0.88</td>
<td>31.33±0.66</td>
<td>34.00±1.52</td>
<td>36.33±0.88</td>
<td>33.33±1.85</td>
<td>34.67±0.88</td>
</tr>
</tbody>
</table>
and *P. aeruginosa*; moderate activity was observed against *E. coli* and least activity was found against *S. aureus* and *S. typhi*. *T. aphylla* mother tincture was found ineffective against *S. sonnei* at all concentrations when compared to standard ciprofloxacin.

These zone of inhibition showed that mother tincture of *B. diffusa* plant has moderate antibacterial activity. Tannins, flavonoids, alkaloids and steroids found in number of medicinal plant might account for antibacterial activity[16]. The mother tincture of *H. europeum* plant has moderate antibacterial activity. Tannins, favonoids, alkaloids and steroids found in number of medicinal plant might account for antibacterial activity[16]. The mother tincture of *C. plicata* plant has both highly effective as well as moderate activities. The presence of polyphenolic and flavonoids in *C. plicata* might be responsible for effectiveness against pathogenic bacteria[18]. The mother tincture of *E. echinatus* plant exhibited both highly effective and moderate antibacterial activities. Aerial parts of *E. echinatus* plant contain alkaloids, echinopsine, echinopside and echinozolinone. Apigenin and its derivatives, echinacin and echinacinic are responsible for antimicrobial activity[19]. These results indicated that mother tincture of *H. europeum* plant has moderate to highly effective and its antibacterial activities might be due to pyrrolizidine alkaloids. *T. aphylla* plant has moderate and high antibacterial activity against different species. Flavonoids and polyphenolic in arial parts of *T. aphylla* might be responsible for its antibacterial activity.

As compare to all medical plants results of *H. europeum* showed highly effective activity against three bacterial strains *B. subtilis*, *P. aeruginosa* and *S. typhi*. *C. plicata* exhibited maximum zone of inhibition against three bacterial strains *S. aureus*, *P. aeruginosa* and *E. coli*. Tincture of *B. diffusa* was highly effective only against *E. coli*. Maximum activity of *E. echinatus* was against *S. typhi* bacterial strain. However, *T. aphylla* had shown activity against *B. subtilis* and *P. aeruginosa*. So it is concluded that *H. Europeum*, *C. plicata* and *T. aphylla* are most effective plants against pathogenic microorganisms. However, *B. diffusa* and *E. echinatus* can also be utilised as antibacterial agents but their activity is comparatively lesser.

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**REFERENCES**