INTRODUCTION

The different systems of medicine practised in India, Ayurveda, Siddha, Unani, Amchi and local health traditions, utilize a large number of plants for the treatment of human diseases. Around 400 plants were mentioned having therapeutic values were mentioned by Rigveda, Yajurveda, Atharvana veda (Rajasekarapandiyan et al., 2007). Infectious disease is one of the leading causes of death worldwide, particularly, in developing countries. Infections due to a variety of bacterial etiologic agents, such as pathogenic Escherichia coli, Vibrio cholerae, Shigella sp., Salmonella sp. (Kamruzzaman, et al., 2013,Dubey, 2013).

The natural products not only possess significant pharmacological properties but also provide a source of very important lead compounds. The natural products discovered from medicinal plants have provide numerous clinically used medicinal plants. The natural products discovered from medicinal plants have provide numerous clinically used medicinal plants. The natural products discovered from medicinal plants have provide numerous clinically used medicinal plants. The natural products discovered from medicinal plants have provide numerous clinically used medicinal plants. The natural products discovered from medicinal plants have provide numerous clinically used medicinal plants. The natural products discovered from medicinal plants have provide numerous clinically used medicinal plants.

Preparation of plant extracts

The leaves of the plants were air dried at room temperature for 10 days then powdered using a mixer grinder. The powdered leaves (100 g) were extracted in a Soxhlet apparatus for 72 h with Hexane, chloroform, ethyl acetate, acetone and methanol extracts. Leaf extracts were evaluated against enteric bacterial pathogens by using standard disc diffusion, determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). Methanol extract of Vitex negundo leaves showed potent antibacterial activity (inhibition zone: 7.8-20.7 mm, MIC: 15.62 – 31.25 μg/mL, MBC: 31.25 -62.5 μg/mL) against all the pathogenic enteric bacteria (Escherichia coli, Salmonella typhi, Vibrio cholera, Vibrio fluvialis, Shigella flexneri, Proteus vulgaris and Proteus mirabilis) tested. For the first time it was observed that methanol extract of V. negundo leaves exhibited strong vibriocidal activity in vitro conditions. Therefore, it will be useful to identify and isolate the active compounds of this extract that could be a good alternative medicine to treat cholera.

Evaluation of antibacterial activity

The test solution was prepared with 10mg/mL of crude extract solutions in 5% Dimethyl sulfoxide (DMSO). A Sterile Disc (HiMedia) 6mm were impregnated with different concentration 10μl, 20μl and 30μl of the extract to obtain 100μg, 200μg and 300μg/disc and allowed to dry at room temperature. Ciprofloxacin (5μg/disc) was used as the positive control and 5% DMSO was used as blind (negative) control (Bauer et al., 1966). Petri plates were prepared by pouring 20 mL of Mueller Hinton agar and allowed to solidify. Plates were dried and 0.1 of standardized inoculum suspension was poured and uniformly spread. After drying the disc impregnated the extract disc were placed on the surface of the plate. The inoculated plates were incubated at 37 °C for 24 h and the zone of inhibition was observed and measured in millimeters.

Minimal Inhibitory Concentration (MIC)

500μl of various concentrations (500, 250, 125, 62.50, 31.25, 16.12, 8.06 and 4.03μg/mL) of extract stock solutions were mixed with 500μl of Mueller Hinton broth and 50 μl of bacterial pathogens suspensions individually. Mueller Hinton broth alone served as negative control. Whole setup in duplicate was incubated at 37°C for 24 h. The MIC was the lowest
concentration of the synthetic compounds that did not permit any visible growth of bacteria during 24 h of incubation after inoculation examined on the basis of turbidity (Ravikumar et al., 2010a).

Minimum Bactericidal Concentration (MBC)
To avoid the possibility of misinterpretations due to the turbidity of insoluble compounds if any the MBC was determined by sub-culturing the above (MIC) serial dilutions after 24 h in nutrient agar plates using 0.01 mL culture inoculate and incubated at 370°C for 24 h. MBC was regarded as the lowest concentration that prevents the growth of bacterial colony on this solid media (Ravikumar et al., 2010b).

Statistical analysis
All the data antibacterial activities were examined as mean ±SD. One – sample t test was carried out to determine the significant differences (P<0.05) between the means, the analysis was carried out using Statistical Package of Social science (SPSS package software, version 11.5, Chicago, IL, USA).

RESULTS AND DISCUSSION
The antimicrobial activity of crude leaf extracts of Calotropis gigantea, Lantana camara, Thevetia peruviana, Thespesia populnea and Vitex negundo and the solvent hexane, chloroform, ethyl acetate, acetone and methanol. Antibacterial potential of leaf extract was assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities carried out in 100, 200 and 300 mg/mL of each leaves was used for antimicrobial screening. The antibacterial activity of the extract increased linearly with increase in volume of extract (mg/mL). The methanol extract have shown more sensitive in all plant compared to other solvent.

Antibacterial activity of methanol extract of Thevetia peruviana extracted highest activity in Shigella flexneri (7mm - 11mm) followed by Vibrio cholera (7.7mm - 10mm) and Salmonella typhi (7.5mm – 9.8mm) (Table-1). Highest antibacterial activity of Thespesia populnea was observed in Shigella flexneri (7.5mm – 11mm) and Vibrio cholera (7.2mm – 10mm) and Salmonella typhi (7.5mm – 9.8mm) (Table -2).

Table: 1 Antimicrobial activity of methanol extract of Thevetia peruviana

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of inhibition (mm)</th>
<th>MIC</th>
<th>MBC</th>
<th>Ciprofloxacin (5 µg/disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>200</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7.0 ± 0.6</td>
<td>8.2 ± 0.6</td>
<td>9.0 ± 0.5</td>
<td>125</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>7.5 ± 0.6</td>
<td>8.0 ± 0.6</td>
<td>9.8 ± 0.9</td>
<td>125</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>7.2 ± 0.6</td>
<td>8.8 ± 0.7</td>
<td>10 ± 1.0</td>
<td>62</td>
</tr>
<tr>
<td>Vibrio fluvialis</td>
<td>7.7 ± 0.6</td>
<td>8.2 ± 0.6</td>
<td>9.2 ± 0.7</td>
<td>125</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>7.0 ± 0.6</td>
<td>7.8 ± 0.5</td>
<td>11 ± 1.0</td>
<td>62</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>6.7 ± 0.6</td>
<td>7.5 ± 0.5</td>
<td>8.0 ± 0.5</td>
<td>250</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>7.0 ± 0.5</td>
<td>7.9 ± 0.6</td>
<td>9.0 ± 0.9</td>
<td>250</td>
</tr>
</tbody>
</table>

Table: 2 Antibacterial activity of methanol extract of Thespesia populnea

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of inhibition (mg/mL)</th>
<th>MIC</th>
<th>MBC</th>
<th>Ciprofloxacin (5 µg/disc)</th>
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<tbody>
<tr>
<td></td>
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<td>300</td>
<td></td>
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<tr>
<td>Escherichia coli</td>
<td>6.5 ± 0.6</td>
<td>8.3 ± 0.6</td>
<td>9.5 ± 0.5</td>
<td>125</td>
</tr>
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<td>Salmonella typhi</td>
<td>7.5 ± 0.6</td>
<td>8.0 ± 0.6</td>
<td>9.8 ± 0.9</td>
<td>125</td>
</tr>
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<td>8.8 ± 0.7</td>
<td>10 ± 1.0</td>
<td>62</td>
</tr>
<tr>
<td>Vibrio fluvialis</td>
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<td>8.5 ± 0.6</td>
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<td>125</td>
</tr>
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<td>Shigella flexneri</td>
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</tr>
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<td>250</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>7.0 ± 0.5</td>
<td>7.8 ± 0.6</td>
<td>9.0 ± 0.9</td>
<td>250</td>
</tr>
</tbody>
</table>

Microorganism       | Zone of inhibition (mm) | MIC | MBC  | Ciprofloxacin (5 µg/disc) |
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<td>7.9 ± 0.6</td>
<td>9.0 ± 0.8</td>
<td>125</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>7.4 ± 0.6</td>
<td>8.7 ± 0.7</td>
<td>10 ± 1.0</td>
<td>31.25</td>
</tr>
</tbody>
</table>
Antibacterial activity of methanol extract of *Vitex negundo* presented in (Table 5). Highest activity was observed for *Vibrio cholera* (8.4mm-20.7mm) followed by *Escherichia coli* (8.9mm-19mm) and *Vibrio fluvialis* (8.4mm-18.3mm).

**Table 5 Antibacterial activity of Methanol extract of Vitex negundo**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of inhibition (mg/mL)</th>
<th>MIC</th>
<th>MBC</th>
<th>Ciprofloxacin (5 µg/disc)</th>
</tr>
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<tbody>
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<td>13.8</td>
<td>20.7</td>
<td>31.25</td>
</tr>
<tr>
<td>Vibrio fluvialis</td>
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<td>12.8</td>
<td>18.3</td>
<td>31.25</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>9.0</td>
<td>12.7</td>
<td>17.3</td>
<td>31.25</td>
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<td>Proteus vulgaris</td>
<td>8.1</td>
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</tr>
<tr>
<td>Proteus mirabilis</td>
<td>7.8</td>
<td>11.6</td>
<td>16.7</td>
<td>62.5</td>
</tr>
</tbody>
</table>

Since disc diffusion assay has some limitations and this assay only indicate the growth inhibition ability of the extracts against certain microorganisms, it could not tell whether the inhibition zone is for bactericidal or bacteria static activity of the certain extracts (Kumar et al, 2010). Therefore to confirm the nature of activity and specific dose against certain microorganisms, we determined the MIC and MBC, the values ranged between 31.25 to 500 µg/mL for all enteric bacteria. *Vitex negundo* leaf could kill all types of enteric bacteria tested a range of 31.25 to 62.5 µg/mL of the extract was sufficient to kill the *V. cholera*.

In vitro antibacterial activity of *Vitex negundo* leaf methanol extract (500 µg/mL) at different time interval suggested that it has very strong killing activity against all the *Vitex* species. These results suggested that the compounds of *Vitex negundo* leaf responsible for antibacterial activity in vitro are effective to exert its activity in vivo also. This result convinced us to examine the potentiality of this extract whether it can be able to protect mice from the pathogenic *V. cholera* infection.

**Conclusion**

In this study, we have shown the antibacterial activity of five different plants and five solvent leaf extract on a wide range of enteric pathogens. *Vitex negundo* leaf methanol extract is high activity equally in vitro conditions to kill the cholera causing bacteria. If we identified and isolate the active compounds from *Vitex negundo* leaf. Therefore, further studies should be undertaken to study the active compounds remaining in *Vitex negundo* leaves.

**REFERENCE**