In vitro inhibitory effect of selective plants against clinical strains of Escherichia coli isolated from diarrheal samples from malnourished children

Savak Jasmina S, Vaidya Shashikant P, Deshpade Sunita D and Kar Saraswathy

Abstract
A diarrheal disease is a major public health problem among children. The emergence of infection caused by multiple drug resistant enteric pathogens has now necessitated the search for alternative parenteral agents and introduction of natural plant products. Ayurvedic literature indicates decoction of processed medicinal plant parts as an anti-diarrheal home remedy. Hence systemic study of medicinal plants is very important. This study attempts to screen etiological agent of chronic diarrhoea in malnourished patients and to study in vitro effect of plants on the clinical isolates specifically to Escherichia coli isolates. The study and control group in the study included 100 patients each. A random collection of stool samples and its inoculation on culture media was done. The minimum battery of tests was performed for identification of isolates obtained. Culture positivity was 100% and 94% in control and study group patients studied. E.coli remained the predominant isolate followed by Klebsiella pneumoniae. Antibacterial effect of selective plants was checked on E.coli isolates obtained from study group. The extracts were prepared by hot water decoction method of plant parts like roots of sunthi (Zingiber officinale), seeds of Jeera (Cuminum cyminum), roots of musta (Cyperus rotundus) and it’s in vitro antibacterial activity was carried out by agar well diffusion method against standard strain and clinical isolates of E.coli. Sunthi showed a in vitro antibacterial activity, while musta jeera, as well as combination of all three-plants failed to show in vitro antibacterial activity, when tested against standard strain and clinical isolates of E.coli. Though in literature these plants were documented as one of the promising household remedy as antidiarrheal agents towards diarrhoea, in vitro results of the present study does not show them as effective antidiarrheal agent, except Sunthi.

Keywords: Zingiber officinale, Cuminum cyminum, Cyperus rotundus, diarrhoea, antidiarrhoeal agent, antibacterial activity

1. Introduction
In India, diarrhoeal diseases is a major public health problem among children under the age of five years [1]. In health institution, up to third of total Pediatric admission is due to diarrhoeal diseases and up to 17 per cent of all deaths in indoors pediatrics patients are diarrhoeal related. Acute diarrhoea lasts for less than two weeks, which is nearly always be presumed to be of infective type [2, 3]. Detection of etiological agents of infective diarrhoea is important for therapeutic aspects and for implementation of appropriate control strategies. In developing countries, the bacterial pathogens are most commonly associated with endemic forms of diarrhoea. The emergence and widespread distribution of drug resistant enteric bacteria have imposed serious limitations on successful antibiotics treatment. Spontaneous acquisition of drug resistance among enteric pathogens is due to selective pressure of antibiotic therapy [4]. This problem of antimicrobial resistance in microorganisms causing diarrhoeal diseases in both developed and developing countries continues to be alarming. These multiple drug resistance strains have caused major disease outbreaks with high mortality and morbidity in developing countries [5]. The emergence of infection caused by multiple drug resistant enteric pathogens has now necessitated the search for alternative parenteral agents and the introduction of natural plant products [6]. This is a very important replacement for the resistance. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic values. Recently the acceptance of traditional medicine as an alternative form of
health care and the development of microbial resistance to the available antibiotics has led us to investigate the antimicrobial activity of medicinal plants [6]. Ayurveda describes certain plant drugs, which can be used in treating or reducing severity of diarrhoea [7]. Ayurveda literature search indicates that the decoction of processed Zingiber officinale (Zo) (sunthi), Cyperus rotundus (Cr) (musta), Cuminum cyminum (Cc) (jeera) can be used as an anti-diarrhoeal home remedy [8]. In order to find out their activity, a systemic study of medicinal plants is very important. Scientific study towards various Ayurvedic plant drugs and its effect on various pathogens in vitro as well as in vivo are not well documented. Hence, it is need of today to establish such documents for its regular use as remedy, which will be useful for the control and cure of the various infectious conditions like diarrhoea. Keeping in mind the above facts, we have made an attempt to screen etiological agent of chronic diarrhoea in malnourished patients and to study in vitro effect of selective plant drugs on the clinical isolates specifically to E. coli isolates, as E. coli is probably very common cause of diarrhoea.

2. Material and methods

This prospective longitudinal study was undertaken at the Department of Microbiology, T.N. Medical College and B.Y.L. Nair Charitable. Hospital, Mumbai. Selection of subjects for the study and control group was done on the basis of standard classification of malnourished and chronic diarrhoea. The subjects in the present study were of age group of more than 1 month up to 12 years old. Study group and Control group included 100 patients each. Random collection of stool sample was done in a sterile wide mouth container before starting any empirical treatment from diarrhoeal home remedy [7]. In order to find out their effect of selective plant drugs on the clinical isolates obtained from study group patients using agar dilution method. Details about combination of drug are as follows: (a) Zo + Cc; (b) Zo + Cr; (c) Cc + Cr; (d) Zo + Cc + Cr

3. Results

Table 1 shows the bacterial isolates obtained from study and control group. Culture positivity was 100% and 94% in control and study group patients studied. E. coli remained the predominant isolate among study and control group patients that is 65% and 77%, followed by Klebsiella pneumoniae, as 18% and 13% respectively.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Bacteria isolated</th>
<th>Groups</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Escherichia coli(E.coli)</td>
<td>65</td>
<td>77</td>
<td>71.00</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Klebsiella pneumoniae (K. pneumonia)</td>
<td>18</td>
<td>13</td>
<td>15.50</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas aeruginosa (P. aeruginosa)</td>
<td>0</td>
<td>3</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Proteus vulgaris (P. vulgaris)</td>
<td>1</td>
<td>0</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Staphylococcus aureus (S. aureus)</td>
<td>0</td>
<td>2</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Shigella flexneri (S. flexneri)</td>
<td>4</td>
<td>2</td>
<td>3.00</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Salmonella paratyphi A (S. paratyphi A)</td>
<td>3</td>
<td>0</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Salmonella typhi (S. typhi)</td>
<td>3</td>
<td>0</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Vibrio cholera (V. cholera)</td>
<td>0</td>
<td>3</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>No bacterial isolate</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Key: n1 = number of Cases, n2 = number of controls

Table 2: In vitro effect of plants on E. coli ATCC 25922 strain

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Dilutions</th>
<th>Zone of inhibition in diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zo</td>
<td>Cr</td>
</tr>
<tr>
<td>1</td>
<td>Undiluted (stock-100mcg/ml)</td>
<td>30.33</td>
</tr>
<tr>
<td>2</td>
<td>1:2</td>
<td>26.66</td>
</tr>
<tr>
<td>3</td>
<td>1:4</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>1:8</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>1:10</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>1:12</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>1:14</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>1:16</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>1:32</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Positive control(Gentamicin)</td>
<td>45</td>
</tr>
</tbody>
</table>
Sunthi showed a dose dependent in vitro antibacterial activity up to 1:14 dilution, when tested against E. coli ATCC 25922 strain. Zone diameter was confirmed by taking averages of 3 tests. Musta, Jeera as well as combination of all three-plant drugs failed to show in vitro antibacterial activity, when tested against E. ATCC 25922 Strain.

<p>| Table 3: In vitro effect of combination of plants on E. coli ATCC 25922 strain |</p>
<table>
<thead>
<tr>
<th>No. Dilutions</th>
<th>Zone of inhibition in diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Undiluted Stock (100mcg/ml)</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>2  1:2</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>3  1:4</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>4  1:8</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>5  1:10</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>6  1:12</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>7  1:14</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>8  1:16</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>9  1:32</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>10 Positive control (Gentamicin)</td>
<td>45 45 45 45</td>
</tr>
</tbody>
</table>

Table 4: In vitro effect of plants on clinical isolates of E. coli. n = 65

<table>
<thead>
<tr>
<th>No. Dilutions</th>
<th>No of E. coli isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  1:10</td>
<td>17 - - -</td>
</tr>
<tr>
<td>2  1:12</td>
<td>17 - - -</td>
</tr>
<tr>
<td>3  1:14</td>
<td>17 - - -</td>
</tr>
<tr>
<td>4  1:16</td>
<td>17 - - -</td>
</tr>
<tr>
<td>5  1:32</td>
<td>17 - - -</td>
</tr>
<tr>
<td>6 Control</td>
<td>+ + + +</td>
</tr>
</tbody>
</table>

Key: +: growth; - : no growth; n = no of clinical isolates of E. coli

None of the combination of plants showed in vitro antibacterial activity against E. coli ATCC 25922 strain.

Table 5: In vitro effect of plants in various combinations against clinical isolates of E.coli n = 65

<table>
<thead>
<tr>
<th>No. Dilutions</th>
<th>No of E. coli isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  1:10</td>
<td>Zo+ Cc</td>
</tr>
<tr>
<td>2  1:12</td>
<td>- - - -</td>
</tr>
<tr>
<td>3  1:14</td>
<td>- - - -</td>
</tr>
<tr>
<td>4  1:16</td>
<td>- - - -</td>
</tr>
<tr>
<td>5  1:32</td>
<td>- - - -</td>
</tr>
<tr>
<td>6 Control</td>
<td>+ + + +</td>
</tr>
</tbody>
</table>

Key: +: growth; - : no growth; n = no of clinical isolates of E. coli

None of the combination of plants showed in vitro antibacterial activity against clinical isolates of E.coli from study group by agar dilution method.

4. Discussion

In India, traditional uses of many household remedies are seen to be useful in diarrhoea and are found to be effective without any side effects. But there is no scientific support and documentation to use these remedies as a routine treatment. Hence we have made an attempt to screen etiological agent of chronic diarrhoea in malnourished patients and to study in vitro effect of selective plants like Sunthi, Musta, Jeera individually and in various combination against clinical isolates specifically to E. Coli isolates which are predominant. E.coli constitutes a diverse group of organisms, including non-pathogenic strains, which are among the most common bacteria in the normal flora of the human intestine and pathogenic strains. These Diarrhoeagogenic E.coli have been studied extensively and are classified on the basis of serogrouping or pathogenic mechanisms into five major groups. Enter pathogenic E.coli (EPEC), an important cause of diarrhoea in infants in developing countries. Enter otoxigenic E.coli, a cause of diarrhoea in infants in developing areas of the world and a cause of traveler’s diarrhoea in adults. Enter oivnses E.coli, which cause either a watery Enteroxigenic like illness or less commonly, a dysentery-like illness. Enter ohemorrhagic E.coli, which cause hemorrhagic colitis and Hemolytic Uremic Syndrome. Enter eaggreagative E.coli and diffuse-adherent E.coli, which along with EPEC have been implicated as cause of persistent diarrhoea.

In the present study, comparison between acute and chronic diarrhoea cases revealed that E.coli is the commonest etiology in acute as well as chronic diarrhoea cases. Though, exact demarcation between types of E.coli was not observed, but finding of this study suggest that multiple acute diarrhoeal episodes in children leads to develop malnourished condition which decreases the immunity in children and may support to develop normal communal flora as etiological agents for repeated prolonged diarrhoeal condition in pediatric cases. For confirmation of EHEC E.coli Hicrome agar was used in this study, while EPEC serotyping was carried out using ‘O’ type standard antisera. Children less than 1 yr were found to be predominant group associated with infective chronic diarrhoea more prone to severe malnutrition.

Sixty five clinical isolates of E.coli from study group were tested for in vitro antibacterial activity of Sunthi, Jeera and Musta by agar dilution method. Among selected plants Sunthi was found to be effective. It showed antibacterial activity against 26% of clinical isolates of E. coli obtained from study group cases. Jeera and Musta did not show any in vitro antibacterial activity against any clinical isolate of E. coli. Also the combination of all three plants did not show any activity against clinical isolates.

Ballal M et al. in 2005* from India reported that Sunthi extract found to be effective as antifungal at 7 mg/ml
concentration, while the study in 2010 [13], focuses the significant antibacterial activity of garlic extract on streptomycin-resistant strains solely and in synergism with streptomycin. Statistical comparison of sole extract and streptomycin synergism with streptomycin control had proved it significant. The study in 2016 [14], showed that standard S. aureus strain and E. coli strain was completely inhibited by 10 mg/ml and 15 mg/ml of garlic in agar media respectively and their clinical isolates were completely inhibited by 25 mg/ml, indicating that standard isolates were most sensitive and clinical isolates were least sensitive. The study recommended that garlic could be used as effective antibacterial agent for these pathogens.

Jeera was found to be ineffective against clinical isolates of E.coli in our study. Study in 2013 [15] showed antimicrobial properties of methanolic extract of cumin seeds on four enteropathogenic and food-spoiler bacterial strains. Minimum concentrations of cumin extract effective against E.coli, P.aeruginosa, S.aureus and B.pumilus were found to be 12.5, 6.25, 25.0 and 6.25 mg dry weight per ml respectively.

Musta was found to be ineffective against clinical isolates of E.coli in our study. Daswani PG et al [16] carried out study with Musta on EPEC using HEP-2 cells by tissue culture technique to check the production of both toxin and found to be effective only at very high concentrations. Activity of that plant was not necessarily dose dependent. In this study, effect on adherence of enteropathogenic E. coli and invasion of enteroinvasive E. coli and Shigella flexneri to HEP-2 cells was evaluated as a measure of effect on colonization. Effect on enterotoxins such as enterotoxigenic E. coli heat labile toxin (LT), heat stable toxin (ST) and cholera toxin (CT) was also assessed. The decoction showed reduced bacterial adherence to and invasion of HEP-2 cells and affected production of CT and action of LT. The decoction of Musta does not have marked antimicrobial activity and exerts its anti-diarrhoeal action by mechanisms other than direct killing of the pathogen. In another study [17], the attempt was made to evaluate the antimicrobial activity of various solvent extracts of contents of Balchaturbhadra Yoga against different gram positive and gram negative bacteria. In the present antimicrobial study of ingredients of Balchaturbhadra Yoga, the Pippali and Karkatshringi showed promising antimicrobial activity against E. coli, E. faecalis, and V. cholera but antimicrobial activity of Musta and Ativisha were not found.

Dichotomy in the activities of these plants in various studies indicate that the extraction method and in vitro testing method needs to be validated against standard protocol.

5. Conclusion

The study can be thus concluded as follows. Bacterial infection is more common with chronic diarrhoea in malnourished children. E.coli is the predominant etiological agent for chronic infective diarrhea in malnourished children. As per suggestive mechanisms of chronic infective diarrhoea in malnourished children, normal commensal E.coli can be one of the predominant etiological agent for chronic infective diarrhoea in malnourished children. In vitro antibacterial activity of Sunthi was observed but Jeera and Musta did not show any antibacterial activity towards E.coli isolates from study group. None of the combination of three drugs showed any in vitro antibacterial effect on E.coli isolates from study group. In vitro results of these plants did not show them as effective antibacterial agent, except Sunthi. But in literature these plants were documented as one of the promising household remedy as anti-diarrhoeal agents. Hence the mode of action of these plants needs to be evaluated further as anti-diarrhoeal agent.

6. Recommendations

More research work on these plants to combat chronic and acute diarrhoea in malnourished children with respect to its mode of action and decoction methods are needed.

7. Acknowledgement

The authors are extremely thankful to Microbiology Department of T.N. Medical College and B.Y.L. Nair Charitable Hospital, India for providing research facilities to conduct this work.

8. References

5. Ballal M. Screening of Medicinal plants used in Rural Indian folk Medicine for treatment of diarrhea, Read. 2005; 14:685