INTRODUCTION

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, many efforts have been made to ascertain novel antimicrobial compounds from diverse kinds of sources such as microorganisms, animals and minerals.1 In India Ayurveda is one of the noteworthy systems of traditional medicine practice that uses mainly certain plants for the treatments of ailments in both man and other animals.2-4 Plants are used directly as therapeutic agents, as well as starting material for the synthesis of drugs or as models for pharmacologically active compounds.5 Now a day herbal medicines are gaining growing interest because of their cost-effective, eco-friendly attributes and true relief from disease condition. The increasing prevalence of multidrug resistant strains of bacteria and the recent emergence of strains with reduced susceptibility to antibiotics raises the spectre of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies.6 Consequently plants as single herb and compound drugs prepared from it should be probed to comprehend their properties, safety and efficacy for a search of new potent antimicrobial compounds.7 Bilwadi Agada is mentioned in Ashtanga Hridaya chapter 36 / 84-858 comprises the effect in versatile infective conditions like Vischika, Jwara, Garavisha and various poisonous conditions including venomous bites. So it is very much essential to evaluate the effects of this compound preparation against certain microbes. Present paper highlights the results of contents of Bilwadi Agada analyzed both as potential antimicrobial and anti-fungal in the form of compound drug as well as a source for natural compounds that act as new anti-infection agents.

Need and Significance

In last three decades, numbers of new antibiotics have produced, but clinical efficacy of these existing antibiotics is being threatened by the emergence of multi drug-resistant pathogens. In Ayurvedic literature various modalities as well as drugs are prescribed for the diseases which have infective focii according to modern medical science. Principal internal medicines administered in Ayurveda for such conditions are herbo-mineral in origin. Contrary to the synthetic drugs, antimicrobial substances of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious disease. Most of the higher plants produce a large number of diverse chemical compounds with different biological activities. Therefore such plants should be investigated to understand their properties, safety and efficacy and for a search of new potent antimicrobial compounds and fractions.

Aim and Objectives

The aim of this study was to evaluate the antimicrobial activity of compound herbal preparation, ‘Bilwadi Agada’

- To evaluate anti-microbial effect of Bilwadi Agada on standard bacterial strains.
To evaluate anti-fungal effect of Bilwadi Agada on certain standard fungal strains.

**MATERIALS AND METHODS**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the content</th>
<th>Latin Name</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Bilva (moola)</td>
<td>Aegle marmelos corr</td>
</tr>
<tr>
<td>2</td>
<td>Surasa (pushpa)</td>
<td>Ocimum sanctum linn</td>
</tr>
<tr>
<td>3</td>
<td>Karanja (phala)</td>
<td>Pongamia pinnata Perri</td>
</tr>
<tr>
<td>4</td>
<td>Tagara</td>
<td>Valeriana wallichii DC</td>
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<td>5</td>
<td>Devadaru</td>
<td>Cedrus deodara Roxb</td>
</tr>
<tr>
<td>6</td>
<td>Haritaki</td>
<td>Terminalia chebula Retz</td>
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<tr>
<td>7</td>
<td>Vibhitaki</td>
<td>Terminalia bellirica Roxb</td>
</tr>
<tr>
<td>8</td>
<td>Amalaki</td>
<td>Emblica officinalis Gaertn</td>
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<td>9</td>
<td>Shunthi</td>
<td>Zingiber officinale Rosc</td>
</tr>
<tr>
<td>10</td>
<td>Maricha</td>
<td>Piper nigrum Linn</td>
</tr>
<tr>
<td>11</td>
<td>Pippali</td>
<td>Piper longum Linn</td>
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<td>12</td>
<td>Haridra</td>
<td>Curcuma longa Linn</td>
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<tr>
<td>13</td>
<td>Daruharidra</td>
<td>Berberis aristata DC</td>
</tr>
<tr>
<td>14</td>
<td>Bhavana with Aja mutra (Goat’s urine)</td>
<td>Capra indica - Urine</td>
</tr>
</tbody>
</table>

Photo Gallery of the Ingredients (Bilwadi Agada)

Figure 1: *Aegle marmelos*

Figure 2: *Ocimum sanctum*

Figure 3: *Pongamia pinnata*

Figure 4: *Valeriana wallichii*

Figure 5: *Cedrus deodara*

Figure 6: *Terminalia chebula*

Figure 7: *Terminalia bellirica*

Figure 8: *Emblica officinalis*
Plan of Study
The antimicrobial activity was assessed qualitatively and quantitatively by the presence or absence of inhibition zones. Micro-organisms strains of *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi* were used for assessing the anti microbial activity with standard Cefpodoxime (10 mg / ml). Fungal strains *Aspergillus niger* and *Candida albicans* were used for anti-fungal activity with control Fluconazole in the concentration of (10 mg / ml).

Study Protocol
Antimicrobial activity will be determined by Agar well method. Muller Hinton and Saboured Dextrose Broth were used as medium for bacterial and fungal strains respectively. Positive control for bacterial culture was carried out under the similar condition by using Cefpodoxime dispersible tablets (10 mg / ml) and control for fungal culture was Fluconazole tablet (10 mg / ml). The petri-dishes with the bacterial and fungal cultures will be incubated at 37 ± 20 C for 24 h and 27 ± 20 C for 48 h respectively. The assessment of anti microbial activity was based on the measurement of diameter of inhibition zone formed. The experiment was repeated twice and the results were taken as mean of two readings.

Preparation of Extract
Bitwadi Agada – herbo-mineral formulation was subjected to simple percolation process. Distilled water was used for preparation of extracts required for the study.

OBSERVATION AND ANALYSIS
RESULTS
The potential sensitivity of Bilwadi Agada was obtained against all the microorganisms tested as zone of inhibition. The final results are based on the measurement of mean diameter of inhibition zone formed on the completion of study.

In Table 2 the drug displayed various activities against microbes inhibiting it at various concentrations ranging from 60 % to 100 %. The inhibition zones against Staphylococcus aureus were 1 mm and 2 mm at 90 % and 100 % concentration respectively comparable to the standard antibiotic Cefpodoxime with inhibition zone of 5 mm. Bilwadi Agada also showed activity against Salmonella paratyphi – B with inhibition zone of 2 mm, 4 mm, 6 mm and 8 mm in concentration of 70, 80, 90 and 100 % respectively. However it has no activity against E. coli and Bacillus subtilis.

Table 2: Antimicrobial Activity of Bilwadi Agada

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Organism</th>
<th>Bilwadi Agada</th>
<th>Control</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Concentrations</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 %</td>
<td>70 %</td>
</tr>
<tr>
<td>1</td>
<td><em>Bacillus subtilis</em></td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td><em>Staphylococcus aureus</em></td>
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</tr>
<tr>
<td>3</td>
<td><em>Escherichia coli</em></td>
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<td>--</td>
</tr>
<tr>
<td>4</td>
<td><em>Salmonella paratyphi – B</em></td>
<td>--</td>
<td>2 mm</td>
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Table 3: Anti-fungal Activity of Bilwadi Agada

<table>
<thead>
<tr>
<th>Organism</th>
<th>Bilwadi Agada</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentrations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 %</td>
<td>70 %</td>
</tr>
<tr>
<td><em>Candidia albicans</em></td>
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<td>--</td>
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<tr>
<td><em>Aspergillus niger</em></td>
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Table 4: Total Microbial Load Count

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
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</thead>
<tbody>
<tr>
<td>Total Plate count</td>
<td>14 x 10³</td>
<td>Cuf/ g</td>
</tr>
<tr>
<td>Total fungal count</td>
<td>3 x 10⁴</td>
<td>Cuf/ g</td>
</tr>
</tbody>
</table>

DISCUSSION
Reviews of activities of individual ingredients of Bilwadi Agada showed potent anti-microbial as well as antifungal activities. Crude extracts of *Aegle marmelos* (hexane, cold methanol and hot methanol extracts at a concentration of 100 mg / ml) showed positive results especially against *E. coli*.° another study showed that ethanolic leaf extracts of *Aegle marmelos* exhibited significant activity towards bacterial strains like *B. subtilis* and *E. coli* were more sensitive towards the treatment when compared to *S. aureus*.¹
Similarly O. sanctum displayed marked antibacterial efficacy against E. coli, S. aureus and B. subtilis. Likewise Pongamia pinnata, Valeriana wallichii, Cedrus deodara, Terminalia chebula as anti-microbial and anti-fungal activity. The antibacterial activity of Emilia of E. chebula has also exhibited remarkable activity against various gram +ve and gram –ve bacteria in number of studies conducted by scholars earlier. One of the noteworthy finding observed in present study is that, ‘Bilwadi Agada’ does not demonstrate any activity against E. coli which are one or the other way beneficial to the gut for its normal functioning comparable to the control drug which may affect intestinal flora and disturb G.I. functioning.

CONCLUSION

The antimicrobial and anti-fungal activity of Bilwadi Agada at higher concentration is comparable with Cefpodoxime and Fluconazole with concentration of 10 mg / ml each. Present study has confirmed the antimicrobial potentials of compound herbino-mineral preparation supporting its application may be as a preventive remedy for various microbial diseases. It also exhibited moderate to mild antimicrobial activity against most of the tested bacteria and fungi. Nevertheless authentic use of Bilwadi Agada may require further fundamental study vis-à-vis higher concentrations and samples.

REFERENCES


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