Antibacterial activity of herbal mouth wash against clinical isolates of oral bacteria

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Running Title: Antibacterial activity of herbal mouth wash

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Abstract
Viridans Group Streptococci (VGS) are the normal oral flora and etiological agents of serious infections such as infective endocarditis (IE), septicemia, meningitis and are often associated with infections in neutropenic and immunocompromised patients. The reported isolation of Staphylococcus sp from the oral cavity of healthy adults has changed the perception of looking Staphylococcus as pathogen. They could well be an oral colonizer and may associate with various diseases. The increasing resistance has shifted the treatment towards macrolides and other higher forms. In the present study an oral mouth wash was tested for its antibacterial activity against oral bacteria such as VGS and Staphylococcus sp by agar well diffusion method and minimum inhibitory concentration (MIC) assay. At 100% concentration, the oral mouth wash showed the highest zone of inhibition (25 mm) against S.mutans. MIC of the oral mouthwash against S.mutans and S.oralis was 9.37 mg/ml. To conclude our mouth wash had an excellent antibacterial activity against both the tested oral bacterias. This in turn reflects that our mouth wash could be effective against oral bacteria and can be used regularly in day to day life.

Keywords: Herbal mouth wash, Antibacterial, Oral bacteria

Introduction
Viridans Group Streptococci (VGS) are the normal oral flora and etiological agents of serious infections such as infective endocarditis (IE), septicemia, meningitis [1] and are often associated with infections in neutropenic and immunocompromised patients [2]. They are gram positive, catalase negative, spherical or oval shaped cocci in chains. They exhibit alpha hemolysis or no hemolysis when grown on blood agar. They are facultative anaerobes and are fastidious requiring media enriched with blood or tissue fluids [3]. VGS has been classified into five groups’ viz. mutans, salivarius, anginosus, sanguinus and mitis [4]. Among the various groups S.mutans and S.oralis have been frequently isolated from dental plaques. It has been found to cause dental caries and also associated with infective endocarditic. Incidence of infections due to VGS has been increasing in recent years and they are being associated with several life threatening diseases. Unlike VGS, Staphylococci are not generally considered to be the part of normal oral flora, and considered to be transient colonisers [5]. However, Murphy [6] has isolated Staphylococcus from the oral cavities of 86% of a group of 100 students, of which 17 were harboring Staphylococcus aureus. The reported isolation of Staphylococcus sp from the oral cavity of 94% of healthy adults [7] has changed the perception of looking Staphylococcus as pathogen. They could well be an oral colonizer and may be associated with various diseases.

Beta-lactam especially cephalosporin antibiotics are the drugs of choice for the treatment of VGS and
Antibacterial activity screening

Anti bacterial activity of the herbal mouth wash was screened by agar well diffusion method. Briefly, to the Mueller Hinton agar (MHA) plates supplemented with 5% blood were inoculated with 24 hrs culture of the respective bacterial strains. Using a sterile 6 mm agar well cutter 4 wells were made. 20 µL of the herbal mouth wash at different concentrations were loaded on to the respective marked wells. The plates were kept undisturbed for 30 min to allow the diffusion of the mouth wash in to the agar. Then the plates were incubated at 37°C for 24 hrs with 5% CO₂ for VGS and without CO₂ for Staphylococcus. The plates were then observed for the zone of inhibition around the wells.

MIC & MBC:

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by agar dilution method using MHA supplemented with 5% sheep blood. Briefly, MHA supplemented with 5% sheep blood with different concentration (0.58 to 300mg/ml)of the herbal mouth wash were prepared. Fresh 24 hrs grown culture was used in the study. The culture turbidity was adjusted to match with 0.5 Mac Farland’s standard and 10 µL were spot inoculated on to the respective agar plates. The plates were incubated at 37°C for 24 hrs with 5% CO₂ for VGS and without CO₂ for Staphylococcus. After incubation the plates were observed for the inhibition of growth. The minimum concentration at which growth is inhibited is termed as the MIC value; since the extract is colored MBC values were also obtained.

Results

Agar well diffusion

At 300mg/ml concentration the oral mouth wash showed the highest zone of inhibition, the zone of inhibition increases as the concentration increases (Table 1). At 300 mg/ml concentration the oral mouth wash showed an inhibition zone of 25 mm against S.mutans and 11 mm against S.oralis. However, S.aureus was completely resistant to the oral mouth wash which fails to show inhibition zone even at highest concentration (300 mg/ml). Ampicillin was used as the control drug.
Table 1: Results of agar well diffusion assay

<table>
<thead>
<tr>
<th>Organism</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300 mg/ml</td>
</tr>
<tr>
<td><em>Streptococcus mutants</em></td>
<td>25</td>
</tr>
<tr>
<td><em>Streptococcus oralis</em></td>
<td>11</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>R</td>
</tr>
</tbody>
</table>

R-Resistant, AMP-Ampicillin

Table 2: Results of Minimum Inhibitory Concentration Assay

<table>
<thead>
<tr>
<th>Organism</th>
<th>Minimum inhibitory concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300</td>
</tr>
<tr>
<td><em>Streptococcus mutants</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Streptococcus oralis</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
</tr>
</tbody>
</table>

Presence of growth (+), Absence of growth (-)

Table 3: Results of Minimum Bactericidal Concentration Assay

<table>
<thead>
<tr>
<th>Organism</th>
<th>Minimum bactericidal concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300</td>
</tr>
<tr>
<td><em>Streptococcus mutants</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Streptococcus oralis</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
</tr>
</tbody>
</table>

Presence of growth (+), Absence of growth (-)

Discussion

The implication of oral bacteria has been well documented in various infections such as IE, septicemia meningitis. The increasing resistance towards different antibiotics by various bacterial species especially normal oral flora such as VGS requires greater attention. These bacteria may act as a potential reservoir in transmitting resistance genes to various susceptible organisms. The frequent exposure of antibiotics and reported overuse and abuse of antibiotics has led to these conditions in transmitting these resistance genes. The need of hour is to search for an effective antibacterial drug which can effectively kill the bacteria without resistance development. Herbal drugs have a great potential to one of its kind which can efficiently kill and remove microbial colonization in the oral cavity. In our study a poly herbal mouth wash showed a comparable result when tested against VGS with known antibiotic viz., ampicillin. In a study pudina extract at 50% concentration showed a comparable zone of inhibition to that of our
result against S. mutans [12]. Babu et al. [13] reported that clove showed an inhibitory zone of 18 mm against S. pyogenes which exactly corroborates with our result. To conclude our mouth wash had an excellent antibacterial activity against both the tested oral bacteria. This in turn reflects that our mouth wash could be effective against oral bacteria and can be used regularly in our day to day life.

Conflict of interest
The authors declare that there is no conflict of interest to reveal.

References