In vitro comparison of the effect of shallot extract and chlorhexidine mouthwash on oral pathogens

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Chlorhexidine is the most common mouthwash that we can compare the effect of new products with it as a gold standard. This study is designed to compare the effects of shallot extract with chlorhexidine on oral pathogens. The water extract of shallot was mixed with ethyl acetate in 50:50 proportions for a period of 24 h; the upper organic layer was separated and dried in a rotary evaporator, dissolved in methanol and subjected to antimicrobial activity. The tested bacteria were Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius and Lactobacillus casei. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of shallot extract and chlorhexidine were measured by E test and tube dilution method respectively. The growth of all tested microorganisms was stopped by shallot extract. The least MIC of shallot extract was for S. mutans, 0.4 µg/ml and the most was for L. casei, 1.4 µg/ml. The MIC of chlorhexidine for these bacteria was 0.62 and 5 µg/ml respectively. Regarding the results, shallot extract has good effects on oral pathogens as compared with chlorhexidine and can be used as a new mouthwash but its side effects must be investigated.

Key words: Shallot extract, chlorhexidine, oral pathogens, mouthwash.

INTRODUCTION

The primary way of prevention of oral diseases is plaque control and prevention of plaque accumulation on tooth and gingival surfaces. In fact mechanical plaque removal is the most effective way of preventing caries, gingivitis, periodontitis, and microbial systemic diseases (Carranza, 2007). Although people try to develop their oral hygiene many of them cannot remove plaque favorite (Niklaus and Michel, 1986). So mouthwashes are used to complete the process of mechanical plaque removal (Maza et al., 2002). Chlorhexidine is the first, and the most common mouthwash that its inhibitory effects of plaque accumulation and gingivitis are proven (Lindhe et al., 2003; Abasi, 2001, 2002; Barkvoll and Rolla, 1989; Shiraz, 2000, 2001; Chadwick et al., 1991).

So we can compare the effect of new products with this mouth wash as gold standard. The use of herbal drugs refers back to 6 thousand years ago in Iraq; former people used hollyhock plants for treatment. Now in United States ¼ of drugs in markets are herbal. The plants which have antimicrobial effects are: garlic, onion, thyme etc (Farnsworth and Morris, 1976). Allium ascalonicum is the scientific name of Shallot. This plant is

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The target microbes obtained from the Persian Type Culture Collection. All bacteria were stored in trypticase soy broth containing 25% (v/v) glycerol and refreshed on blood agar medium. Standard strains where included: *Streptococcus mutans* (PTCC: 1683), *Streptococcus sanguis* (PTCC: 1449), *Streptococcus salivarius* (PTCC: 1448) and *Lactobacillus casei* (PTCC: 1608).

### MATERIALS AND METHODS

#### Preparing of shallot extract

About 300 g of white shallot bulbs (collected from Zagros Mountains, 50 Km of Dezful city-south of Iran in spring season) were washed thoroughly in water and mashed properly in a kitchen mixer. The mashed shallot was mixed with 300 ml of distilled water, and soaked with stirring by a magnetic stirrer for a period of 5 h. The suspension was then filtered through Whatman No. 1 filter paper. The water extract was mixed with ethyl acetate in 50:50 proportions and kept for stirring on magnetic stirrer for a period of 24 h; the upper organic layer was separated in separating funnel and centrifuged at 5000 rpm for 10 min. The ethyl acetate layer was then removed and transferred to a clean flask. This work was repeated for 3 times and extracts pooled and dried in a rotary evaporator (Heidolph – Germany) at 50°C and the yield of the extract was measured. The dried extract was dissolved in methanol and subjected to antimicrobial activity (Amin, 2005).

#### Microorganisms and their maintenance

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### RESULTS

According to Table 2 the growth of all tested microorganisms were stopped by shallot extract but *S. mutans* was the most sensitive one. According to Table 1 the efficacy of shallot extract in inhibiting the growth of *S. mutans*, *S. sanguis*, and *L. casei* was more than chlorhexidine and its efficacy on *S. salivarius* was less than chlorhexidine (p<0.05).

The least MIC of shallot concentration was for *S. mutans* 0.4 µg/ml and the most was for *L. casei* 1.4 µg/ml. The MIC of chlorhexidine for target bacteria obtained by E test was ranged from 0.31 to 5.0 µg/ml (Table 1). The results obtained by broth dilution method about the MBC of chlorhexidine showed that this chemical mouth wash has cidal effect more than shallot extract (p<0.05) (Tables 3 and 4).

### DISCUSSION

According to Tables 3 and 4 the MBC of shallot extract is more than that of chlorhexidine. The effect of chlorhexidine.
Table 3. The MBC values of chlorhexidine by broth dilution method.

<table>
<thead>
<tr>
<th>Oral pathogens</th>
<th>MBC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. casei</td>
<td>7.8</td>
</tr>
<tr>
<td>S. salivarius</td>
<td>1.9</td>
</tr>
<tr>
<td>S. mutans</td>
<td>1.9</td>
</tr>
<tr>
<td>S. sanguis</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 4. The MBC values of shallot extract by broth dilution method.

<table>
<thead>
<tr>
<th>Oral pathogens</th>
<th>MBC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. casei</td>
<td>16.8</td>
</tr>
<tr>
<td>S. salivarius</td>
<td>9.6</td>
</tr>
<tr>
<td>S. mutans</td>
<td>4.8</td>
</tr>
<tr>
<td>S. sanguis</td>
<td>10.5</td>
</tr>
</tbody>
</table>

may be due to its chemical properties and may have side effect on epithelial cells of mouth. Although discoloration of tooth and desquamation of oral mucosa by this mouthwash have been proven. We think that the static effect of shallot extract is a positive point for this new antimicrobial agent.

According to MBC of shallot extract (Table 4) S. mutans is the most sensitive bacteria and Lactobacillus is the least sensitive to chlorhexidine; since S. mutans is the main cariogenic bacteria in mouth so this result is satisfying.

The results showed that MIC of shallot extract measured by E test for all 4 bacteria is less than that of chlorhexidine. It means that the antimicrobial activity of shallot extract is more than that of chlorhexidine.

There is no report about study of antifungal effect of shallot extract as a mouth wash. But there are a few reports about antifungal and antimicrobial properties of shallot extract. Amin et al. (2009) purified shallot extract and tested its antimicrobial effects against four bacteria. The MIC for purified shallot extract was about 2-6 µg/ml that is comparable with commercial antibiotics (Amin and Kapadnis 2005; Amin and Kushapoor, 2005).

Azizi et al. (2008) tested the effect of chlorhexidine 0.12% on pathologic oral Streptococcus and normal flora of mouth of 28 volunteers. The results showed that this mouthwash kills a part of normal bacterial flora that is an unfavorable effect of it (Azizi and Lavaf, 2008).

Conclusion

Regarding to the results, shallot concentration has good effect on oral pathogens when compared with chlorhexidine and can be used as a new mouth wash but its pathologic side effects must be investigated.

ACKNOWLEDGEMENTS

This paper is issued from thesis of Zeinab Eftekhar and financial support was provided by Ahvaz Jundishapur University of Medical Sciences.

REFERENCES


