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Full Length Research Paper

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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Table 1. The MIC values of chlorhexidine by E test.

Oral pathogens	MIC (µg/ml)
L. casei	5
S. salivarius	0.31
S. mutans	0.62
S. sanguis	2.5

Table 2. The MIC values of shallot extract by E test.

Oral pathogens	MIC (µg/ml)
L. casei	1.4
S. salivarius	0.8
S. mutans	0.4
S. sanguis	0.8

native in Palestine and grows wild in Syria and Iran, but in some European and American countries is harvested widely (Ghosh, 2000).

Former investigations showed that shallot alcoholic fraction which here is called purified shallot extract has fatal effects on microbes. Since the common mouthwash chlorhexidine is a chemical mouthwash and has side effects like discoloration of tooth, changing oral taste and desquamation of oral mucosa. This study is designed to compare the effects of shallot with chlorhexidine on oral pathogens.

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. This 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

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).

Determination of minimal inhibitory concentration (MIC) of shallot extract and chlorhexidine by E test

A colony of freshly grown culture was suspended in 5 ml of Trypticase Soy Broth (TSB) (Merck- Germany) The turbidity was adjusted to that of a Mc Farland 0.5 standard to make a dilution of 1.5×10^8 (Forbes et al., 2007). The plate was inoculated by dipping a sterile cotton swab into the cell suspension and streaking it across the surface of the agar in three directions. The plates were dried at ambient temperature for 15 minutes before applying the discs. 8 sterile discs (diameter 6 mm) were kept on the agar surface in a line. The shallot extract and chlorhexidine mouthwash 0.12% (Shahr Daru Company, Iran) were serially diluted in methanol and distilled water respectively. 10 µl of each dilution was separately used to impregnate the disc. The plates were incubated for 48 h at 37°C. The MIC values were read as the antimicrobial co ncentration at the point where dense colonial growth intersected the disc (Amin, 2005). The test was performed in quadruplicate for each culture.

Determination of minimal bactericidal concentration (MBC) of shallot extract and chlorhexidine

The broth dilution method was used for determination of MBC. Eight concentrations of chlorhexidine and shallot extract was prepared and poured in 16 test tubes then bacterial suspension with the concentration of 5×10^6 bacteria per ml was added to each tube and incubated in 37°C for 24 h. The optical brig htness of tubes shows the inability of bacteria to growth. The tube with the minimum concentration of antibiotic which had stayed turbid showed the MIC of that antibiotic. For determination of MBC, 0.01 ml of the suspension of the other tubes (which were clear and apparently no bacteria have grown in them) was cultured in solid medium to study which concentration of antimicrobial substance prevented the growth of the bacteria. The minimum concentration which showed growth of less than 10% of original concentration of bacteria (5×10^6) monitoring the MBC of the antibiotic (Forbes et al., 2007). Both E test and tube test were repeated 3 time for all 4 bacteria to standard the results.

RESULTS

According to Table 2 the growth of all tested micro-organisms 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.

Oral pathogens	MBC (µg/ml)
L. casei	7.8
S. salivarius	1.9
S. mutans	1.9
S. sanguis	15

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

Oral pathogens	MBC (µg/ml)
L. casei	16.8
S. salivarius	9.6
S. mutans	4.8
S. sanguis	10.5

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 antimicrobial 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.

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