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An *in vitro* antimicrobial comparison of miswak extract with commercially available non-alcohol mouthrinses

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Abstract: The aim of this study was to assess the antimicrobial activity of eight commercially available mouthrinses and 50% miswak extract against seven microorganisms. Corsodyl, Alprox, Oral-B advantage, Florosept, Sensodyne, Aquafresh Mint, Betadine and Emoform mouthrinses were used while 50% aqueous extract of miswak (*Salvadora persica*) was used against *Streptococcus faecalis*, *Streptococcus pyogenis*, *Streptococcus mutans*, *Candida albicans*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The ditch plate method was used to test the antimicrobial activity. Inhibition zones of microorganisms around ditches were measured in millimetres. Range, mean and standard deviations were used for comparison of antimicrobial activity. Mouthrinses containing chlorhexidine was with maximum antibacterial activity, while cetylpyridinium chloride mouthrinses were with moderate and miswak extract was with low antibacterial activity. Further research is needed for the substantivity of these mouthrinses and further *in vivo*/*in vitro* studies are needed using Biofilm model to substantiate present findings. Dental professionals must exercise caution and provide guidance in assisting their patients in making informed choices regarding their use of mouthrinses for clinical efficacy.

Key words: miswak (chewing stick); *Salvadora persica*; mouthrinses; antimicrobial activity; herbal extract; chlorhexidine

Introduction

To maintain oral health, contemporary dentistry emphasizes the control of dental plaque and of microorganisms in the mouth (1).

The need for disease prevention logically leads to the removal of plaque, as this is where the problem originates. Yet many patients have neither the requisite dexterity nor the willpower to maintain good oral hygiene. Everyone agrees however, on the importance of keeping bacterial plaque under control, first by scaling and root planning, then by daily, thorough maintenance by the patient at home (2).

Ordinary easy to use mouthrinses have proved to be most helpful to patients attempting to maintain good oral hygiene. However, consumers are faced with such a wide variety of products to choose from that professional assistance in selecting an appropriate mouthrinse has become a necessity (2).

On the global market, oral hygiene products with therapeutic claims have been sold with or without proper guidelines or control for their safety and efficacy. Mouthrinse products have provided another approach towards expanding the therapeutic benefits provided by routine oral hygiene procedures (3, 4).

In addition to containing antimicrobial agents, mouthrinses often contain many different ingredients including alcohol, detergents, emulsifiers, organic acids and dyes. The clinical applications for antimicrobial mouthrinses fall into three broad categories: they are useful as preventive agents, as therapeutic agents, and they assist certain professional procedures (5).

Dentists and consumers are exposed to advertising campaigns that highlight the benefits of a variety of over-the-counter oral hygiene products. Many of these products claim to ameliorate a range of oral diseases or disease-related conditions. As a result, consumers purchase these products for self-treatment. As these products gain public acceptance, false or misleading therapeutic (disease) claims become a concern, especially when these claims emphasize the simple reduction of plaque mass without regard to any therapeutic result. Ultimately, it is the consumers who are adversely affected by these claims (6).

In recent years, there is a growing interest in the herbal products as mouthwash or oral care products (5, 7–9). Recently, several studies have reported on the antibacterial effects of miswak (chewing sticks) on cariogenic bacteria and periodontal pathogens (9–11), and inhibitory action on dental plaque formation (12) and immediate effect of a toothbrush and miswak on cariogenic bacteria (13). A review of the literature has shown that no previous investigation has assessed the comparative antimicrobial activity of commercially available mouthrinses with miswak crude extract.

So, the aim of this study was to assess the antimicrobial activity of commercially available mouthrinses in the Middle East (Saudi Arabia) with miswak crude extract.

Materials and methods

The study was carried out at Laboratory of Oral Microbiology, College of Dentistry, King Saud University.

Microorganisms used in the study

The following human isolate microorganisms were used for the *in vitro* antimicrobial activity namely: *Streptococcus faecalis*, *Streptococcus pyogenis*, *Streptococcus mutans*, *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermis*.

Preparation of miswak crude extract

A sample of the most commonly used chewing sticks in Saudi Arabia of which the colour and scent indicated that it came from an arak tree (*Salvadora persica*), was collected from Gizan Province in Saudi Arabia. The fresh miswak was cut into small pieces and allowed to dry at room temperature for 2 weeks. Then it was ground to powder in a ball mill. Successive 10 g quantity was put into sterile screw capped bottle to which 100 ml of sterile deionized distilled water was added. The extract was allowed to soak for 48 h at 4°C and then centrifuged at 2000 rpm for 10 min. The supernatant was passed through filter paper (0.45 mm μm pore size) and the extract was prepared at 50% concentration. The extract was stored at 4°C and used within 1 week (11).

Mouthrinses

Eight commonly used non-alcohol mouthrinses, commercially available in the market were obtained i.e. namely: Corsodyl, Alprox, Oral-B advantages, Florosept, Sensodyne, Aquafresh, Betadine and Emoform-F (Table 1).

Test for antimicrobial activity

The ditch plate method was used to test the antimicrobial activity. Four to five colonies were suspended in 3 ml of sterile distilled water and a lawn culture was produced on the blood agar plate. Ditches were made at the centre of Petri dishes and 0.1 ml mouthrinse were pipetted into the ditches. The plates were left for 1 h at room temperature and incubated at 37°C for 24 h. Then they were examined for inhibition zones of microorganisms around the ditches. The experiment was performed twice with 3 days apart. First and second reading of measurements in mm were calculated.

Table 1. **The composition of mouthrinses**

Corsodyl mouthwash (mint): pH 6.0, Smith Kline Beecham, UK, 0.2% w/v chlorhexidine gluconate
Alprox solution for mouth rinsing: pH 5.0, licenser, ALPRO Dental Products, GMBH Germany Arabian Products for Medical Disinfectants (APMD), Riyadh. Ingredients: phenylalanine, polyaminopropyl biguanides, tosylchloromide sodium, sorbitol, ethylenediaminetetra acetate, allantoin, aroma
Oral B advantage™ mouthrinse: Oral B Laboratories Tooth and Gum Care Ireland pH 6.0. Ingredients: aqua, glycerin, alcohol, aroma, propylparaben, methylparaben, poloxamer 407, cetylpyridinium chloride, sodium fluoride, sodium saccharin, CI4205, CI47005
Florosept mouthwash with fluoride protection: pH 6.0, Spimaco Al-Qaseem, Saudi Arabia. Ingredients: cetylpyridinium chloride, sodium fluoride, zinc chloride
Sensodyne: pH 6.0, Stanford-Miller, UK, daily mouthrinse, daily fluoride protection for your teeth, fluoride protection, prevents caries, freshens breath. Ingredients: sodium fluoride
Aquafresh Mint monthwash: pH 6.0, for a clean, fresh feeling Smith Kline Beecham, UK. Ingredients: sodium fluoride, cetylpyridinium chloride
Betadine mouthwash gargle: pH 6.0, Licenser: Monde Pharma Basel, Switzerland, Manufactured in Cairo, Egypt. Ingredients: povidone–iodine
Emoform F mouth bath concentrate: pH 7.0, WILD Basel, Switzerland. Against sensitivity teeth, inflamed and slightly bleeding gums. Ingredients: sodium monofluorophosphate, sodium chloride, sodium sulphate anhydrate, potassium sulphate, sodium saccharine, menthae pip (mint flavour) aetherole, colour aroma

Statistical analysis

The data was entered and analysed by Statistical Package for Social Sciences (SPSS) version 10. Ranges, minimum and maximum, mean and standard deviations were used for comparison of different mouthrinses and 50% miswak extract.

Results

The pH of mouthrinses ranged from 5.0 to 7.0. The pH of miswak extract was 5.5. Zones of microbial inhibition were measured in millimetre. Seven of eight mouthrinses demonstrated various level of antimicrobial activity against seven different microorganisms. A 50% extract of miswak showed antimicrobial activity against only two microorganisms, e.g. *Strep. faecalis* and *Staph. mutans*. Corsodyl (chlorhexidine) mouthrinse showed the maximum microbial inhibition, while Sensodyne mouthrinse showed no antimicrobial activity. Miswak extract showed minimum mean antimicrobial activity (Tables 2 and 3 and Figs 1 and 2).

Discussion

This study indicated that all of the mouthrinses tested produced antimicrobial zones of inhibition except of Sensodyne against the selected oral microorganisms. Miswak extract was also effective against *Strep. mutans* and *Staph. faecalis*.

The selection of mouthrinses for the study was based on a convenience sample commercially available in the market. Mouthrinses with high alcohol content were avoided for the experiment. It should be noted that alcohol has long been used for its antiseptic properties, although in mouthrinses preparations it mainly functions as a vehicle that dissolves and delivers mouthrinses ingredients. Mouthrinses increase the time of mucosa being in contact with alcohol and it has been proved that those with a high content of alcohol do cause hyperkerastosic lesions both in human beings and in laboratory animals (14). In this study, 0.2% chlorhexidine containing mouthrinse (Corsodyl) had maximum antimicrobial activity.

Antimicrobial activity of the mouthrinses can be related to the anionic and cationic potencies of the products, as well as

Table 2. **Mean growth inhibition of different microbes with various mouthrinses (in mm)**

Mouthrinse	pH	*Streptococcus faecalis*	*Streptococcus pyogenis*	*Streptococcus mutans*	*Candida albicans*	*Staphylococcus aureus*	*Staphylococcus epidermidis*
Corsodyl	6.0	+7.0	+11.0	+10.0	+10.0	+10.0	+7.0
Alprox	5.0	+1.0	+11.0	+4.0	+2.0	+3.0	+3.0
Oral-B	6.0	+4.0	+3.0	+5.0	+4.0	+4.0	+4.0
Florosept	6.0	+4.0	+8.0	+5.0	+4.0	+3.0	+3.0
Sensodyne	6.0	−0.0	−0.0	−0.0	−0.0	−0.0	−0.0
Aqua Fresh	6.0	−4.0	+4.0	+4.0	+5.0	+3.0	+5.0
Betadine	5.0	+2.0	+1.0	+1.0	+3.0	+3.0	+4.0
Emoform F	7.0	+4.0	+6.0	+6.0	+0.0	+3.0	+3.0
Miswak extract	5.5	+7.0	−0.0	+3.0	−0.0	−0.0	−0.0

−, no microbial inhibition; +, microbial inhibition in mm.

Table 3. **Comparison of mean and ranges of microbial inhibition**

Mouthrinse	Number	Range	Minimum	Maximum	Mean	SD
Corsodyl	6	7.00	4.00	11.00	8.4286	2.5071
Alprox	6	10.00	1.00	11.00	4.0000	3.2660
Oral-B	6	2.00	3.00	5.00	4.0000	0.5774
Florosept	6	5.00	3.00	8.00	4.5714	1.7182
Sensodyne	6	0.00	0.00	0.00	0.0000	0.0000
Aqua Fresh	6	2.00	3.00	5.00	4.2857	0.7559
Betadine	6	3.00	1.00	4.00	2.2857	1.1127
Emoform F	6	10.00	0.00	10.00	3.6660	3.1547
Miswak extract	6	4.00	3.00	7.00	1.4286	2.6992

their effect on changing cellular osmotic pressure and cell metabolism. In addition, the antimicrobial ingredients may absorb into bacterial surfaces and disrupt the cell membrane. The bactericidal effect of chlorhexidine is largely because of disruption of cell integrity and the precipitation of the cytoplasmic content (15).

At present, chlorhexidine still remains the gold standard of chemotherapeutic agent for plaque and gingivitis control (16–18). It has been widely used for years in Europe and elsewhere in over-the-counter oral products such as mouthrinse (0.2%), varnish, gel, dentifrice, and chewing gum (16, 19). Mouthrinse containing chlorhexidine (0.12%) is available only as a prescription drug in the United States. Recent studies have shown that 0.20% chlorhexidine is better than 0.12% of chlorhexidine mouthrinses (20, 21). The other mouthrinses with cetylpyridinium chloride proved effective against different microbes in the study. Cetylpyridinium Chloride is a quaternary ammonium compound with aliphatic chain and is classified as a cationic surface active agent. It has demonstrated antimicrobial activity against a broad spectrum of oral bacterial (22). It can interact with the bacterial cell membrane, resulting in a leakage of cellular components, disruption of cellular metabolism, inhibition of cell growth and cell death (23).

Cetylpyridinium chloride containing mouthrinses have been marketed in the United States since 1940. As the positively charged hydrophilic region of cetylpyridinium chloride is critical to antimicrobial activity, mouthrinse formulations should not contain ingredients that diminish or compete with the activity to this cationic group (6).

The selection of miswak from the salvadora persica tree for the present study was based on a number of factors. It is most common in the Middle East Region. Its taste is not unpleasant and it is cheap. Furthermore, it manifests antiplaque and many

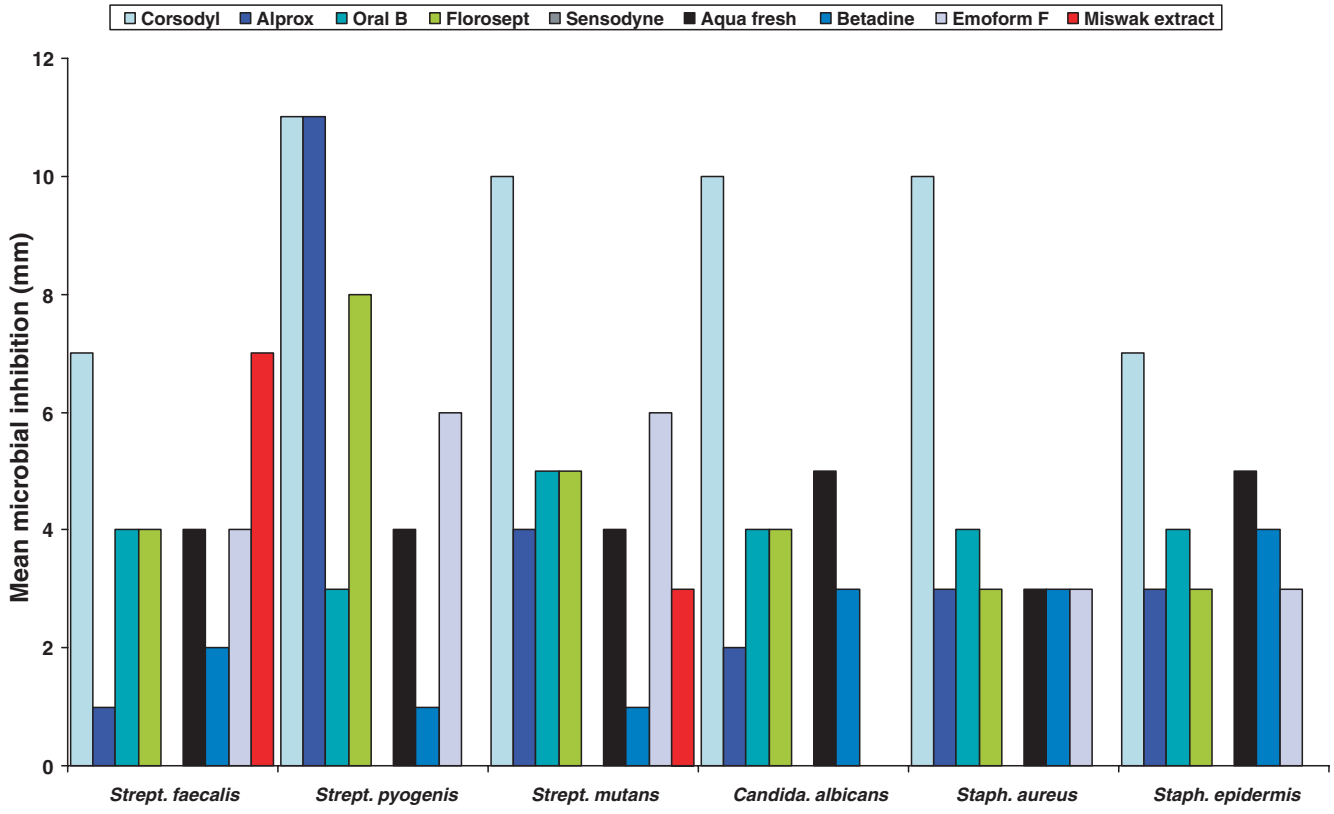

Fig 1. Bacteria versus mouthrinses.

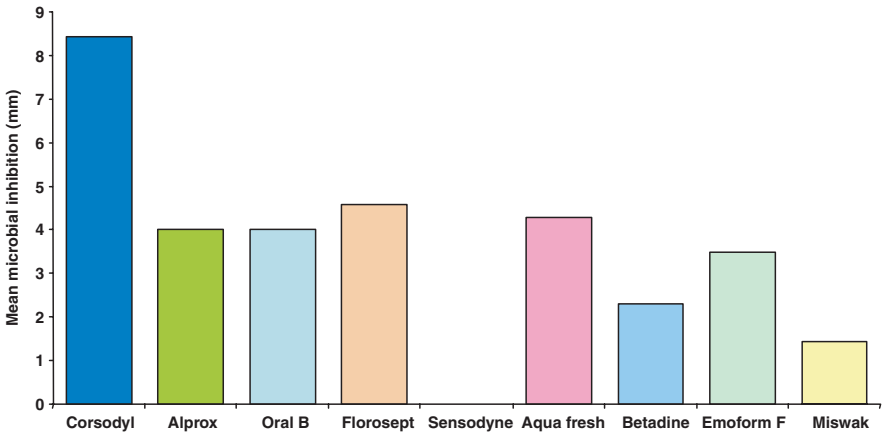

Fig 2. Mean microbial inhibition versus mouthrinses.

pharmacological properties (24). Chewing sticks contain trimethylamine, salvadorine chlorides, fluoride in large amounts, silica, sulphur, vitamin C and small quantities of tannins, saponins flavenoids and sterols (25). In a recent study (26), it was found that in addition to above-mentioned chemicals, miswak extract also contains cyanogenic glycoside and benzylisothiocyanate. The results of present study, the antimicrobial activity on *Strep. mutans* with miswak extract is in accord with previous studies (27, 28).

Recently, it has been found that there is marked reduction in *Strep. mutans*, using miswak and 50% miswak extract (13). Within the limits of this study, it is concluded that mouthrinse containing chlorhexidine was maximally effective against different microbes used in the study. Secondly, cetylpyridinium chloride mouthrinses showed moderate levels of microbial inhibition, while 50% miswak extract was mildly effective against *Strep. mutans*.

The Shapiro *et al.* (29) found that the herbal- and phenolic-based products tested were less effective than most chlorhexidine-containing mouthrinses. The results of our study is in accord with their findings, although the testing methods were different in both studies.

Antimicrobial efficacy is usually determined by examining minimum inhibitory concentration, bactericidal effects and other test that commonly utilize various microbial culture techniques (1, 30, 31). Cultural methods offer several advantages such as selective quantification of microorganisms (32) but are laborious and only enumerate bacteria that grow on agar (1, 30, 31). The limitations of laboratory tests and discrepancies between *in vitro* and *in vivo* results have been described (1, 31). In an effort to improve these methods, newer approaches for microbial estimation have been investigated, including estimation of microbial components such as adenosine triphosphate (33), electrical impedance (34), fluorescent dyes to examine microbial viability (32, 35) and many molecular approaches, as well as reporter genes (36). Another recent study has demonstrated the utility of alamar blue to examine the antimicrobial effects of oral care formulations in laboratory and clinical studies (37). The choice of use of ditch plate method on blood agar, in the present study was because of the limited availability of any other antimicrobial laboratory technique at the facility.

The true target for oral chemotherapeutics is dental plaque biofilm (38–40). It is established that the microbiota in resident biofilms is much less susceptible to antibacterial compounds than so-called 'planctonic' bacteria (41, 42).

'Classical' *in vitro* measures of antimicrobial potency utilizing planctonic monocultures and prolonged contact times are poorly predictive of the clinical efficacy of antiseptic mouthrinses because of the bacterial colonization in biofilms within the oral cavity and the way in which mouthrinses are applied prophylactically and therapeutically (43). Shapiro *et al.* (29) have developed an *in vitro* oral biofilm model for comparing the efficacy of antimicrobial mouthrinses.

So in future, further studies should be conducted to find out clinical efficacy of mouthrinses *in vitro* and *in vivo* with special emphasis on their substantivity (duration of action *in vivo*) and polyspecies biofilm model for preclinical testing of antiplaque formulations.

Dental professionals must exercise caution and discretion in interpreting the therapeutic claims made by these products and provide guidance in assisting patients in making informed decisions regarding their use (6). Dentists should also emphasize that if people could mechanically remove plaque with tooth brushing and flossing, antimicrobial mouthrinses would have minimal importance to the oral hygiene regime. Rinsing and/or irrigating with an antimicrobial mouthrinse can be important adjunct to maintaining oral hygiene.

Conclusion

1 Corsodyl mouthrinse produced the largest zones of microbial inhibition than any other mouthrinse.

2 All the mouthrinses tested demonstrated antimicrobial activity *in vitro*, except Sensodyne.

3 Miswak extract had mild antimicrobial activity against *Strep. mutans*.

4 Further *in vitro* and *in vivo* studies are needed using biofilm model to substantiate the present findings.

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