Original article

The antimicrobial potential of ten often used mouthwashes against four dental caries pathogens

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Abstract

Introduction and objective: Increasing number of people are using mouthwashes for general and oral health care. Few of these mouthwashes, however, have undergone rigorous testing, as evidenced by the limited amount of information on their safety and efficacy in the literature. The aim of this study was to determine the antimicrobial properties of ten commonly available mouthwashes against four oral pathogens related to caries and to oral fungal infections, to verify the claims made by the manufacturers to provide information to dental professionals about the efficacy of their products *in vitro* and to use these mouthwashes as a base for the evaluation of antimicrobial plant products.

Materials and methods: The authors used two different techniques: microbial growth in nutrient broth by turbidity measurement and an agar well diffusion method to evaluate the antimicrobial effectiveness of ten often used mouthwashes against four microorganisms: *Streptococcus mutans* and *Staphylococcus aureus* (bacteria), *Candida albicans* and *Saccharomyces cerevisiae* (fungi). Nutrient broth without mouthwash and sterile distilled water served as the control respectively in the two techniques.

Results: Hexidine mouthwash emerged as the most effective mouthwash [maximum mean diameter of inhibition zone against *S. aureus* (28.3mm to 33.9mm) followed by *S. mutans* (23.6mm to 26mm), *S. cerevisiae* (20.6mm to 26.3mm) and minimum against *C. albicans* (11.9mm to 22.9mm)] followed by Chlohex and Triguard, all of which had excellent level of activity. Following Triguard were Zytee, Chlohexplus, Hexnor and Chlorhexidine that showed good antimicrobial activity and finally, displaying very little antimicrobial activity was Listerine while Toss-K and Senquel-AD totally lacked antimicrobial activity.

Conclusion: Hexidine mouthwash (ICPA Health Products Ltd., Ankleshwar, India) showed excellent antimicrobial activity against the four dental caries causing microorganisms *in vitro.* The six mouthwashes found to be effective against all the four tested microorganisms at all the four concentrations, comprising of Chlorhexidine gluconate as the basic constituent, presented different antimicrobial activities.

Key words: Dental caries, Antimicrobial activity, Zone of inhibition, Microbial growth inhibition, Mouthwashes, Chlorhexidine gluconate

Introduction

Despite great improvements in the global oral health status, dental caries still remains one of the most prevalent diseases [1]. The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are by-products of carbohydrate metabolism by *Streptococcus mutans,* a cariogenic bacterium [2]. Colonization of teeth by cariogenic bacteria is one of the most important risk factors in the development of dental diseases [2]. *S. mutans* and *Candida albicans* are the two microbes often implicated in oral diseases, *C. albicans* is the most common yeast isolated from the oral cavity and a common cause of oral thrush, endocarditis, septicemia, vaginitis and infection of skin, nails and lungs [3-5]. It is by far the fungal species most commonly isolated from infected root canals, showing resistance to intercanal medication [6,7]. *Staphylococcus aureus* is a major human pathogen, responsible for a number of hospital-acquired infections, initially colonizes several locations in the human body, but the mouth and hands are the main reservoirs for propagation of this pathogen in the hospital environment [8-10].

Individuals heavily colonized by cariogenic bacteria are considered to be at high risk for dental caries. Hence eradication of these microorganisms is important for dental treatment [11]. Prevention of oral diseases is easier than a cure. The widespread use of mouthwashes as an aid to oral hygiene is a relatively recent phenomenon in the developing countries of the world. Development work on the mouthwashes has been done mostly by the manufacturers, and the little work that has been done relates to the individual ingredients they contain rather than to their complete formulations [12-14].

While their primary appeal is as an aid to breath freshness and cleansing the mouth, the majority of the newer mouthwashes also claim to have antiseptic properties [5,13,15-17]. While many manufacturers claim that their mouthwashes have antimicrobial properties, the aim of this study was to determine the antimicrobial properties of ten commonly available mouthwashes against four oral pathogens related to caries and to oral fungal infections {since these organisms have now gained more importance due to the increased incidence of AIDS/HIV [18]}, to verify the claims made by the manufacturers to provide information to dental professionals about the efficacy of their products *in vitro* and to use these mouthwashes as a base for the evaluation of antimicrobial plant products.

Materials and methods

Collection of mouthwashes

Ten often used mouthwash products (Table 1) were purchased from the drug stores of Kurukshetra and Gurgaon, Haryana, India.

Test microorganisms

The test microorganisms *S. mutans* (MTCC *497), *S. aureus* (MTCC 740), *C. albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170) were procured from MTCC, IMTECH, Chandigarh. These were subcultured on specific media, procured from HiMedia Laboratory Pvt. Ltd., Bombay, India, recommended for different microorganisms such as Brain Heart Infusion Agar, BHI (*S. mutans*), Nutrient Agar (*S. aureus*) and Malt Yeast Agar (*C. albicans* and *S. cerevisiae*) and incubated aerobically at 37^{o}C. The identification of all the microbes was confirmed by standard biochemical and staining methods [19-21].

Screening for antimicrobial activity

Antimicrobial effectiveness of various mouthwashes was assessed by using two techniques:

Turbidity measurement by spectrophoto-meter: One percent of nutrient broth

(HiMedia Ltd.) was prepared containing a 10% concentration of the mouthwashes. After autoclaving, the broth and mouthwashes were inoculated with 100µl of the microbial inoculums adjusted equal to 10^6cfu/ml (with turbidity equating to a McFarland standard of 0.5) and were incubated aerobically at 37°C for 24h. The inoculated broths were suspended and their optical density was measured by spectrophotometer at a wavelength of 490nm as a guide to microbial growth. The experiments were performed in triplicates for each mouthwash and the mean for each test microorganism was calculated. Broth without mouthwash was used as control [13].

Table 1: Ingredients of various mouthwashes tested for antimicrobial potential

Name	Batch number	Expiry date	Manufacturer	Ingredients as listed on packages
Listerine (cool mint)	7208	December 2010	Pfizer Limited, Kolhapur, India	Thymol 0.06%, Eucalyptol 0.09%, Menthol 0.04%, Ethanol 21.6%v/v.
Chlorhexidine	ZM-701	March 2010	Blue Cross Laboratories Ltd., Nasik, India	Chlorhexidine gluconate 0.2%w/v in pleasantly flavored aqueous base
Toss-K	TK-4316	March 2010	Ind-Swift Limited, Chandigarh, India	Potassium nitrate 3%w/v, sodium fluoride 0.2%w/v in pleasantly flavored aqueous base
Zytee	W6021	December 2010	Raptakos, Brett and Co. Ltd., Mumbai, India	Clove oil 1%, Mentha oil 1%, Menthol 1%, Chamomile oil 0.015%, Sodium benzoate 2%, Ethanol 31%v/v
Hexnor	EHN-001	December 2010	Dynor Pharmaceuticals Pvt. Ltd., Delhi, India	Chlorhexidine gluconate 0.2%w/v, Sodium fluoride0.05%w/v, Zinc chloride 0.09%w/v
Senquel-AD	BSD 6028	December 2012	Dr. Reddy's Laboratories Ltd., Hyderabad, India	Potassium nitrate 3%w/v, Sodium fluoride 0.2%w/v in pleasantly flavored aqueous base
Hexidine	AU8059	December 2012	ICPA Health Products Ltd., Ankleshwar, India	Chlorhexidine gluconate 0.2%w/v in pleasantly flavored aqueous base
Chlohex	BCX 6023	March 2011	Dr. Reddy's Laboratories Ltd., Hyderabad, India	Chlorhexidine gluconate 0.2%w/v in pleasantly flavored aqueous base
Chlohex plus	BCP 7008	March 2011	Dr. Reddy's Laboratories Ltd., Hyderabad, India	Chlorhexidine gluconate 0.2% w/v, Sodium fluoride 0.05%w/v, Zinc vhloride 0.09%w/v in pleasantly flavored aqueous base
Triguard	RKR8022	December 2010	FDC Ltd., Aurangabad, India	Chlorhexidine gluconate 0.2%, Sodium fluoride 0.05%, Zinc chloride 0.09% in pleasantly flavored base

Agar well diffusion method: The mouthwashes were tested at four different concentrations: 1:4(25%), 1:1(50%), 3:4(75%) and full strength (100%), taking sterile distilled water as the diluent, using agar well diffusion method or cup plate method [22-23]. In this method, pure isolate of each microbe was subcultured on the

recommended specific media for each microorganism at 37°C for 24h. From each inoculated agar plate, a minimum of four colonies were touched with a sterile loop and transferred into a tube containing normal saline (0.85%) and density of each microbial suspension was adjusted equal to that of 10^6 cfu/ml (standardized by 0.5McFarland standard) and was used as the inoculum [23-27].

A 100µl volume of each mouthwash concentration (full strength, 3:4, 1:1, 1:4) and the control was propelled directly into the wells (in triplicates) of the inoculated specific media agar plates for each test organism. The plates were allowed to stand for ten minutes for diffusion of the mouthwash to take place and incubated at 37°C for 24h, 48h and 72h [28-29]. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the mouthwash, was recorded if the zone of inhibition was greater than 8mm [30]. The experiments were performed in triplicates and the mean values of the diameter of inhibition zones with ± standard deviation were calculated. Nutrient broth without mouthwash in turbidity measurement method and sterile distilled water in agar well diffusion method were used as negative control.

Results

The mouthwashes were measured at 10% concentration for turbidity by spectrophotometer, Hexidine showed no turbidity at all thus having excellent activity, while Chlohex and Triguard showed very little turbidity thus having equally good activity against all the microorganisms. Zytee and Chlohex plus possessed comparatively lesser potential while Hexnor, Chlorhexidine and Listerine showed average ability to inhibit the microbial growth. Toss-K and Senquel-AD showed even more turbidity than the control, thus showing total inability to control the dental caries pathogens. Figure 1 shows the antimicrobial activity of ten mouthwashes against *S. mutans* in BHI broth by measuring the optical density.

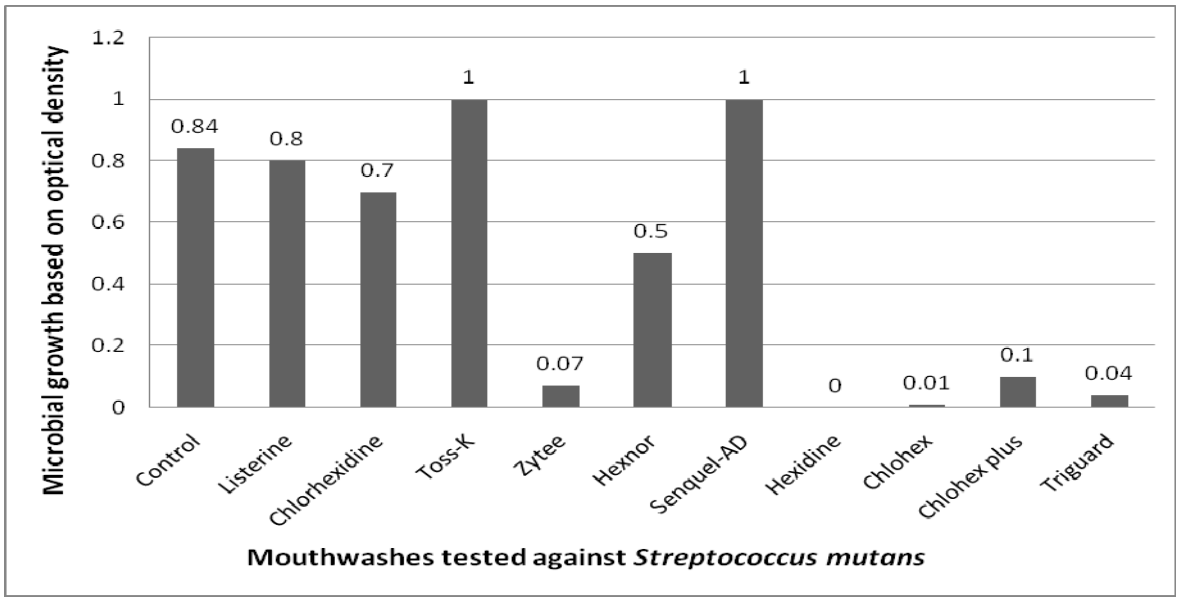

Fig. 1: Microbial growth of *S. mutans* in Brain heart infusion broth with different mouthwash substitutes: summary of optical density data (three sets of experiments)

Although few negligible changes in the inhibition zones were observed in some mouthwashes after 48h and 72h, most of the antimicrobial activity was observed, in all the ten mouthwashes tested, during the initial 24h of incubation when tested by agar well diffusion method. Six test mouthwashes, namely Chlorhexidine (Blue Cross Laboratories Ltd., Nasik, India), Hexnor (Dynor Pharmaceuticals Pvt. Ltd., Mumbai, India), Hexidine (ICPA Health Products Ltd., Ankleshwar, India), Chlohex and Chlohex plus (Dr. Reddy's Laboratories Ltd., Hyderabad, India) and Triguard (FDC Ltd., Aurangabad, India) produced consistent antimicrobial activity against all the four test organisms i.e. *S. mutans, S. aureus, S. cerevisiae* and *C. albicans* at all the four test concentrations-full strength, 3:4, 1:1 and 1:4 (Fig. 2). At 3:4, 1:1 and 1:4 dilutions, the differences among the ten test mouthwashes shown to inhibit the growth of microorganisms at full strength became less evident (Table 2).

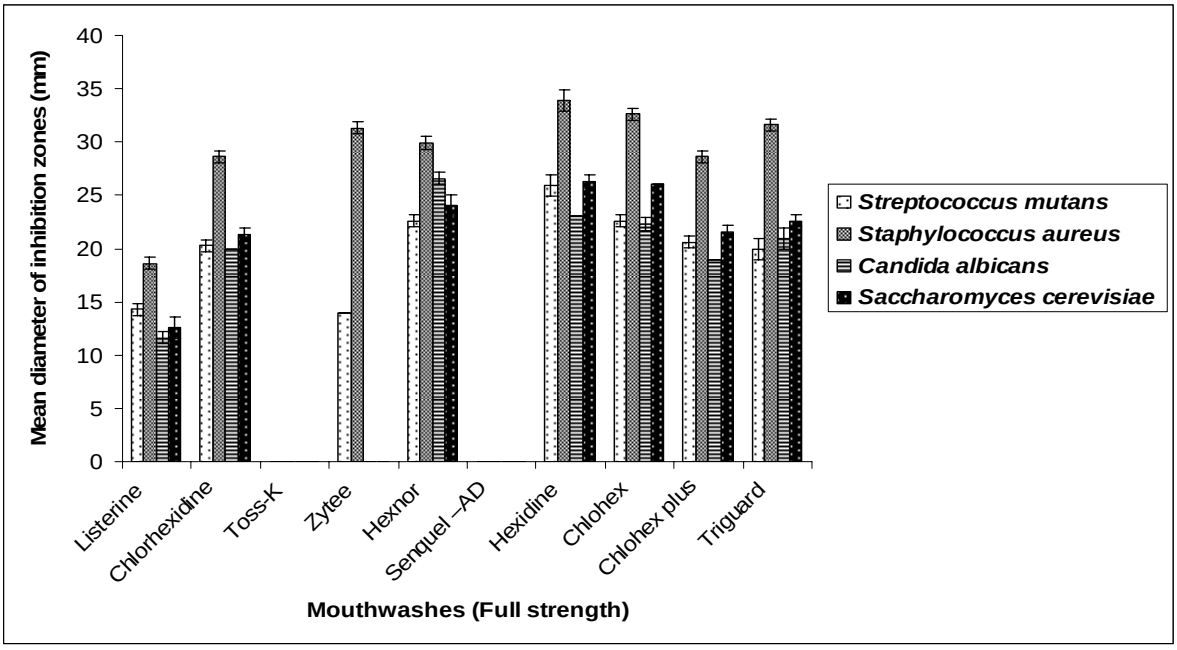

Fig. 2: Mean diameter and standard deviation of zones of microbial inhibition exhibited by ten mouthwashes after 24h at full strength (100%concentration) against four microorganisms (Bar indicates standard deviation)

Out of these, Hexidine showed the highest antimicrobial activity against all the four microorganisms, the maximum inhibition zone produced against *S. aureus* (28.3mm to 33.9mm) followed by *S. mutans* (23.6mm to 26mm), *S. cerevisiae* (20.6mm to 26.3mm) and minimum against *C. albicans* (11.9mm to 22.9mm), at different concentrations with the maximum inhibition zone being produced at full strength (Fig. 3(a, b, c, d)). One of the tested mouthwashes, Zytee (Raptakos, Brett and Co. Ltd., Mumbai, India) showed inhibition of *S. aureus* at all the four concentrations ranging between 14mm and 31.3mm at 24h which gradually reduced to 25.9mm at 48h and further 20.6mm at 72h, thus showing the bacteriostatic nature of this mouthwash. Zytee showed inhibitory activity against *S. mutans* at 1:1, 3:4 and full strength ranging between 10.3mm and 13.9mm but had no inhibitory effect on the

two fungi *C. albicans* and *S. cerevisiae*. Another tested mouthwash Listerine cool mint (Pfizer Ltd., Kolhapur, India) showed inhibitory zones against the two bacteria *S. mutans* and *S. aureus* ranging between12.6mm and 18.6mm at all the four concentrations. It produced zones of inhibition ranging between 10.3mm and 12.6mm against *C. albicans* and *S. cerevisiae* at 1:1, 3:4 and full strength but no zone of inhibition against the two yeasts at 1:4 concentrations (Table 2).

Two of the ten tested mouthwashes, Toss-K (Ind-Swift Ltd., Chandigarh, India) and Senquel-AD (Dr. Reddy's Laboratories Ltd., Hyderabad, India) did not show any inhibitory effect against any of the four microorganisms at any of the four tested concentrations. The inhibitory activity of Hexnor against *S. aureus* was found to increase from 27.3mm to 30.3mm at 3:4 concentration and from 29.9mm to 33.0mm at full strength when measured after 72h of incubation. Rest all mouthwashes (except Zytee) showed almost the same zone diameter after 24h, 48h or 72h, while the zone diameter increased slightly in all the mouthwashes when moving from 1:4 concentration to full strength showing that full strength is the most effective concentration against all the tested microorganisms.

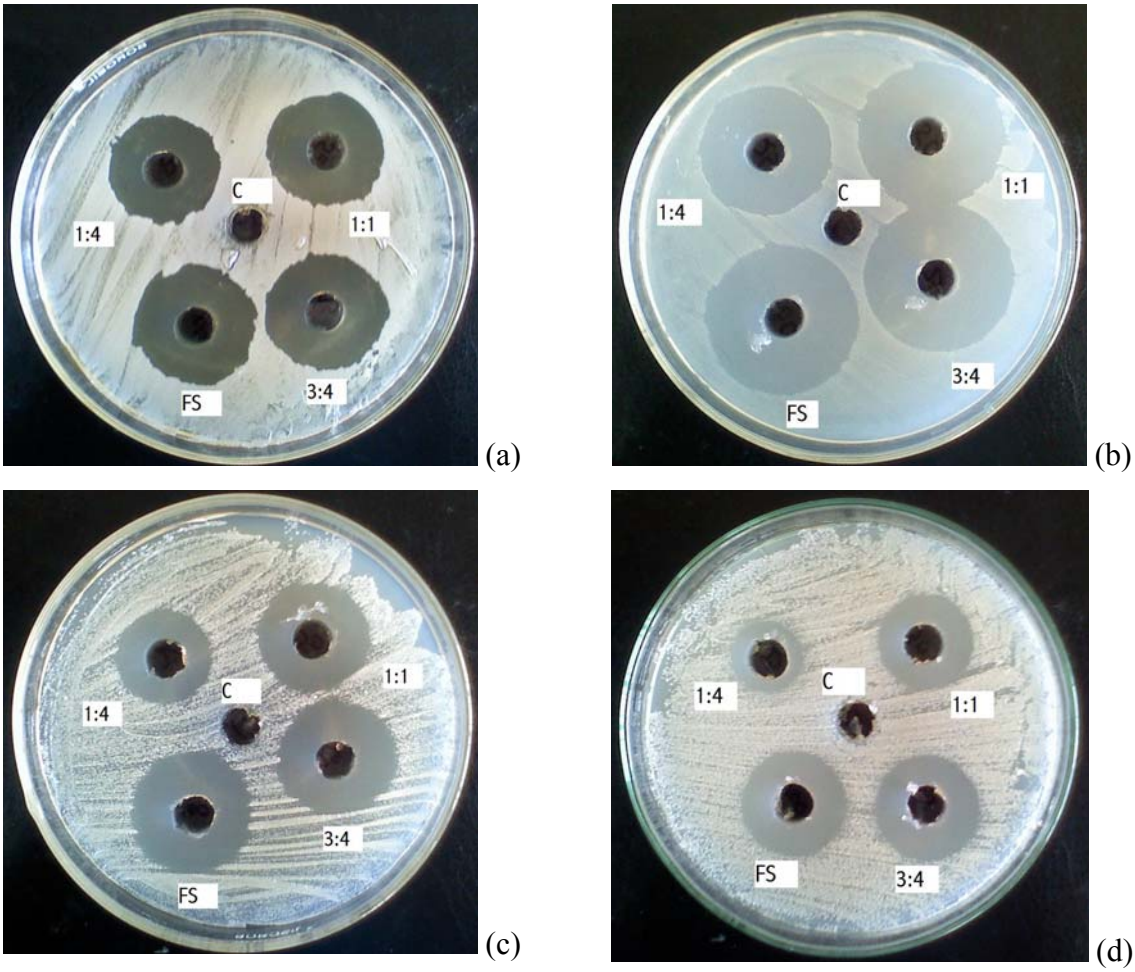

Fig. 3: Zones of inhibition produced by Hexidine mouthwash at 24h against the four tested microorganisms at four different concentrations and control (a) *S. mutans*, (b) *S. aureus*, (c) *S. cerevisiae* and (d) *C. albicans*, (C= Control, F.S. = Full Strength)

Table 2: Antimicrobial activity of ten mouthwashes against four dental caries pathogens (bacteria and yeasts) determined by agar well diffusion method

Mouthwash	Concentrations tested	Mean diameter of growth of inhibition zones (mm)											
		S. mutans			*S. aureus*			*C. albicans*			*S. cerevisiae*		
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
Control		-	-	-	-	-	-	-	-	-	-	-	-
Listerine (cool mint)	**1:4**	12.6[a] ± 0.57[b]	12.6 ± 0.57	12.5 ± 0.57	15.6 ± 0.57	15.6 ± 0.57	15.6 ± 0.57	-	-	-	-	-	-
	1:1	12.6 ± 0.57	12.6 ± 0.57	12.6 ± 0.57	16.2 ± 1.52	16.2 ± 1.52	15.9 ± 1	10.3 ± 0.57	10.3 ± 0.57	10.3 ± 0.57	10.6 ± 0.57	10.6 ± 0.57	10.6 ± 0.57
	3:4	13.6 ± 0.57	13.6 ± 0.57	13.6 ± 0.57	16.3 ± 0.57	16.3 ± 0.57	16.3 ± 0.57	10.6 ± 0.57	10.6 ± 0.57	10.6 ± 0.57	11.9 ± 1	11.9 ± 1	11.9 ± 1
	F.S.	14.3 ± 0.57	14.3 ± 0.57	14.3 ± 0.57	18.6 ± 0.57	18.6 ± 0.57	18.6 ± 0.57	11.6 ± 0.57	11.6 ± 0.57	11.6 ± 0.57	12.6 ± 1	12.6 ± 1	12.6 ± 1
Chlorhexidine	**1:4**	18.3 ± 0.57	18.3 ± 0.57	17.9 ± 1	23.6 ± 0.57	23.6 ± 0.57	23.6 ± 0.57	13.3 ± 0.57	13.3 ± 0.57	13.3 ± 0.57	15.9 ± 1	15.9 ± 1	16.0 ± 0
	1:1	19.6 ± 0.57	19.6 ± 0.57	19.6 ± 0.57	25.3 ± 0.57	25.3 ± 0.57	25.3 ± 0.57	16.3 ± 0.57	15.9 ± 1	15.9 ± 1	18.3 ± 0.57	18.3 ± 0.57	18.3 ± 0.57
	3:4	20.3 ± 0.57	20.3 ± 0.57	20.3 ±0.57	26.3 ± 0.57	26.9 ± 1	27.3 ± 0.57	18.0 ± 0	18.0 ± 0	17.9 ± 0.57	20.6 ± 0.57	20.6 ± 0.57	20.6 ± 0.57
	F.S.	20.3 ± 0.57	20.3 ± 0.57	20.3 ± 0.57	28.6 ± 0.57	28.6 ± 0.57	28.6 ± 0.57	20.0 ± 0	20.0 ± 0	19.6 ± 0.57	21.3 ± 0.57	21.3 ± 0.57	21.3 ± 0.57
Toss-K	**1:4**	-	-	-	-	-	-	-	-	-	-	-	-
	1:1	-	-	-	-	-	-	-	-	-	-	-	-
	3:4	-	-	-	-	-	-	-	-	-	-	-	-
	F.S.	-	-	-	-	-	-	-	-	-	-	-	-
Zytee	**1:4**	-	-	-	14.0 ± 0	14.0 ± 0	14.0 ± 0	-	-	-	-	-	-
	1:1	10.3 ± 0.57	10.3 ± 0.57	10.3 ± 0.57	17.0 ± 0	16.9 ± 1	16.3 ± 0.57	-	-	-	-	-	-
	3:4	12.6 ± 0.57	12.6 ± 0.57	12.6 ± 0.57	19.6 ± 0.57	19.6 ± 0.57	19.6 ± 0.57	-	-	-	-	-	-
	F.S.	14.0 ± 0	14.0 ± 0	13.9 ± 1	31.3 ± 0.57	25.9 ± 1	20.6 ± 0.57	-	-	-	-	-	-

Table 2 (continued)

Mouthwash	Concentrations tested	Mean diameter of growth of inhibition zones (mm)											
		S. mutans			*S. aureus*			*C. albicans*			*S. cerevisiae*		
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
Hexnor	**1:4**	21.3 ± 0.57	21.3 ± 0.57	21.3 ± 0.57	24.0 ± 0	24.0 ± 0	24.0 ± 0	21.6 ± 1.15	21.6 ± 0.57	21.6 ± 0.57	19.3 ± 0.57	19.3 ± 0.57	19.4 ± 0.57
	1:1	21.6 ± 0.57	21.6 ± 0.57	21.6 ± 0.57	25.6 ± 0.57	25.6 ± 0.57	25.6 ± 0.57	23.3 ± 0.57	23.3 ± 0.57	23.3 ± 0.57	22.6 ± 0.57	22.6 ± 0.57	23.0 ± 0
	3:4	22.6 ± 0.57	22.6 ± 0.57	22.6 ± 0.57	27.3 ± 0.57	28.0 ± 0	30.3 ± 0.57	25.9 ± 1	25.9 ± 1	25.9 ± 1	23.3 ± 0.57	23.3 ± 0.57	23.3 ± 0.57
	F.S.	22.6 ± 0.57	22.6 ± 0.57	22.6 ± 0.57	29.9 ± 1	30.3 ± 0.57	33.0 ± 0	26.6 ± 0.57	26.6 ± 0.57	27.3 ± 0.57	24.0 ± 0	23.9 ± 1	23.9 ± 1
Senquel-AD	**1:4**	-	-	-	-	-	-	-	-	-	-	-	-
	1:1	-	-	-	-	-	-	-	-	-	-	-	-
	3:4	-	-	-	-	-	-	-	-	-	-	-	-
	F.S.	-	-	-	-	-	-	-	-	-	-	-	-
Hexidine	**1:4**	23.6 ± 0.57	23.6 ± 0.57	23.6 ± 0.57	28.3 ± 0.57	28.3 ± 0.57	28.3 ± 0.57	11.9 ± 1	12.6 ± 0.57	13.0 ± 1	20.6 ± 0.57	20.6 ± 0.57	20.6 ± 0.57
	1:1	25.3 ± 0.57	25.3 ± 0.57	25.3 ± 0.57	32.6 ± 0.57	32.6 ± 0.57	32.6 ± 0.57	20.3 ± 0.57	20.3 ± 0.57	20.3 ± 0.57	23.6 ± 0.57	23.6 ± 0.57	23.6 ± 0.57
	3:4	25.6 ±0.57	25.6 ± 0.57	26.0 ± 0	33.6 ± 0.57	33.6 ± 0.57	33.6 ± 0.57	23.6 ± 0.57	23.6 ± 0.57	22.9 ± 1	25.0 ± 0	25.0 ± 0	25.0 ± 0
	F.S.	25.9 ± 1	25.9 ± 1	26.0 ± 0	33.9 ± 1	33.9 ± 1	33.9 ± 1	23.0 ± 0	23.0 ± 0	22.9 ± 1	26.3 ± 0.57	26.3 ± 0.57	26.3 ± 0.57
Chlohex	**1:4**	18.0 ± 0	18.0 ± 0	18.3 ± 0.57	26.3 ± 0.57	26.3 ± 0.57	26.3 ± 0.57	16.3 ± 0.57	16.3 ± 0.57	15.9 ± 1	20.0 ± 0	20.0 ± 0	20.0 ± 0
	1:1	20.6 ± 0.57	20.6 ± 0.57	20.6 ± 0.57	29.3 ± 0.57	29.3 ± 0.57	29.3 ± 0.57	19.3 ± 0.57	19.3 ± 0.57	18.9 ± 1	23.3 ± 0.57	23.3 ± 0.57	23.3 ± 0.57
	3:4	21.3 ± 0.57	21.3 ± 0.57	21.3 ± 0.57	30.6 ± 0.57	30.6 ± 0.57	30.6 ± 0.57	20.0 ± 0	20.0 ± 0	20.0 ± 0	25.3 ± 0.57	25.3 ± 0.57	25.3 ± 0.57
	F.S.	22.6 ± 0.57	22.6 ± 0.57	22.6 ± 0.57	32.6 ± 0.57	32.5 ± 0.57	33.0 ± 0	22.3 ± 0.57	22.3 ± 0.57	22.3 ± 0.57	26.0 ± 0	26.0 ± 0	26.0 ± 0

Table 2 (continued)

Mouthwash	Concentrations tested	Mean diameter of growth of inhibition zones (mm)											
		S. mutans			*S. aureus*			*C. albicans*			*S. cerevisiae*		
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
Chlohex plus	**1:4**	17.9 ± 1	17.9 ± 1	17.9 ± 1	23.3 ± 0.57	23.3 ± 0.57	23.3 ± 0.57	13.3 ± 0.57	12.9 ± 1	10.6 ± 0.57	17.6 ± 0.57	17.6 ± 0.57	18.3 ± 0.57
	1:1	18.3 ± 0.57	18.3 ± 0.57	18.3 ± 0.57	27.6 ± 0.57	27.6 ± 0.57	28.0 ± 0	15.3 ± 0.57	14.9 ± 1	14.6 ± 0.57	20.0 ± 0	20.0 ± 0	21.3 ± 0.57
	3:4	19.9 ± 1	19.9 ± 1	20.0 ± 0	27.9 ± 1	27.9 ± 1	28.0 ± 0	17.6 ± 0.57	17.6 ± 0.57	16.9 ± 1	21.3 ± 0.57	21.3 ± 0.57	22.6 ± 0.57
	F.S.	20.6 ± 0.57	20.6 ± 0.57	20.6 ± 0.57	28.6 ± 0.57	29.3 ± 0.57	30.0 ± 0	19.0 ± 0	19.0 ± 0	18.9 ± 1	21.6 ± 0.57	21.6 ± 0.57	21.5 ± 0.57
Triguard	**1:4**	16.3 ± 0.57	16.3 ± 0.57	16.3 ± 0.57	27.6 ± 0.57	27.6 ± 0.57	27.6 ± 0.57	14.3 ± 0.57	14.3 ± 0.57	14.3 ± 0.57	18.0 ± 0	18.0 ± 0	18.0 ± 0
	1:1	19.6 ± 0.57	19.6 ± 0.57	19.6 ± 0.57	28.0 ± 0	28.0 ± 0	28.0 ± 0	17.3 ± 0.57	17.3 ± 0.57	17.3 ± 0.57	22.3 ± 0.57	22.3 ± 0.57	22.3 ± 0.57
	3:4	19.6 ± 0.57	19.6 ± 0.57	20.0 ± 0	30.3 ± 0.57	30.3 ± 0.57	30.3 ± 0.57	19.0 ± 0	19.0 ± 0	19.0 ± 0	22.6 ± 0.57	22.6 ± 0.57	22.6 ± 0.57
	F.S.	19.9 ± 1	20.0 ± 0	20.0 ± 0	31.6 ± 0.57	31.6 ± 0.57	31.6 ± 0.57	20.9 ± 1	20.9 ± 1	20.9 ± 1	22.6 ± 0.57	22.6 ± 0.57	22.6 ± 0.57

F.S. = Full Strength, (-) = No Zone,[a] Values including diameter of the well (8mm) are means of three replicates, [b]± Standard deviation.

Discussion

Following the completion of the two different techniques to assess the antimicrobial potential of the mouthwashes, statistical ranking procedures were used to place the ten different mouthwashes in order of antimicrobial effectiveness. The results reveal wide variations in their effectiveness against the four tested microorganisms. Of the ten mouthwashes tested, Hexidine mouthwash emerged as the most effective antimicrobial mouthwash, based on the optical density in liquid nutrient media and the mean diameter of the zones of microbial inhibition produced by the mouthwashes in agar well diffusion method, against all the four tested microorganisms followed by Chlohex and Triguard, all of which showed excellent level of activity. Following Triguard were Zytee, Chlohexplus, Hexnor and Chlorhexidine that showed good antimicrobial activity and finally, displaying very little antimicrobial activity was Listerine while Toss-K and Senquel-AD totally lacked antimicrobial activity.

Interestingly, all the three mouthwashes that showed excellent antimicrobial activities had Chlorhexidine gluconate as the active ingredient. Chlorhexidine gluconate is a cationic biguanide with broad-spectrum antimicrobial action, whose effectiveness in decreasing the formation of dental biofilm (plaque) and gingivitis has been demonstrated in several clinical studies [31-34]. Its mechanism of action is that the cationic molecule binds to the negatively-charged cell walls of the microbes, destabilising their osmotic balance [16,35].

Its substantivity, the ability of an agent to be retained in particular surroundings, is due to its ability to bind to the carboxyl groups of the mucin that covers the oral mucus and be steadily released from these areas in an active form, displaced by the calcium ions segregated by the salivary glands [36]. Chlorhexidine formulations are considered to be the "gold standard" antiplaque mouthrinses due to their prolonged broad spectrum antimicrobial activity and plaque inhibitory potential [16, 17].

It is known that a balance exists in a person's oral microbial population. If this balance is lost, opportunistic microorganisms can proliferate, enabling the initiation of disease processes. Therefore, the mouthwash identified as having the largest microbial inhibition zone-and thus probably the strongest antibacterial and antifungal properties-may not be necessarily superior to those found to have smaller diameter inhibition zones. Because a mouthwash used *in vivo* likely is diluted by saliva, the level to which antimicrobial properties are buffered or lost in dilution *in vitro* is of interest [37]. In addition, dentists should keep in mind that the mean average inhibition zone of one mouthwash may not be directly comparable with that of another mouthwash because different mouthwashes are constituted of different active ingredients and may diffuse at different rates.

This testing method also functioned as a screening method, and it may not have been able to detect the effects of a chemical that does not diffuse through the agar matrix. More importantly, the test was conducted *in vitro*, so it cannot be assumed that the results of antimicrobial efficacy could be proportional or transferable to the oral cavity and translated into clinical effectiveness. Studies have demonstrated the effectiveness of rinsing with an antimicrobial mouthrinse in significantly reducing both salivary [38-40] and mucosal [41-42] levels of bacteria. Thus, from the overall results obtained, it is evident that various mouthwashes listing Chlorhexidine gluconate as the active ingredient presented different antimicrobial activities.

This is probably due to the different formulations in different mouthwashes in association with other ingredients. The possible explanation may be the active product concentration and its interaction with other constituents, in addition to differences in the formulations, might be responsible for different effects. The result justifies the antimicrobial claims of the mouthwashes, made by earlier workers [13, 43-44].

Conclusion

Hexidine mouthwash (ICPA Health Products Ltd., Ankleshwar, India) showed excellent antimicrobial activity against the four dental caries causing microorganisms *in vitro.* The six mouthwashes found to be effective against all the four tested microorganisms at all the four concentrations, comprising of Chlorhexidine gluconate as the basic constituent, presented different antimicrobial activities. The possible explanation may be the active product concentration and its interaction with other constituents, in addition to differences in the formulations, would be responsible for different effects.

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