

## Original paper

# The *in vitro* activity of selected mouthrinses on *Candida* strains isolated from the oral cavity

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**ABSTRACT.** The purpose of this *in vitro* study was to determine the effects of 13 mouth rinses on one hundred *Candida* strains isolated from the oral cavity of patients. Five of the rinses had no antifungal activity. Chlorhexidine, Colgate and Dentosept acted on all isolated strains; Corsodyl did not act on *C. glabrata* (2 strains), Octenidol on *C. glabrata* and *C. guilliermondii* (1 each), Sylveco on *C. tropicalis* (2), Oral B on *C. parapsilosis*, Curasept did not affect 17 strains, including on *C. albicans* (5), *C. guilliermondii* (4), *C. dubliniensis* (3), *C. humicola* and *C. parapsilosis* (2 each) and *C. glabrata* (1). The lowest MIC (the strongest potential activity), was observed for chlorhexidine, followed by Sylveco, Corsodyl and Colgate; in contrast, the highest MIC (the lowest activity) was calculated for Octenidol and Curasept. Eight of the mouthrinses inhibited the *in vitro* growth of potentially pathogenic fungal strains isolated from the oral cavity at varying dilutions of up to 1:16, depending on the rinse. Chlorhexidine demonstrated strong antifungal efficiency. The antifungal effect of a mouthrinse depends on its composition and the species of fungus. Mouthrinse use is an important complement to procedures ensuring proper oral hygiene and preventing certain oral diseases.

**Keywords:** *Candida* strains, patients, mouthrinses, antifungal activity

## Introduction

The oral microbiome is one of the most complex found in humans and has been widely studied in both healthy and sick subjects. Some of the microorganisms which settle in the oral cavity are considered as „normal” flora, but may become pathogens under certain conditions. As general health is built upon a symbiotic relationship between the oral microbiota and the host immune system, proper oral hygiene not only prevents oral disease, but can also affect human health [1–3].

Brushing teeth with appropriate brushes, powders, pastes or gels, serves as basic hygiene treatment. In addition, it is recommended to use floss (waxed, unwaxed) and dental tape, as well as interdental brushes (cleaning rods). In order to maintain proper oral hygiene, tongue brushes and scrapers (cleaners), and various types of rinses can also be used to complement brushing, loosen food residue, and remove components of the microbiota from the area. Mouthwash should be an integral part

of daily hygiene procedures [4,5].

Our previous papers have demonstrated the effects of selected mouthrinses on reference strains of *Trichomonas vaginalis* (ATCC 30207) and *Entamoeba gingivalis* (ATCC 30927), as well as on eight reference strains of fungi: *C. albicans* (CBS 2312), *C. albicans* (L 45), *C. albicans* (ATCC 24433), *C. dubliniensis* (CBS 7987), *C. glabrata* (CBS 862), *C. krusei* (CBS 573), *C. parapsilosis* (CBS 10947) and *C. tropicalis* (CBS 2424) [6,7].

The purpose of this study was to investigate the effects of mouthrinses on *Candida* strains isolated from the oral cavity of patients from the Barlicki Memorial University Hospital and Department of Diagnostics and Treatment of Parasitic Diseases and Mycoses, Medical University of Lodz.

## Materials and Methods

One hundred strains isolated from patients were used, including *C. albicans*, *C. glabrata* (24 each), *C. guilliermondii*, *C. parapsilosis* (14 each), *C. du-*

Table 1. Mean diameter of *Candida* growth inhibition by particular mouthrinses

Solution	Mean diameter of growth inhibition (in mm)							
	CHX	Dentosept A	Sylveco	Colgate	Corsodyl	Curasept	Octenidol	Oral B
WD*	16.7	22.4	17.0	15.2	15.4	13.9	15.0	15.2
1:2	14.3	17.4	12.6	12.8	13.0	11.6	12.9	12.9
1:4	11.9	12.6	11.8	11.3	11.5	10.1	11.0	10.9
1:8	10.5	10.4	10.9	11.1	–	–	10.4	10.8
1:16	–	10.2	–	11.0	–	–	10.3	10.5

\* WD - without dilution

*bliniensis*, *C. tropicalis*, *C. famata* (7 each), *C. humicola* (2 each) and *C. krusei* (1 strain).

Thirteen mouthrinses were used in the study, including pure chlorhexidine (CHX), and 12 commercially-available varieties: Azulan, Colgate Plax Complete Care Sensitive, Corsodyl 0,2%, Curasept ADS 205, Dentosept, Dentosept A, Eludril Classic, Listerine Total care, Octenidol, Oral-B Pro-Expert Clinic Line, Sylveco and *Tinctura salviae*.

The procedure for performing antifungal susceptibility tests, plotting activity curves and calculating of MIC (minimal inhibitory concentration), as well as methods of estimation of obtain results were presented in our previous paper [7].

## Results and Discussion

Among the 12 commercially-available mouthrinses examined in the study, the following

were not found to show antifungal activity: Azulan, Dentosept, Eludril Classic, Listerine Total care, *Tinctura salviae*.

The largest inhibition zones were produced by Dentosept A (mean scores without dilution – 22.4 mm, 1:2 – 17.4 mm, 1:4 – 12.6 mm, 1:8 – 10.4 mm and 1:16 – 10.5 mm) and Colgate (mean scores without dilution – 15.2 mm, 1:2 – 12.8 mm, 1:4 – 11.3 mm, 1:8 – 11.1 mm and 1:16 – 11 mm). The smallest inhibition zones were demonstrated by Curasept (mean scores without dilution – 15.4 mm, 1:2 – 13.0 mm and 1:4 – 11.5 mm). Detailed data are summarized in Table 1.

Chlorhexidine, Colgate and Dentosept acted on all isolated strains; Corsodyl did not act on *C. glabrata* (two strains), Octenidol on *C. glabrata* and *C. guilliermondii* (one each), Sylveco on *C. tropicalis* (two), Oral B on *C. parapsilosis*, Curasept did not affect 17 strains, including on *C. albicans* (five), *C. guilliermondii* (four), *C. dubliniensis* (three), *C. humicola* and *C. parapsilosis* (two each)

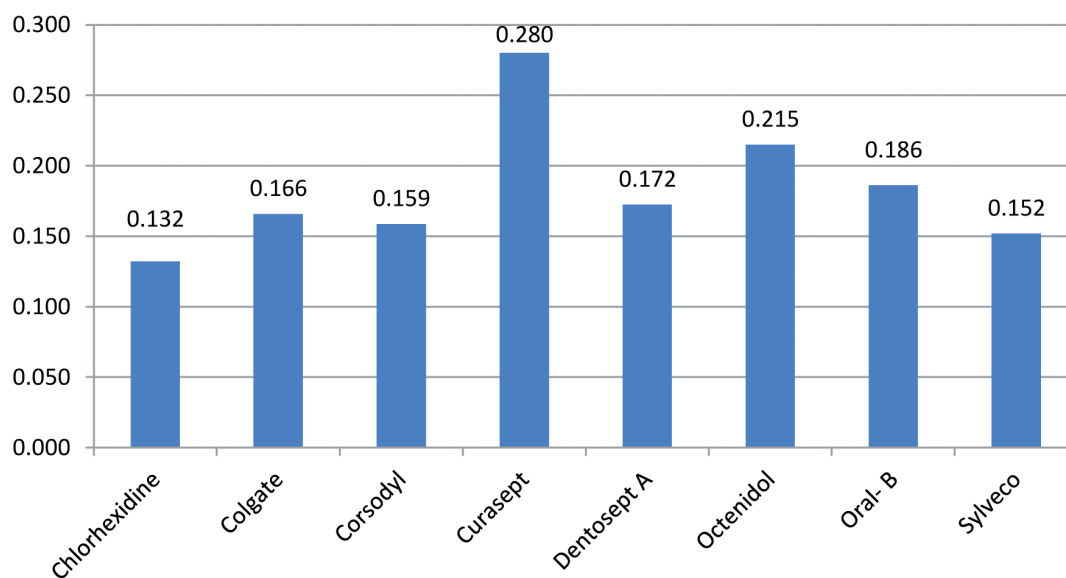


Figure 1. Overall MIC value for particular mouthrinses for patient strains

Table 2. Mean diameter of growth inhibition (in mm) for particular species under different concentration/dilution

Species	Mean diameter of growth inhibition (in mm)									
	CHX			Dentosept A				Sylvecco		
Concentration/dilution	0.2%	0.1%	0.05%	WD*	1:2	1:4	WD*	1:2	1:4	1:8
1. <i>C. famata</i>	16.4 <sup>4,7</sup>	14.2	12.1	27.0 <sup>4,6,7,9</sup>	20.0 <sup>4,6,7</sup>	15.4 <sup>6,7,9</sup>	20.2 <sup>6,7,9</sup>	16.9 <sup>6,7,9</sup>	14.2 <sup>6-9</sup>	12.6
2. <i>C. humicola</i>	15.7	13.3	11.5	25.8	19.0	11.7	19.0	16.0	13.7	11.2
3. <i>C. krusei</i>	16.3	14.0	–	25.3	20.0	–	17.0	14.3	–	–
4. <i>C. dubliniensis</i>	14.5 <sup>6,7-9</sup>	12.2 <sup>1,6,7-9</sup>	–	21.4	16.2	10.8 <sup>1,5,8</sup>	21.5 <sup>5,9</sup>	16.7 <sup>6,7-9</sup>	13.5 <sup>6-9</sup>	10.4
5. <i>C. tropicalis</i>	15.3 <sup>7,8</sup>	13.1 <sup>5-9</sup>	10.5 <sup>7,8</sup>	26.4 <sup>6</sup>	21.8 <sup>4,6,7</sup>	14.4 <sup>6</sup>	17.3	14.7	13.7 <sup>8</sup>	11.0
6. <i>C. guilliermondii</i>	16.7	14.3	11.8	21.3	16.2	11.7	16.7	14.2	11.5	10.2
7. <i>C. parapsilosis</i>	18.5 <sup>6,8,9</sup>	15.9 <sup>1,2,6,9,10</sup>	13.5 <sup>6,9,10</sup>	22.3	16.5	13.0	14.2 <sup>2,5,6,8,9</sup>	11.7 <sup>2,5,6,8,9</sup>	10.9	–
8. <i>C. albicans</i>	17.2	14.3	11.7	23.6	18.8 <sup>6</sup>	13.0	17.1	14.5	11.1	10.2
9. <i>C. glabrata</i>	16.4	14.3	12.1	19.2 <sup>1,2,5,7,8</sup>	15.4 <sup>1,5,8</sup>	11.6 <sup>5,8</sup>	16.2	13.9	11.4	11.3
Statistically significant differences between species	<b>Z:</b> 3.045-7.725 <b>P</b> <0.01	<b>Z:</b> 3.553-7.838 <b>P</b> <0.01	<b>Z:</b> 3.444-5.804 <b>P</b> <0.02	<b>Z:</b> 3.552-7.335 <b>P</b> <0.01	<b>Z:</b> 3.541-6.212 <b>P</b> <0.01	<b>Z:</b> 3.5-5.549 <b>P</b> <0.01	<b>Z:</b> 3.384-8.825 <b>P</b> <0.02	<b>Z:</b> 3.461-7.842 <b>P</b> <0.02	<b>Z:</b> 3.295-5.179 <b>P</b> <0.03	ND**

Table 2 continue

Species	Mean diameter of growth inhibition (in mm)							
	Colgate		Corsodyl		Curasept			
Concentration /dilution	WD*	1:2	1:4	WD*	1:2	1:4	WD*	1:2
1. <i>C. famata</i>	15.0 <sup>3,4,7-9</sup>	14.9	12.1 <sup>5,7</sup>	15.0	14.4	12.6 <sup>4,8</sup>	13.4	10.9
2. <i>C. humicola</i>	15.0	13.3	11.5	15.0	12.2	12.3	13.0	–
3. <i>C. krusei</i>	19.3 <sup>4,5,7</sup>	14.0	–	19.3	13.0	–	15.0 <sup>4</sup>	13.0
4. <i>C. dubliniensis</i>	14.7 <sup>5-9</sup>	12.2 <sup>1,3,6-9</sup>	–	14.7	13.2	10.5	12.1	–
5. <i>C. tropicalis</i>	16.6 <sup>6,8,9</sup>	13.1 <sup>1,3,6-9</sup>	10.5 <sup>6-9</sup>	16.6	14.1	11.7	13.6	11.3
6. <i>C. guilliermondii</i>	13.2	14.3	11.8	13.2	13.2	12.0	12.9	10.2
7. <i>C. parapsilosis</i>	15.1 <sup>2,6,8,9</sup>	15.9 <sup>1-3,6,9,10</sup>	13.5 <sup>2,6,8,9</sup>	16.2 <sup>4,6</sup>	15.1 <sup>4,6</sup>	13.7 <sup>4,5,8</sup>	15.0 <sup>1,4-6</sup>	13.0 <sup>1,4,6</sup>
8. <i>C. albicans</i>	14.5 <sup>9</sup>	14.3	11.7	14.5 <sup>1,5-7</sup>	11.6 <sup>1,5-7</sup>	10.3	14.1 <sup>4,6,7</sup>	11.7 <sup>4,6,7</sup>
9. <i>C. glabrata</i>	16.5	14.3	12.1	16.5 <sup>1,5-7</sup>	11.7 <sup>1,5-7</sup>	–	14.1 <sup>4,6,7</sup>	11.9 <sup>4,6</sup>
Statistically significant differences between species	<b>Z:</b> 2.012-6.423 <b>P</b> <0.04	<b>Z:</b> 2.051-6.423 <b>P</b> <0.04	<b>Z:</b> 2.143-6.095 <b>P</b> <0.03	<b>Z:</b> 3.381-7.725 <b>P</b> <0.03	<b>Z:</b> 3.306-7.943 <b>P</b> <0.03	<b>Z:</b> 3.249-8.054 <b>P</b> <0.04	<b>Z:</b> 3.505-6.946 <b>P</b> <0.02	<b>Z:</b> 3.208-7.076 <b>P</b> <0.04

Table 2 continue

Species	Mean diameter of growth inhibition (in mm)			
	Octenidol		Oral B	
Concentration /dilution	WD*	1:2	WD*	1:2
1. <i>C. famata</i>	14.8	12.6	14.9	12.8
2. <i>C. humicola</i>	14.0	11.5	15.0	13.0
3. <i>C. krusei</i>	15.0	12.3	13.3	11.3
4. <i>C. dubliniensis</i>	14.9	12.8	16.6 <sup>7,8</sup>	13.9
5. <i>C. tropicalis</i>	16.7 <sup>2,7</sup>	13.9 <sup>7</sup>	17.2 <sup>7,8</sup>	14.3 <sup>7</sup>
6. <i>C. guilliermondii</i>	14.9	12.9	15.5	13.3
7. <i>C. parapsilosis</i>	14.9	12.8	14.2	11.9
8. <i>C. albicans</i>	14.9	12.8	14.3	12.0
9. <i>C. glabrata</i>	15.0	13.1	15.9 <sup>7,8</sup>	13.9
Statistically significant differences between species	Z: 3.553-7.838 P<0.01	Z: 3.444-5.804 P<0.02	Z: 3.553-7.838 P<0.01	Z: 3.444-5.804 P<0.02

\* WD - without dilution

\*\* ND - no differences

and *C. glabrata* (one).

For all fungal species together, statistically significant differences ( $p=0.0000$ ) were observed for Chlorhexidine (CHX) (2%, 0.1% and 0.05% – value of  $H = 67.51-88.97$ ), for Dentosept A, Sylveco, Corsodyl, Octenidol and Oral B (WD, 1:2

and 1:4 – values of  $H = 36.05-119.72$ ) as well as for Curasept, and Colgate (WD, and 1:2 – values of  $H = 85.98-117.27$ ).

Statistically significant differences were observed in most cases between particular species of fungi and the type of mouthwash at different dilutions. More detailed data, including the mean diameter of growth inhibition and Z and p-values, are presented in Table 2.

Linear regression found the lowest mean MIC values, indicating the strongest potential activity, for chlorhexidine ( $\bar{x} = 0.037$ ), followed by Sylveco (0.152), Corsodyl (0.159) and Colgate (0.166). In contrast, the highest mean MIC, and hence the lowest activity, was calculated for Octenidol ( $\bar{x} = 0.215$ ) and Curasept (0.28); detailed data is presented in Table 3 and Figure 1. Interestingly, *C. tropicalis* ( $\bar{x} = 0.1564$ ) was found to be the most sensitive to the examined mouthrinses, while *C. guilliermondii* ( $\bar{x} = 0.2396$ ) and *C. dubliniensis* (0.2349) were the most resistant. More detailed data is given in Figure 2.

Different oral microorganisms, also fungi, may play a relevant role in the onset of carious lesions and periodontal diseases. One way to maintain proper oral hygiene is to use mouthrinses that exhibit bacteriostatic, fungistatic, protozostatic or/and bactericidal, fungicidal or protozoicidal action against a wide range of potentially invasive pathogens [3,8].

Five of the tested mouthrinses (Azulan, Dentosept, Eludric Classic, Listerine Total care and

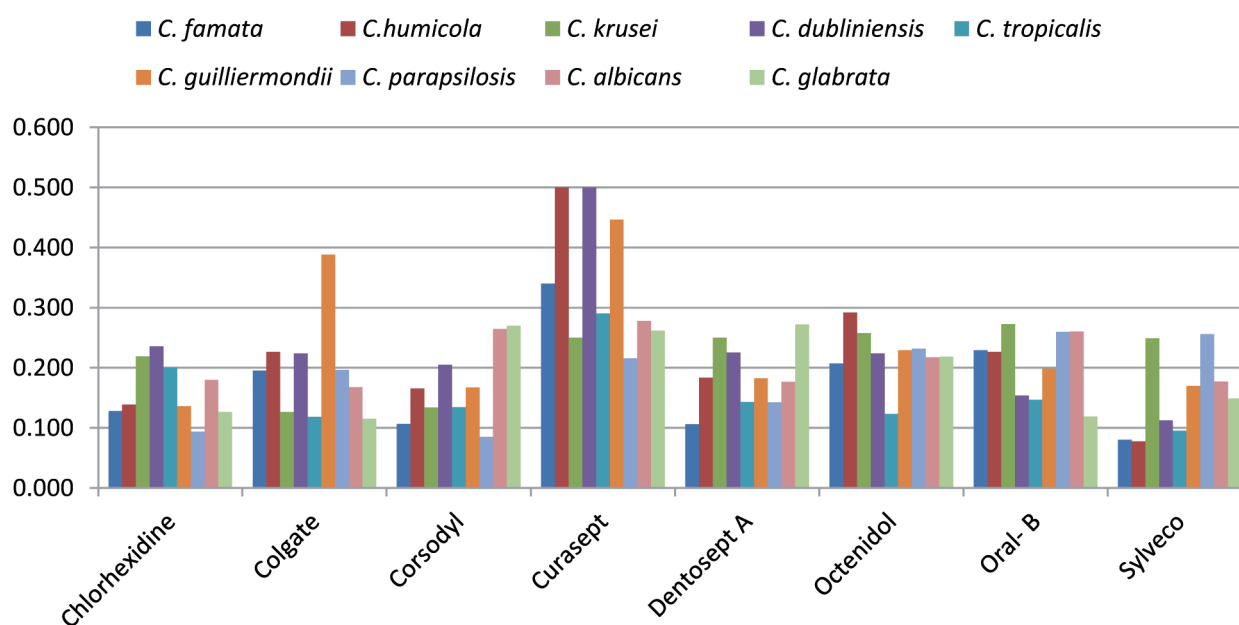


Figure 2. MIC value of mouthrinses for different species of Candida (study group)

Table 3. MIC average values of mouthrinses for different species of *Candida* isolated from oral cavity of patients

Species	MIC average values									Statistically significant differences between species
	CHX (a)	Colgate (b)	Corsodyl (c)	Curasept (d)	Dentosept A (e)	Octenidol (f)	Oral B (g)	Sylveco (h)	Value of Z and p	
1. <i>C. famata</i>	0.1277 <sup>d,f,g</sup>	0.1953 <sup>d</sup>	0.1066 <sup>d,f,g</sup>	0.3399	0.1061 <sup>f,g</sup>	0.2073	0.2290	0.0800 <sup>d,f,g</sup>	<b>Z: 3.187-6.293; P&lt;0.01</b>	
2. <i>C. humicola</i>	0.1387	0.2264	0.1652	0.5	0.1835	0.2916	0.2264	0.0778 <sup>b,e,f,g</sup>	<b>Z: 3.187-6.293; P&lt;0.01</b>	
3. <i>C. krusei</i>	0.2188	0.1263	0.1339	0.25	0.25	0.2576	0.2726	0.2489	ND*	
4. <i>C. dubliniensis</i>	0.2356	0.2239	0.2049	0.5 <sup>b,c,e-g</sup>	0.22549	0.2239	0.1535	0.1125 <sup>a-g</sup>	<b>Z: 3.578-7.961; P&lt;0.01</b>	
5. <i>C. tropicalis</i>	0.2004 <sup>b,c,f</sup>	0.1184 <sup>e</sup>	0.1345 <sup>e</sup>	0.2902 <sup>b,c,f,h</sup>	0.1427	0.1231	0.1465	0.0954	<b>Z: 3.221-4.332; P&lt;0.01</b>	
6. <i>C. guilliermondii</i>	0.1360	0.3879 <sup>a,c,e-h</sup>	0.1668	0.4550 <sup>a,c,e,f,g,h</sup>	0.1824	0.2293	0.1984	0.1696	<b>Z: 3.668-7.409; P&lt;0.01</b>	
7. <i>C. parapsilosis</i>	0.0938 <sup>b,d-h</sup>	0.1965	0.0848 <sup>b,d-h</sup>	0.2156	0.1426 <sup>d,f,g+</sup>	0.2316	0.2594	0.2559	<b>Z: 4.774-8.518; P&lt;0.01</b>	
8. <i>C. albicans</i>	0.1799 <sup>c,d,f,g</sup>	0.1673 <sup>c,d,g</sup>	0.2646	0.2815	0.1767 <sup>c,d,g</sup>	0.2175	0.2600	0.1771 <sup>c,d,g</sup>	<b>Z: 3.378-6.454; P&lt;0.01</b>	
9. <i>C. glabrata</i>	0.1264	0.1150	0.2698 <sup>a,b,c-h</sup>	0.2620 <sup>a,b,g,h</sup>	0.2719	0.2185	0.1186 <sup>c,f</sup>	0.1489	<b>Z: 3.138-8.108; P&lt;0.01</b>	

\*ND - no differences

*Tinctura salviae*) did not act on the fungal strains isolated from patients. In a previous study, in which the same 13 mouthrinses were tested against eight reference strains of fungi (*C. albicans* – CBS 2312, *C. albicans* – L 45, *C. albicans* – ATCC 24433, *C. dubliniensis* – CBS 7987, *C. glabrata* – CBS 862, *C. krusei* – CBS 573, *C. parapsilosis* – CBS 10947 and *C. tropicalis* – CBS 2424), Dentosept A, Chlorhexidine and Colgate had the strongest effect [7]. In the present study, Chlorhexidine, Sylveco and Corsodryl had the strongest while Octenidol and Curasept demonstrated the weakest effects on examined strains.

Chlorhexidine (CHX) was found to be the most effective mouthrinse out of four, including 0.2% chlorhexidine and Oral-B, against 68 strains from six species of *Candida* (*C. albicans* – 51; *C. glabrata* – 8; *C. kefyr* – 4; *C. parapsilosis*, *C. tropicalis* – 2 each; *C. intermedia* – 1) [9]. This findings is consistent with those obtained in the present study: CHX demonstrated the lowest mean MIC against strains isolated from patients ( $\bar{x}$  = 0.132).

The high antifungal activity of CHX has been confirmed by Aoun et al. [10], who compared the effectiveness of 0.1% hexetidine with that of 0.12% chlorhexidine in the elimination of *C. albicans* from dentures *in vivo* (measured as CFU/ml). For chlorhexidine, the CFU number dropped from 2911 on the first day to eight on the fourth day, whereas after hexetidine, these values fell from 52860 to 6576. The respective control values for water were 252717 and 253568.

Welk et al. [11] compared the effects of rinsing for three days, twice a day, with Octenidol with those of 0.12% chlorhexidine and Listerine (CFU/sample). The best results were obtained with Octenidol, which demonstrated zones of inhibition ranging from 14–15 mm.

In addition, 0.2% CHX was found to more effectively inhibit the adhesion of *C. albicans* cells to epithelial cells of the oral cavity than 0.05% CPC (cetylpyridinium chloride) and 0.045% triclosan [12], although no statistically significant difference was observed between CHX and CPC.

Similar results were obtained by Fathilah et al. [13], who examined the effect of CHX and CPC on *C. tropicalis* and *C. krusei*. Both mouthrinses act separately on the tested species and reduce the number of fungal populations. Dasgupta et al. [14] report a significantly smaller number of fungal colonies to be present in the oral cavity after scaling and washing with chlorhexidine compared to

essential oils or povidoniodine.

Carvalho et al. [15] found CHX (0.12–0.2%), hexetidine (0.1%) and alcohol (7 and 14%). CHX 0.2% with alcohol to demonstrate stronger antifungal activity than alcohol and hexetidine against forty *C. albicans* strains isolated from the mouth.

Nacamoto et al. [16] found out of seven mouthrinses, including chlorhexidine, Listerine and Viadent, those containing cetylpyridinium showed the strongest effects against *Candida albicans*. However, Bugno et al. [17] found turmeric to demonstrate high antifungal activity, with CHX in second place, followed by cetylpyridinium. This is in line with our present findings, in which the best results were not obtained for Colgate lotion containing cetylpyridinium.

A comparison of Listerine Antiseptic, Tatar Control Listerine Antiseptic, Peridex and 0.2% chlorhexidine found all to be active against the following tested strains in commercially-available concentrations *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, *C. tropicalis* and *Saccharomyces cerevisiae* strains isolated from patients, as well as *Candida* ATCC and *Candida* NCPF. These results show that the macro dilution method demonstrates high sensitivity according to the NCCL [18].

In conclusion, eight of the tested mouthrinses inhibited the *in vitro* growth of fungal strains isolated from patients, either undiluted or at 1:2, 1:4, 1:8 and even 1:16 dilutions, depending on the type of mouthrinse, with chlorhexidine demonstrating high efficiency at removing potentially pathogenic fungi. The antifungal effect of a mouthrinse depends on its composition and the species of fungus. The use of mouthrinses is an important complement to procedures ensuring proper oral hygiene and preventing certain oral diseases.

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