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Evaluation of antibacterial activity against *Candida albicans* according to the dosage of various denture cleansers

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PURPOSE. The purpose of this study is to compare the antibacterial activity of currently purchasable denture cleansers against *Candida albicans*. **MATERIALS AND METHODS:** This study used tablet-type denture cleansers, Polident®, Coolingdent® and Fittydent®, along with liquid denture cleansers, Hexamedine®, Listerine® and Apple vinegar®. The antibacterial activities of denture cleansers were evaluated based on the number of *C. albicans* and concentrations of the denture cleansers. **RESULTS.** In the 0.5×10^6 cfu/㎖ culture medium, the *C. albicans*' death rate of Polident® was significantly lower than those of Fittydent®, Hexamedine®, Listerine®, and Apple vinegar®(P<.05). In the 0.5×10^7 cfu/, the *C. albicans*' death rates of Polident® and Coolingdent® were significantly lower than those of Fittydent®, Hexamedine®, Listerine® and Apple vinegar®(P<.05). The *C. albicans*' death rates of Polident® and Coolingdent® were significantly decreased at 0.02 g and 0.01 g. The *C. albicans*' death rate of Fittydent® was significantly decreased at 0.005 g (P<.05). The *C. albicans*' death rate of Hexamedine® was significantly decreased at 1/16 dilution. The *C. albicans*' death rate of Listerine® was decreased at 1/8 dilution, and the antibacterial activity of Apple vinegar® was decreased at 1/4 dilution (P<.05). **CONCLUSION.** As the number of *C. albicans* increased, the antibacterial activities of the denture cleansers decrease. In the tablet-type denture cleanser, all denture cleansers showed 100% *C. albicans*' death rate when used at a dose of 1 tablet. One denture cleanser showed the same antibacterial effect with only 1/3 of a tablet. In the liquid type denture cleanser, the level of dilution required was different for each denture cleanser. [J Adv Prosthodont 2021;13:100-6]

KEYWORDS
Antibacterial activity; *C. albicans*; Liquid denture cleanser; Tablet type denture cleanser

INTRODUCTION

Denture cleaning and disinfection are essential for preventing denture stomatitis and halitosis, as well as for maintaining good oral health.[1-3] However,

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many patients wear dentures without knowing the proper cleaning methods.[2,4] Dentists should fully educate their patients on how to clean dentures[2,5] and should know the effects and mechanisms of denture cleaning.

Denture cleaning methods can be categorized as mechanical and chemical.[4,6-8] Mechanical methods include brushing and ultrasonic cleaning. Chemical methods use denture cleansers, which can be classified as alkaline peroxides, alkaline hypochlorites, dilute acids, disinfectants and enzymes.[4,8,9] The peroxide-based solutions showed favorable antimicrobial activity.[10] Chemical denture cleansers are convenient to use and are effective in cleaning the areas that cannot be reached using a toothbrush, especially in patients who have difficulties with brushing, such as the weak and elderly and those with mental or physical disability.[2] However, these have the disadvantage of being expensive. Denture stomatitis is the most common inflammatory disease experienced by about 60 - 65% of all denture wearers.[11-14] Although the exact cause of this disease has not been identified,[15,16] *Candida albicans* is believed to be the main causative agent responsible for the onset, persistence, and exacerbation of denture stomatitis.[15-17] Consequently, eliminating *C. albicans* can help prevent denture stomatitis.[18]

A variety of denture cleansers are commercially available today. This study aimed to compare the antibacterial activities against *C. albicans* of tablet-type denture cleansers (Polident®, Coolingdent® and Fittydent®) versus liquid cleansers (Hexamedine®, Listerine® and Apple vinegar®) currently sold in Korea, and to present the proper dose of denture cleansers to the patients who use them based on the findings.

MATERIALS AND METHODS

This study used tablet-type denture cleansers with enzymes, Polident® and Coolingdent®, and without enzyme, Fittydent®, along with liquid denture cleansers, Hexamedine®, Listerine® and Apple vinegar® (Table 1). Table 2 shows the main ingredients of the tablet-type cleansers used in the experiment.

In the study, the antibacterial activities of denture cleansers were evaluated based on the number of *C. albicans*. A spectrophotometer (Epoch, Bio-tek, Winooski, VT, USA) was used to measure the absorbance of *C. albicans* broth, and broth with concentration of 1×10^7 cfu/ml was prepared. Considering the applica-

Table 1. Denture cleansers

	Commercial name	Chemical composition	Manufacturer	Enzyme (Everlase 6.0T)
Tablet-type denture cleansers	Polident®	Alkaline peroxides	GlaxoSmithKline, Brentford, England	+
	Coolingdent®	Alkaline peroxides	Greenworldpharm, Hwaseong, Korea	+
	Fittydent®	Alkaline peroxides	Fittydent International GmbH, Pinkafeld, Austria	-
Liquid denture cleansers	Hexamedine®	Chlorhexidine	Bukwang R&D, Ansan, Korea	-
	Listerine®	Diluted acid	Johnson&Johnson, New Brunswick, NJ, USA	-
	Apple vinegar®	Diluted acid	Ottogi, Anyang, Korea	-

Table 2. Major components of tablet-type denture cleansers

Denture cleansers	Major components
Polident®	Oxone (persulfate), sodium perborate, monohydrate, everlase 6.0T
Coolingdent®	Oxone (persulfate), sodium perborate, monohydrate, everlase 6.0T
Fittydent®	Oxone (persulfate), sodium perborate, monohydrate, sodium hydrogen carbonate

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tion methods of the tablet-type and liquid cleansers, the broth for the tablet-type cleansers was diluted additionally by 1/2, meaning it was diluted using the 1/10, 1/100, 1/1000, and 1/10000 to prepare the tablet-type cleansers with 0.5×10^7, 0.5×10^6, 0.5×10^5, 0.5×10^4 and 0.5×10^3 cfu/ml concentrations, and the liquid cleansers with 1×10^7, 1×10^6, 1×10^5, 1×10^4 and 1×10^3 cfu/ml concentrations.

To match the recommended dose given by the manufacturer, tablet-type cleansers, Polident®, Coolingdent® and Fittydent®, were prepared in powder form, 0.04 g each. Each powder sample was admixed with 2 ml of tablet-type cleanser broth and reacted for 5 min. After vortex mixing, 10 ml of each solution was drawn using a micropipette and applied twice on BHI solid medium. Considering the number of *C. albicans*, 10 ml of the original solution was used for broth with 0.5×10^3 and 0.5×10^4 cfu/ml concentrations, while for broth with 0.5×10^5, 0.5×10^6 and 0.5×10^7 cfu/ml concentrations, 10 ml drawn was diluted with 1/10, 1/100, and 1/1000, respectively, before being applied. After culturing for 24 h at 37°C, the number of colonies formed were visually inspected (Fig. 1).

Liquid cleansers, Hexamedine®, Listerine® and Apple vinegar®, were prepared using 1 ml of each cleanser and admixed with 1 ml of broth and reacted for 5 min. After vortex mixing, 10 ml of each solution was drawn and diluted, and subsequently placed on a solid medium for culturing and inspection.

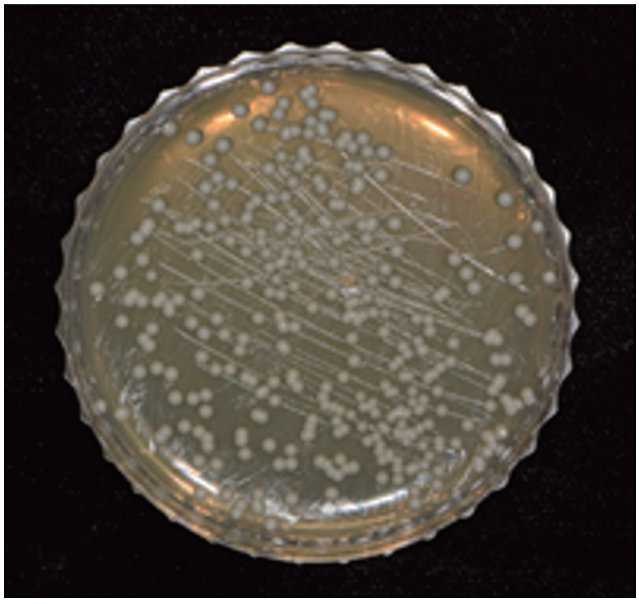

Fig. 1. *Candida albicans* grown on brain heart infusion agar.

For the negative control group, 10 ml of each tablet-type cleanser broth was drawn and diluted, and then subsequently placed on a solid medium for culturing and inspection. The experiment was repeated three times.

In the next experiment, the antibacterial activities were evaluated based on the concentrations of the denture cleansers. A spectrophotometer was used to prepare a broth with 1×10^6 cfu/ml concentration. The broth for the tablet-type cleansers was diluted additionally by 1/2. Tablet-type cleansers prepared in powder form weighed 0.01 g, 0.02 g, 0.04 g, and 0.08 g, corresponding to 1/4, 1/2, 1, and 2 tablets, respectively. Fittydent® was prepared, additionally, weighing 0.005 g and 0.03 g, corresponding to 1/6 and 1 tablet, respectively. Each tablet-type cleanser sample was admixed with 2 ml of tablet-type cleanser broth and reacted for 5 min. After vortex mixing, 10 ml of each solution was drawn and diluted by 1/100, and then applied twice on a BHI solid medium. After culturing for 24 h at 37°C, culturing of colonies formed were inspected.

For liquid cleansers, 1 ml of each original solution and solutions diluted by 1/2, 1/4, and 1/8 were prepared and admixed with liquid cleanser broth for 5 min. After vortex mixing, 10 ml of each solution was drawn and diluted by 1/100, and then applied twice on a BHI solid medium, for culturing and inspection. For the negative control group, 10 ml of each tablet-type and liquid cleanser broth was drawn and diluted by 1/100, and then applied twice on a BHI solid medium, for culturing and inspection. The experiment was repeated three times.

The antibacterial activities of the denture cleansers were evaluated and compared by calculating the cell death rate of *C. albicans*. Analysis was performed via ANOVA. Post hoc analysis was performed via Scheffe test using the SPSS Ver. 22. 0 (SPSS Inc., Chicago, IL, USA).

Cell death rate (%) = {(number of control bacterial strains – number of test broth bacterial strains) / number of control bacterial strains} × 100

RESULTS

In the experiment on antibacterial activities of denture cleansers based on the number *C. albicans* strains, *C. albicans* broth with concentrations 0.5×10^3, 0.5×10^4 and 0.5×10^5 cfu/ml showed excellent cell death rate of ≥ 98% for all six denture cleansers. At broth concentration of 0.5×10^6 cfu/ml, Polident® showed cell death rate of approximately 70%, which was significantly lower than those of the Fittydent®, Hexamedine®, Listerine® and Apple vinegar® ($P < .05$). At broth concentration of 0.5×10^7 cfu/ml, Polident® and Coolingdent® showed approximately 22% and 23% cell death rates, respectively, which were significantly lower than those of the Fittydent®, Hexamedine®, Listerine®, and Apple vinegar® ($P < .05$) (Table 3).

In the experiment on antibacterial activities based on the concentrations of the denture cleansers, Polident® showed cell death rate of approximately 69% and -10% at 0.02 g (1/2 tablet) and 0.01 g (1/4 tablet), respectively, which represented statistically significant decrease ($P < .05$). Coolingdent® also showed cell death rate of -4% and -5% at 0.02 g and 0.01 g, respectively, which also represented significant decrease. Meanwhile, Fittydent® showed cell death rate of approximately 44% at 0.005 g (1/6 tablet), revealing a significant decrease ($P < .05$) (Table 4, Table 5).

Hexamedine® showed cell death rate of 89% at concentration diluted by 1/16, which was a statistically significant decrease ($P < .05$). Cell death rate of Listerine® at concentration diluted by 1/8 and Apple vinegar® at concentration diluted by 1/4 were approximately 40% and 44%, respectively, which represented significant decreases ($P < .05$) (Table 6).

Table 3. *C. albicans*' death rate of denture cleansers in relation to *C. albicans* broth concentrations

Concentration (cfu/ml)	Death rate (%)					
	Polident®	Coolingdent®	Fittydent®	Hexamedine®	Listerine®	Apple vinegar®
0.5×10^3	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a
0.5×10^4	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a	98.8 ± 1.7^a
0.5×10^5	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a	98.8 ± 1.8^a
0.5×10^6	70.4 ± 29.9^c	97.8 ± 3.1^b	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a	99.4 ± 0.9^a
0.5×10^7	22.0 ± 9.3^d	22.8 ± 14.6^d	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a

$^{a-d}$: Values with the different letters are significantly different ($P < .05$) except a between b and b between c

Table 4. *C. albicans*' death rate of Polident® and Coolingdent® by dosage

Dosage (g)	Death rate (%)	
	Polident®	Coolingdent®
0.01 (1/4 n)	-10.5 ± 8.0^a	-3.5 ± 8.7^d
0.02 (1/2 n)	69.2 ± 27.2^b	-4.7 ± 11.0^d
0.04 (n)	100.0 ± 0.0^c	100.0 ± 0.0^e
0.08 (2 n)	100.0 ± 0.0^c	100.0 ± 0.0^e

(n = amount correspond to manufacturer's recommended dosage)
$^{a-e}$: Values with the different letters are significantly different ($P < .05$)

Table 5. *C. albicans*' death rate of Fittydent® by dosage

Dosage (g)	Death rate (%)
	Fittydent®
0.005 (1/6 n)	44.2 ± 39.7^a
0.01 (1/3 n)	100.0 ± 0.0^b
0.02 (2/3 n)	100.0 ± 0.0^b
0.03 (n)	100.0 ± 0.0^b
0.04 (4/3 n)	100.0 ± 0.0^b
0.08 (8/3 n)	100.0 ± 0.0^b

(n = amount correspond to manufacturer's recommended dosage)
$^{a-b}$: Values with the different letters are significantly different ($P < .05$)

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Table 6. *C. albicans*' death rate of Hexamedine®, Listerine® and Apple vinegar® by concentration

Concentration	Death rate (%)		
	Hexamedine®	Listerine®	Apple vinegar®
1/16 dilution	89.2 ± 1.9[a]	35.6 ± 3.4[c]	36.0 ± 2.1[e]
1/8 dilution	99.0 ± 1.0[b]	39.8 ± 5.3[c]	40.2 ± 2.1[f]
1/4 dilution	100.0 ± 0.0[b]	94.4 ± 4.0[d]	44.2 ± 6.3[g]
1/2 dilution	100.0± 0.0[b]	100.0 ± 0.0[d]	89.4 ± 3.7[h]

[a-h] : Values with the different letters are significantly different ($P < .05$)

DISCUSSION

Many patients are burdened by the cost of using denture cleansers and are unsure of the dose for single use. According to the findings in this study, Polident® showed good antibacterial activities at a dose of 0.08 g (2 tablets) and 0.04 g (1 tablet). At a dose of 0.02 g (1/2 tablet), the antibacterial activities decreased significantly. Therefore, it is recommended that one whole tablet of Polident® should be used, as recommended by the manufacturer. Coolingdent® also showed significant decrease in antibacterial activities at 0.02 g (1/2 tablet), and thus, using one whole tablet is recommended. In the experiment on antibacterial activities of denture cleansers based on the number *C. albicans* strains, Fittydent® showed better antibacterial activities than Polident® and Coolingdent®. However, comparing their weight of 1 tablet, Fittydent® was lighter than Polident® and Coolingdent®. Therefore, our experiment included manufacturer's recommended dose of 0.03 g (1 tablet) and 0.005 g (1/6 tablet) for Fittydent®.

Fittydent® showed significant decrease in antibacterial activities at 0.005 g. Therefore, ≥ 1/3 of a tablet of Fittydent® can be used as a single dose. Hexamedine® showed significant decrease in antibacterial activities at 1/16 diluted concentration. Therefore, Hexamedine® can be used by diluting up to 1/8. Listerine® and Apple vinegar® can be used with concentrations diluted up to 1/4 and 1/2, respectively.

In the experiment on antibacterial activities of denture cleansers based on the number *C. albicans* strains, all six products showed ≥98% cell death rate against *C. albicans* at *C. albicans* broth concentrations of 0.5×10^3, 0.5×10^4 and 0.5×10^5 cfu/ml. No significant differences in this value were observed. However, at broth concentration of 0.5×10^6 cfu/ml, Polident® showed statistically significant decrease in cell death rate of approximately 70%. At broth concentration of 0.5×10^7 cfu/ml, Polident® and Coolingdent® showed significant decrease in cell death rate of approximately 22% and 23%, respectively. This demonstrated that the antibacterial activities of Polident® and Coolingdent® decreased against relatively high concentration of *C. albicans* and that the efficacy of the cleanser is reduced when a large number of bacteria are present. Therefore, proper cleaning and disinfection before the accumulation of bacteria on the dentures are necessary to keep the dentures clean.

Because of the method used in this study, liquid denture cleansers reacting with *C. albicans* broth would inevitably reduce their concentrations. Therefore, liquid denture cleansers, Hexamedine®, Listerine®, and Apple vinegar®, were evaluated for their antibacterial activities at 1/2 diluted concentrations. However, the liquid denture cleansers still showed high cell death rate of ≥ 98%. This indicated that Hexamedine®, Listerine®, Apple vinegar®, and Fittydent® have higher antibacterial activities than of the Polident® and Coolingdent®.

Table 2 shows the main ingredients of the tablet-type cleansers used in this study. All three products contain oxone (persulfate) that cleans the surface of the denture by creating bubbles and sodium perborate monohydrate that acts as a surfactant. The multiple oxygen bubbles Polident® and Coolingdent® also contain proteolytic enzyme (Esperase 6.0T), whereas Fittydent® does not contain proteolytic enzyme, but contains sodium hydrogen carbonate. A study by Chun *et al*.[15] reported that cleansers containing enzymes tend to show greater ability in decomposing *C. albicans* than those without. However, Fittydent® that does not contain enzymes showed good antibacterial activities in this study. Such result would indicate that the existence of proteolytic enzyme itself does not influence the antibacterial activities of a cleanser. But, a study by Hayran *et al*.[19] reported Polident 3 min® should be suggested over Fittydent® for patients who use any denture resin types. This study

was an experiment of the effective concentration of denture cleanser on some denture base resins. Because it was a study on the cleansing effect of *C. albicans* attached to the resin denture base, the results are different from our study. This result may reflect the limitation of our study using a small amount of powder for the experiment, as well as the effects of other ingredients, such as sodium hydrogen carbonate. Accordingly, additional studies are necessary to investigate and evaluate the contents of each denture cleanser ingredient and the antibacterial activities of each ingredient, respectively.

Lee and Kim[16] studied the prevalence and distribution of *C. albicans* inside the oral cavity and reported that the occurrence of denture stomatitis is closely associated with the quantitative increase in *C. albicans*. However, since *C. albicans*, a resident oral bacterium, may show a positive response rate of 3 - 48% in healthy adults depending on the collection method, measurement methods that can identify the quantitative increase for confirmation of infection are necessary. Exact assessment on the number of *C. albicans* strains within the oral cavity is difficult. In this study, the results from the experiments on antibacterial activities of denture cleansers based on the number *C. albicans* strains demonstrated that Polident® and Coolingdent® showed significant decrease in cell death rate, starting from *C. albicans* broth concentration of 0.5×10^6 cfu/ml. Therefore, the experiment on antibacterial activities based on the concentrations of the denture cleansers used the 0.5×10^6 cfu/ml as the broth concentration.

Denture stomatitis is characterized by several clinical signs but few symptoms. It is rarely associated with discomfort. However, if denture stomatitis is not treated, systematic infections can occur. So, the treatment of denture stomatitis is important. The main causes of denture stomatitis is the level of *C. albicans* measured in the dentures and in the saliva.[20] Therefore, an ideal denture cleanser should have appropriate antibacterial and antifungal activities and should be convenient to use and able to effectively remove coloration as well as the organic and inorganic materials accumulated on the denture, while not damaging it. A denture cleanser should also be non-toxic and non-irritating, while being low cost and easy to store.[2,4] The experiments in this study only evaluated the antibacterial activities of denture cleansers against *C. albicans*. When choosing denture cleansers, appropriate selection based on various conditions is necessary.

Limitations of this study included the fact that it used *in vitro* experiments to evaluate the antibacterial activities of cleansers against *C. albicans*. Secondly, for convenience, the experiments used the powdered form in a small amount of time by grinding the tablet-type cleansers. Consequently, the effects of a single whole tablet were not evaluated. Thirdly, the concentrations of the liquid cleansers were inevitably reduced as they were mixed with the culture broth for reaction. Consequently, the evaluation was performed not on the original solution but on the solutions diluted by 1/2 and others. Therefore, additional studies which address these limitations are necessary in the future.

CONCLUSION

Based on the experiments on antibacterial activities of various denture cleansers against *C. albicans*, the following conclusions were derived.

As the number of *C. albicans* strains increased, the antibacterial activities of the denture cleansers decrease. Therefore, proper cleaning and disinfection before the accumulation of bacteria on the dentures are necessary to keep the dentures clean.

In the tablet-type denture cleanser, all denture cleansers showed 100% death rate when used at a dose of 1 tablet. One denture cleanser showed the same effect with only 1/3 of a tablet.

In the liquid type denture cleanser, the level of dilution required was different for each denture cleanser.

It is necessary to study the appropriate concentration of the each denture cleanser.

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