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ORIGINAL ARTICLE

Effectiveness of non-fluoride and fluoride dentifrices for denture hygiene

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ABSTRACT

Objective: To compare the effectiveness of commonly used herbal/non-fluoride with fluoride dentifrices in order to eliminate pathogenic oral microorganisms from denture base material.

Materials and methods: Heat-polymerized acrylic resin specimens ($n = 288$) were divided into three groups and each group inoculated with three various microorganisms ($n = 96$ for each) *Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa (P. aeruginosa)*. Contaminated specimens were randomly assigned to the application of six herbal/non-fluoride and three fluoride dentifrices. These specimens were divided into two groups: negative and positive control ($n = 3$ for each). All acrylic specimens were incubated at 37 °C for 24 h for samples inoculated with bacterial strains and 37 °C for 48 h for samples inoculated with yeast strains. After the incubation period, all brain–heart infusion broths that contained disinfectant acrylic specimens were cultured on 5% sheep blood agar for bacterial counts and Sabouraud dextrose agar for yeast counts. The number of colony-forming units per millilitre (CFU/mL) were calculated. The results were analysed by Mann–Whitney *U* and Kruskal–Wallis tests ($p = .05$).

Results: Both herbal/non-fluoride and fluoride dentifrices were effective against *Candida albicans*. However, fluoride dentifrices were comparatively better than the herbal/non-fluoride dentifrices against *Staphylococcus aureus* and *P. aeruginosa*.

Conclusions: Herbal dentifrices could be used, especially among the elderly who lack a degree of manual dexterity during the rinsing of dentifrice chemicals from their dentures.

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Introduction

It is well known that the oral cavity is a habitat for a large array of microorganisms. The most commonly encountered pathogens in the oral cavity initiate dental caries and periodontal diseases [1]. Dentures are prone to provide protected colonization niches for a variety of microorganisms due to heterogeneities in their surfaces. Microorganisms on dentures have not only been associated with oral disease but also with systemic diseases such as aspiration pneumonia, cardiovascular disease and diabetes particularly with the elderly [2–4]. It is therefore critical that denture users clean their dentures regularly and correctly.

Denture cleaning methods can be performed chemically and/or mechanically. It has been suggested that the mechanical method represented by brushing with dentifrice is one of the most common methods for maintaining denture hygiene [5].

There has been renewed interest in complementary and alternative medicine (CAM) in recent years. The growing field of alternative medicine has led to the production of dentifrices using plant extracts with antibacterial properties. Commonly used synthetic dentifrices contain chemical agents such as fluoride, which have associated side effects following prolonged use [6]. However, natural products such as herbal dentifrices have numerous properties with health benefits, recently there has been an increase in the use of natural herbal products for general and oral health care. The success of dentifrices is in part determined by their ability to eliminate pathogenic oral microorganisms [7].

In recent years, herbal products have been used for tooth brushing, as they are economic, safe and an effective alternative for the prevention and control of various oral diseases [8]. However, to the best of our knowledge, there are no publications regarding the effect of herbal products as denture cleaners. The present study was conducted to compare the effectiveness of commonly used herbal/non-fluoride and fluoride dentifrices in Cyprus in order to eliminate pathogenic oral microorganisms from denture base material.

Materials and methods

Preparation of denture base material specimens

A heat-polymerized denture base material (Meliodent Heat Cure, Heraeus-Kulzer, Germany) was used in the study. Squares of 10 mm acrylic resin denture base samples with a thickness of 2 mm were fabricated according to the

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Table 1. Origin and morphotype of microorganisms.

Microorganisms	Origin	Morphotype	Code
Candida albicans	ATCC 90028	Yeast	*C. albicans*
Staphylococcus aureus	ATCC 25923	Gram-positive cocci	*S. aureus*
Pseudomonas aeruginosa	ATCC 27853	Gram-negative bacillus	*P. aeruginosa*

manufacturer's recommendations. A total of 550 specimens were prepared and no finishing or polishing procedures were performed in order to simulate the inner surface of complete denture, only remaining excesses were removed with the aid of a 320-grit wet sand paper on all 550 specimens. After preparation of the specimens, their surfaces were washed in water under pressure in order to remove any possible contaminants. Subsequently, all specimens were cleaned ultrasonically in distilled water for 15 seconds, autoclave sterilized for 18 minutes at 1.2 bar/121 °C and stored in distilled water at 37 °C for 24 hours prior to microorganism contamination and adhesion assays [9].

Contamination of denture base material specimens

The origin and morphotype of microorganisms is presented in Table 1.

Preparation of Candida albicans (C. albicans) suspension

C. albicans ATCC 90028 standard strain was cultured on Sabouraud-dextrose agar (SDA) at 37 °C for 48 hours. After 48 hours, the yeast colonies were suspended in 5 mL brain heart infusion broth (BHI). The cell suspension in each tube was adjusted spectrophotometrically to 0.5 McFarland standard (1.6×10^6 CFU/mL). The sterile acrylic specimens ($n = 110$) were placed into the tubes containing 0.5 McFarland standard and incubated at 37 °C for 48 hours.

Preparation of bacterial suspension

Bacterial standard strains (*Staphylococcus aureus (S. aureus)* (ATCC 25923) and *Pseudomonas aeruginosa (P. aeruginosa)* (ATCC 27853)) were cultured on 5% sheep blood agar at 37 °C for 24 hours. After 24 hours, the bacterial colonies were harvested and suspended in 5 mL of BHI broth separately. The cell suspension in each tube was adjusted spectrophotometrically to 0.5 McFarland standard (1.6×10^8 CFU/mL). The sterile acrylic specimens ($n = 110$ for each bacterial suspension) were placed into the tubes containing 0.5 McFarland standard and incubated at 37 °C for 24 hours independently.

Preparation of control group

Six specimens were assessed as an experimental control carried out simultaneously with the treatment groups for each microorganism. The specimens were divided into two groups: negative and positive control ($n = 3$ for each). The positive control group of acrylin resins were cultured with bacteria and yeast separately and not incubated with dentifrices. The negative control group of acrylin resins were not cultured with bacteria and yeasts, rather they were only incubated with sterile BHI broth. Specimens in the control groups were transferred to a sterilized basket and immersed in a container containing saline solution for 24 hours.

Cleaning procedures of dentifrices

Two groups of dentifrices were used for denture cleaning; herbal and non-fluoride dentifrices (Bioplante™, Splat Organic™, Dentistie™, Optme™, Pyorex™, Parodontax™) and fluoride dentifrices (Parodontax Fluoride™, Sensodyne™, Signal™). The particular ingredients included in each dentifrices are presented in Table 2. Dilution of dentifrices was prepared by mixing 1 mg of dentifrice with 1 mL of sterile water. After the preparation of the dentifrices, each acrylic specimens were incubated with dentifrices for 24 h 37 °C. Each acrylic specimen was then washed in a sterile saline solution and transferred to the sterile 5 mL BHI broth tubes. All acrylic specimens were incubated at 37 °C for 24 hours (for bacterial strains) and 37 °C for 48 hours (for yeast strains). Following incubation, a sterile loop was used to transfer and inoculate the suspension on to 5% sheep blood agar for bacterial culturing and SDA for the culturing of yeast.

After the cleaning procedures, the number of colony-forming units per millilitre (CFU/mL) were calculated.

Statistical analysis

Descriptive statistics for each experiment was performed and the data were represented as the mean, standard deviation and median. For each microorganism, the Kruskal–Wallis analysis of variance test was used to compare the effects of the denture-cleaning agents. In the case of statistical significance, the Mann–Whitney *U* test was applied to evaluate pairwise differences. SPSS Software ver. 15.0 (SPSS, Chicago, IL) was used for all calculations. Differences were considered statistically significant at a level of .05.

Results

The median (Min–Max) of each dentifrice, as well as the fluoridated and non-fluoridated dentifrice groups regarding the number of colony forming units (CFU) of microorganisms over a one-hour evaluation period is presented in Tables 3 and 4. Antifungal effects were reported for certain non-fluoridated dentifrices that were approximately equivalent to those containing fluoride. However, fluoridated dentifrices were more effective against *S. aureus* and *P. aeruginosa* than herbal and non-fluoridated dentifrices.

The *p* value matrices of the CFU score pairwise group comparisons of each dentifrices for each three microorganisms are shown in Table 5. There are statistically significant differences between some of the comparisons (Table 5).

The results show that Parodontax Fluoride™, which contains a combination of fluoride and herbal extracts, exhibited higher antimicrobial activity against *S. aureus* than the same formulation containing herbal extracts without fluoride (Parodontax™). However, there is no statistically significant difference between the effectiveness of Parodontax

Table 2. Brand names and ingredients of dentifrices used in the study.

Brand name	Ingredients
Bioplante™, Natrue, Austria	Glycerin, aqua, hydrated silica, *Aloe barbadensis* leaf juice, sodium coco-sulfate, citrus aurantium dulcis peel oil, xanthangum, CI 77891, chondruscrispus extract, citrus bergamia peel oil expressed, *Steviare baudiana* leaf/stem extract, glucose, preserced with sodium benzoate, potassium sorbate, dehydroacetic acid, citric acid, limonene, linalool
Splat Organic™, Organic Pharmaceuticals LTD, Okulovka, Rusya	Sorbitol, Hydrated Silica, Aqua, PEG-8, Sodium Lauryl Sulfate, Aloe Barbadensis Leaf Juice, Calcium Lactate, Glycerin, Aroma, Chitosan, Xanthan Gum, Melaleuca Alternifolia Leaf Oil, Sodium Methylparaben, Papain, Limonene, CI 19140, CI 42090
Dentiste' Whiter Fresh™, Dentiste' Natura Instituto 1912 Dentiste', New York, US	Xylitol, silicone dioxide, peppermint oil, clove oil, menthol, vitamin c, eucalyptus oil, sage extract, chamomile extract(1), fennel extract, glycyrrhiza extract, cinnamon bark extract
AloeDentMiswak™, Optima Health & Nutrition, Bradford, England	Glycerin, sorbitol, hydrated silica, aloe vera, aqua, xylitol, sodium lauroylsarcosinate, peppermint oil, salvadorapersica (miswak) extract, carboxymethyl chitosan, escin, hydroxyethylcellulose, menthol, ubiquinone, tea tree oil, sodium hydroxymethylglycinate, citric acid, CI75810(colour), limonene
Pyorex™, Laboratoire Bailly-Creat, Vernouillet, France	Ethacridine lactate, sodium ricinoleate, mint oil, colouring E127, excipient QS, sodium parahydroxybenzoate
Parodontax™, GlaxoSmithKline, London, England	Sodium bicarbonate, aqua, glycerin, cocamidopropyl betaine, alcohol, *Krameria triandra* extract, *Echinacea purpurea* flower/leaf/steam juice, alcohol denat, xanthan gum, *Chamomilla recutita* extract, *Commiphora myrrha* extract, sodium saccharin, sodium benzoate, salvia officinalis oil, menthe piperita oil, menthe arvensis oil, limonene, CI77491
ParodontaxFluoride™, GlaxoSmithKline, London, England	Sodium bicarbonate, aqua, glycerin, cocamidopropyl betaine, alcohol, *Krameria triandra* extract, *Echinacea purpurea* flower/leaf/steam juice, alcohol denat, xanthan gum, *Chamomilla recutita* extract, *Commiphora myrrha* extract, sodium saccharin, sodium benzoate, salvia officinalis oil, menthe piperita oil, menthe arvensis oil, limonene, CI77491, sodium fluoride
Sensodyne™, GlaxoSmithKline, London, UK	Aqua, sorbitol, hydrated silica, glycerin, potassium nitrate, cocamidopropyl betaine, aroma, xanthan-gum, titanium dioxide, sodium fluoride, sodium saccharin, sodium hydroxide, sucrolase, limonene
Signal™, Unilever, Paris, France	Calcium carbonate, aqua, sorbitol, hydrated silica, sodium laurylsulfate, sodium monofluorophosphate, aroma, cellulosegum, potassium citrate, trisodium phosphate, sodium saccharin, calcium glycero-phosphate, phenylcarbinol, glycerin, limonene, CI 12490

Table 3. Median (min–max) in number of colony-forming units (CFU) of microorganisms over the evaluation period of 1 h.

		Microorganism median (Min–Max)		
	Cleaning agent	*C. albicans*	*S. aureus*	*P. aeruginosa*
Herbal and non-fluoride dentifrices	Bioplante	10^5 (10^4–10^6)	10^6 (10^4–10^8)	10^6 (10^5–10^8)
	Splat Organic	10^5 (10^4–10^6)	10^6 (10^4–10^8)	10^6 (10^5–10^8)
	Dentistie	10^5 (10^4–10^6)	10^7 (10^6–10^8)	10^6 (10^5–10^8)
	Optme	10^5 (10^3–10^6)	10^5 (10^3–10^6)	10^7 (10^5–10^8)
	Pyorex	10^5 (10^3–10^6)	10^7 (10^6–10^8)	10^7 (10^5–10^8)
	Parodontax	10^5 (10^3–10^6)	10^7 (10^5–10^8)	10^7 (10^2–10^8)
Fluoride dentifrices	ParodontaxFluoride	10^4 (10^2–10^6)	10^5 (10^3–10^8)	10^5 (10^3–10^8)
	Sensodyne	10^4 (10^3–10^5)	10^5 (10^3–10^8)	10^5 (10^4–10^8)
	Signal	10^6 (10^5–10^7)	10^7 (10^6–10^8)	10^6 (10^4–10^8)

Table 4. Median (min–max) in number of colony-forming units (CFU) of microorganisms in the two groups and *p* values for each of the three microorganisms.

	C. albicans	*S. aureus*	*P. aeruginosa*
Herbal and non-fluoride	10^5 (10^3–10^6)	10^7 (10^4–10^8)	10^7 (10^4–10^8)
Fluoride	10^5 (10^2–10^7)	10^6 (10^3–10^8)	10^6 (10^2–10^8)
	$p = .088$	$p = 0$	$p = 0$

Fluoride™ and Parodontax™ against *C. albicans* and *P. aeruginosa*.

Discussion

Inadequate denture hygiene habits lead to biofilm accumulation on the surfaces of removable dentures. Dentures have been shown to support the growth of biofilms known as denture plaque, which are complex polymicrobial consortia of bacteria, and yeasts [10]. The use of adequate brushing with auxiliary dentifrice is essential for removing denture plaque and therefore obtaining denture hygiene.

The flora of denture consists not only of normal oral flora but also an unexpected spectrum of pathogenic and opportunistic micro-organisms including a wide range of gram-negative bacteria, gram-positive bacteria and yeasts. *C. albicans*, *S. aureus* and *P. aeruginosa* (gram-negative) are some of the micro-organisms evaluated from the flora in previously worn prostheses [11,12].

In the present study, seven different brands of commonly used herbal and non-fluoride dentifrices were investigated. Of the various herbal dental products considered, Parodontax Fluoride™ (GlaxoSmithKline, Middlesex, UK) has received great attention. It is composed of herbal ingredients with fluoride (1400 ppm) whereas many other herbal dentifrices have no fluoride. The comparison of the antimicrobial activity of Parodontax Flouride™ with Parodontax™ should be the focus of our further studies and to assess the effects of fluoride. The two dentifrices have the same ingredients except fluoride. Therefore, it may be concluded that the form of fluoride found in the dentifrice has an antimicrobial effect against *S. aureus*.

Prodontax Fluoride™, Sensodyne™ and Signal™ were dentifrices investigated in the current study and all contain sodium fluoride [13–15]. Various studies have concluded that fluoride acts as a major antimicrobial ingredient.

Table 5. *p* Value matrixes of CFU score pairwise group comparisons for each of the three microorganisms.

	Bioplante	Splat Organic	Dentistie	Optme	Pyorex	Parodontax	Parodontax Fluoride	Sensodyne	Signal
C. albicans									
Bioplante	1.00	0.62	0.78	0.53	0.95	0.21	0.01[a]	0.05[a]	0.00[a]
Splat Organic		1.00	0.89	0.26	0.67	0.08	0.00[a]	0.01[a]	0.01[a]
Dentistie			1.00	0.39	0.75	0.15	0.00[a]	0.03[a]	0.01[a]
Optme				1.00	0.63	0.54	0.02[a]	0.18	0.00[a]
Pyorex					1.00	0.31	0.01[a]	0.11	0.01[a]
Parodontax						1.00	0.05	0.49	0.00[a]
Parodontax Fluoride							1.00	0.10	0.00[a]
Sensodyne								1.00	0.00[a]
Signal									1.00
S. aureus									
Bioplante	1.00	0.04[a]	0.02[a]	0.04[a]	0.05	0.38	0.10	0.22	0.04[a]
Splat Organic		1.00	0.83	0.86	0.78	0.12	0.00[a]	0.00[a]	0.73
Dentistie			1.00	0.70	0.60	0.08	0.00[a]	0.00[a]	0.52
Optme				1.00	0.89	0.17	0.00[a]	0.00[a]	0.80
Pyorex					1.00	0.18	0.00[a]	0.00[a]	0.97
Parodontax						1.00	0.01[a]	0.04[a]	0.17
Parodontax Fluoride							1.00	0.66	0.00[a]
Sensodyne								1.00	0.00[a]
Signal									1.00
P. aeruginosa									
Bioplante	1.00	0.45	0.10	0.06	0.12	0.47	0.01[a]	0.07	0.24
Splat Organic		1.00	0.22	0.01[a]	0.02[a]	0.86	0.01[a]	0.14	0.54
Dentistie			1.00	0.01[a]	0.01[a]	0.47	0.09	0.54	0.63
Optme				1.00	0.75	0.03[a]	0.00[a]	0.01[a]	0.01[a]
Pyorex					1.00	0.05	0.00[a]	0.01[a]	0.02[a]
Parodontax						1.00	0.06	0.30	0.76
Parodontax Fluoride							1.00	0.41	0.05[a]
Sensodyne								1.00	0.36
Signal									1.00

[a]Difference between the groups.

However, sodium fluoride is an inorganic compound of the naturally occurring, highly poisonous fluorine gas. It is manufactured by neutralizing hydrofluoric acid or hexafluoride silicic acid, which are by-products of fertilizer production and heavy metal manufacturing [16]. *Fluoride* when taken at high doses is detrimental to health. The science clearly demonstrates that fluoride is a toxic chemical that accumulates in the tissues over time, wreaks havoc with enzymes, and produces a number of serious adverse health effects, including neurological and endocrine dysfunction. It is well known that a degree of manual dexterity is often lacking among older patients in particular [17], which suggests that rinsing fluoride from dentures may not always be satisfactory. Therefore, herbal and non-fluoride dentifrices can be recommended for the elderly. In several studies, it has been suggested that ingredients of herbal or botanical origins could be of use as alternative or adjunctive active ingredients in oral hygiene regimes due to their antiplaque or other beneficial properties [17–20]. In some studies, antifungal effects were reported for certain herbal dentifrices that were approximately equivalent to those containing fluoride [6,11]. Our findings are in agreement with these other studies [21,22].

It was evaluated that the effectiveness of fluoridated dentifrices was comparatively better than the herbal and non-fluoride dentifrices against *Staphylococcus aureus* and *P. aeruginosa*. It was indicated that dentures could act as a reservoir for potential respiratory pathogens such as *S. aureus* and *P. aeruginosa* in the oral cavity, thus increasing the theoretical risk of developing aspiration pneumonia [10]. Therefore, it is essential to remove the microorganisms from dentures to help reduce the risk of respiratory infection among the elderly population.

Conclusions

Herbal and non-fluoride dentifrice may be used as an alternative denture cleaning agent. It may be recommended for the elderly to use fluoride dentifrice, only if their manual dexterity is sufficient for rinsing the fluoride remnants from the denture.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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