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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Antifungal activity of toothpastes against oral *Candida* isolates

Activité antifongique de six dentifrices contre diverses espèces de *Candida*

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Abstract Many types of toothpaste have been formulated over recent years to contain antimicrobial compounds with the aim of preventing or reducing plaque, calculus, gingivial inflammation or dental caries. Different brands have their own composition and concentration of ingredients for their efficacy. In this study, the anticandidal activity of six different toothpastes containing the following active ingredients: sodium fluoride, sodium monofluorophosphate, triclosan and herbal compounds were investigated against eight *Candida* species [*Candida glabrata* ($n = 35$), *Candida albicans* ($n = 25$), *Candida krusei* ($n = 10$), *Candida tropicalis* ($n = 9$), *Candida pseudotropicalis* ($n = 7$), *Geotrichum candidum* ($n = 7$), *Candida lipolytica* ($n = 5$), and *Candida lusitaniae* ($n = 4$)], isolated from oral cavities and denture using the agar-diffusion test. Five toothpastes were effective in inhibiting the growth of all *Candida* species, while one toothpaste was not effective against *C. tropicalis*, *C. pseudotropicalis* and *C. lusitaniae*. The herbal toothpaste exhibited good antifungal activity against all *Candida* species.

Résumé Récemment, plusieurs sortes de dentifrices contenant des substances antimicrobiennes ont été mises sur le marché pour prévenir ou réduire la plaque dentaire, l'inflammation gingivale ou les caries dentaires. Chaque marque possède sa propre composition, en vue de son efficacité. Le but de ce travail est d'analyser, par la technique de diffusion en gélose, l'activité antifongique de six dentifrices dont les principaux composés actifs sont le fluorure de sodium, le monofluorophosphate de sodium, le triclosan et diverses subtances végétales contre huit

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espèces de levures : *Candida glabrata* (*n* = 35), *Candida albicans* (*n* = 25), *Candida krusei* (*n* = 10), *Candida tropicalis* (*n* = 9), *Candida pseudotropicalis* (*n* = 7), *Geotrichum candidum* (*n* = 7), *Candida lipolytica* (*n* = 5) et *Candida lusitaniae* (*n* = 4), isolées de la cavité buccale et de prothèses dentaires. On observe qu'un dentifrice est inefficace contre *C. tropicalis*, *C. pseudotropicalis* et *C. lusitaniae*, alors que les cinq autres inhibent la croissance des différentes souches testées. Le dentifrice contenant des substances végétales a montré la meilleure activité antifongique contre toutes les espèces testées.

Introduction

Toothpaste has a history that stretches back nearly 4000 years. Different abrasives, green lead, incense were used to clean stain from teeth until mid-nineteenth century. In middle ages, fine sand and pumice were the primary ingredients in the tooth-cleaning formulas used by Arabs. In 1950 AD, Dr.Washington Wentworth Sheffield, a dental surgeon and chemist, invented the first toothpaste [34]. The regular formulation of modern toothpastes contains abrasive agents, detergents, humectants, thickening agents, flavoring and coloring agents and antimicrobial agents [1]. Currently formulated antimicrobial agents include metal salts (e.g., zinc, stannous, copper), phenols (triclosan), plant extracts (sanguinarine), enzymes (e.g., glucanase, amyloglucosidase/glucose oxidase), essential oils (e.g., thymol, menthol), and bisbiguanides (chlorhexidine). Antimicrobial agents can be used to maintain plaque at levels compatible with oral health by reducing existing plaque, preventing the formation of new plaque, selectively inhibiting those particular bacteria that are associated with disease, and inhibiting the expression of virulence determinants [13,19,22].

Yeasts are opportunistic pathogens, that is, they may cause infections if the enviroment becomes favourable. Oral candidosis is a common opportunistic infection, both in medically compromised and otherwise healthy individuals. Although *C. albicans* is the major pathogen in oral candidosis, infections with *Candida* species other than *C. albicans*, such as *C. krusei*, *C. glabrata* and *C. dubliniensis* have been increasingly described both in compromised and non-compromised hosts. *C. albicans* can cause mucosal infections, such as stomatitis, especially, in denture-wearers. Acrylic dentures play an important role by increasing the risk of candida colonisation, by acting as reservoirs of the microorganisms. *Candida* species has been found in plaque and saliva [14,20,37].

Effective antimicrobial agents are used in toothpaste to produce an inhibitory action on plaque formation and *Candida* colonisation [9,23]. In this study, the anticandidal activity of toothpastes containing antimicrobial agents and herbal ingredients were investigated by in vitro againts oral and denture *Candida* isolates.

Materials and methods

Yeast strains

The study included 102 *Candida* strains, isolated from oral cavity of healthy adults (48 female and 54 male), ages between 24 and 62 years, who were undergoing dental treatment. The clinical specimens were collected with a swab, from saliva, tongue dorsum, palate mucosa, dental biofilm and dentures. They were immersed in 1 ml sterile saline and the tubes were then agitated for 30 s. Aliquots of 100 μl from the clinical specimens were immediately diluted 10-fold and 100-fold in sterile saline and aliquots of 100 μl from each dilution, in duplicate, were inoculated in plates containing Sabouraud dextrose agar (Merc/Germany) added of 0.1 mg/ml chloramphenicol and incubated aerobically at 37 °C for 48 h. After incubation, the isolates were identified by standard-taxonomic procedures (including germ-tube production, typical microscopic appearances on cornmeal agar with Tween-80, the production of chlamydospores, colony morphology and pigment production on chromogenic medium) and the identification was confirmed by the API 32 C AUX (bioMerieux, Marcy-l'Etoile, France) identification system for yeasts.

Toothpastes tested for antifungal activity

Six different toothpastes were tested against oral *Candida* spp. for their antifungal activities. Toothpastes were purchased from local market. The toothpastes and ingredients were listed in Table 1.

Agar-diffusion tests

Yeast isolates were subcultured onto Sabouraud dextrose agar and were incubated at 37 °C for 48 h. Colonies from 48 h cultures were suspended in 5 ml of a sterile-saline solution. The turbidity of the inoculum was adjusted to the turbidity of a McFarland 1 standard and 0.1 ml of the inoculum was dropped on the agar and spread to whole surface by sterile glass applicator. Then, six wells, each in 4 mm depth and 6 mm diameter, were punched on the agar surface with equal distance from each other. Each well was filled with 0.1 mg different toothpaste. Agar plates were incubated at 37 °C for 48 h and the diameter of the zones of inhibition were measured and recorded [17,33] (Figure 1). Fluconazol discs (25 μg, Oxoid) were used as positive control in this study.

Statistical analysis

Statistical analyses were performed using SPSS version 13.0. To identify significant differences, the data were analyzed by analysis of variance using the general-linear model that permits the analysis of sample with different number of replicates. The mean values were separated by Duncan's multiple range test.

Table 1 Toothpastes ingredients.
Composition chimique des différents dentifrices.

Toothpastes	Ingredients as listed on packages
Toothpaste 1	Aqua, sorbitol, hydrated silica, peg-32, sodium lauryl sulfate, titanium dioxide, cellulose gum, zinc citrate, triclosan, sodium fluoride (0,145% fluoride), sodium saccharin, mica, Cl77491
Toothpaste 2	Dicalcium phosphate dihydrate, aqua, glycerin, aluminum hydroxide, sorbitol, sodium citrate, aroma, sodium monofluorophosphate, sodium lauryl sulfate, papain, sodium carrageenan, cocamidopropyl betaine, citric acid, sodium saccharin, methyparaben limonene, Cl42090, Cl19140, natrium monofluorophosphate
Toothpaste 3	Potassium nitrate 5%, sodium fluoride 0,306%, saccharin sodium, sorbitol, titanium dioxide
Toothpaste 4	Sodium bicarbonate, aqua, glycerin, cocamidopropyl betaine, alcohol, *Krameria triandra, Mentha piperita, Mentha arvensis, Echinacea purpurea, Commiphora myrrha, Chamomilla recutita, Salvia officinalis*, xanthan gum, sodium saccharin, Cl77491
Toothpaste 5	Potassium citrate 5,53%, sodium monofluorophosphate 1,14%, sorbitol, aqua, hydrated silica, glycerin, potassium citrate, peg-12, sodium lauryl sulfate, aroma, cellulose gum, sodium saccharin, xanthan gum, limonene, eugenol, Cl42051, Cl77891
Toothpaste 6	Sodium fluoride 0,22%, sorbitol, aqua, hydrated silica, sodium lauryl sulfate, peg-12, aroma, tetrasodium pyrophosphate, cocomidopropyl betaine, sodium saccharin, cellulose gum, sodium fluoride, xanthen gum, hydroxypropyl methylcellulose, menthol, glycerin, limonene, Cl19140, Cl42090, Cl77891

Results

In this study, 102 *Candida* strains were isolated from various oral site and denture of healthy adults and were identified as 35 *C. glabrata*, 25 *C. albicans*, 10 *C. krusei*, nine *C. tropicalis*, seven *G. candidum*, seven *C. pseudotropicalis*, five *C. lipolytica*, and four *C. lusitania*. Fifty-eight of these strains were oral-cavities isolates and 44 were denture isolates.

The anticandidal activities of six different toothpastes were investigated against these *Candida* species using the agar-diffusion test. The highest activity was detected from Toothpaste 4 and Toothpaste 5 was least effective compared with the other test-toothpaste. Activity of test-toothpastes was in decreasing order: Toothpaste 4 greater than Toothpaste 3 greater than Toothpaste 6 greater than Toothpaste 2 greater than Toothpaste 1 greater than Toothpaste 5 (Figure 1 and Table 2).

Figure 1 Inbition zones of different toothpaste against *C. tropicalis* on agar plates inoculated at 35 °C for 48 h. 1: Toothpaste 1; 2: Toothpaste 2; 3: Toothpaste 3; 4: Toothpaste 4; 5: Toothpaste 5; 6: Toothpaste 6.
La zone d'inhibition des différents dentifrices contre C. tropicalis *sur la plaque agar inoculée à 35 °C pour 48 heures. 1 : dentifrice 1 ; 2 : dentifrice 2 ; 3 : dentifrice 3 ; 4 : dentifrice 4 ; 5 : dentifrice 5 ; 6 : dentifrice 6.*

Concerning the anticandidal activity of different toothpastes, there was not a statistically difference ($p > 0.05$) among isolation origins. All of the toothpaste had a similar anticandidal activity against oral-cavity and denture-isolates groups (Table 2). Five of the six toothpastes were effective in inhibiting the growth of all *Candida* species tested in this study. There was a statistically-significant difference ($p < 0.001$) among the anticandidal activities of toothpastes on *Candida* spp. (Table 2).

Discussion

In everybody's life one of the day-to-day chores is plaque removal with a toothbrush using dentifrice. Plaque control means the removal of microbial plaque and the prevention of its accumulation on the teeth and adjacent-gingival surfaces to prevent tooth decay and periodontal disease. By fallowing a proper technique in brushing and using other hygiene aids like floss, toothpaste and mouthrinse, one can effectively maintain good-oral hygiene [7]. Toothpaste, in general, helps in cleaning and polishing tooth surfaces. Besides, toothpastes are now used as vehicles to incorporate chemotherapeutic agents, which can inhibit plaque, calculus, caries or root hypersensitivity. Research studies on antibacterial activity of toothpastes have been focused on their potential to inhibit bacterial growth in the mouth. Little information concerning their antifungal potentials was found in recent literature research [1].

This study was designed to compare the in vitro anticandidal activities of six different thoothpastes against 102 clinically important *Candida* isolates, which may cause oral candidosis in humans. They included 35 *C. glabrata*, 25 *C. albicans*, 10 *C. krusei*, nine *C. tropicalis*, seven

Table 2 Antifungal activity of six toothpastes againts *Candida* species.
Activité antifongique de six dentifrices contre diverses espèces de levures.

Mean diameter of inhibition zones (mm)									
Candada origins	Toothpaste	*C. glabrata*	*C. albicans*	*C. krusei*	*C. tropicalis*	*C. pseudo-tropicalis*	*G. candidum*	*C. lipolytica*	*C. lusitaniae*
Oral-cavities isolates	Toothpaste 1	12.5d[a]	14.6b	10d	12b	15c	15b	15b	10b
	Toothpaste 2	17c	10.6c	20c	10bc	10c	10b	10c	10b
	Toothpaste 3	24.7b	13.6b	30b	9c	20c	15b	10c	10b
	Toothpaste 4	42.7a	38a	48a	48a	35b	37.5a	40a	50a
	Toothpaste 5	—	2.6d	—	—	—	—	—	—
	Toothpaste 6	14.2d	12.6c	22c	10c	10c	15b	10c	10b
Denture isolates	Toothpaste 1	18bc	14b	10d	12.5b	10c	10b	15b	10b
	Toothpaste 2	19.3b	11c	21c	12.5b	15c	10b	10c	10b
	Toothpaste 3	16.6c	12c	30b	7.5c	10c	16.6b	10c	10b
	Toothpaste 4	42.6a	37a	48a	47.5a	50a	36.6a	40a	50a
	Toothpaste 5	4.6e	2.5d	—	—	—	—	3.3d	—
	Toothpaste 6	16c	12c	22c	12.5b	10c	15b	10b	10b
	Fluconazole	21.7	39.7	17.4	37.7	35.8	34.8	37.7	34.8

—: no activity.
— : *inactive*.
[a] Within columns, the statistical difference between two means is given by the letters a, b, c, d and e. If the letters are different the difference is significant ($p < 0.001$) by Duncan test.[a]À l'intérieur de chaque colonne, la difference statistique entre deux moyennes est donnée par les lettres a, b, c, d et e. Si les lettres sont différentes la différence est significative ($p < 0{,}001$) par le test de Duncan.

G. candidum, seven *C. pseudotropicalis*, five *C. lipolytica*, and four *C. lusitania*.

The highest activity was detected from Toothpaste 4 with 50 mm inhibition zones against *C. pseudotropicalis and C. lusitaniae*, whereas the lowest activities were expressed by Toothpaste 5 against all *Candida* spp. with inhibition zones ranging from 0 to 4.6 mm (Table 2).

Toothpaste 1 was effective in inhibiting the growth of all *Candida* species and produced wider inhibition zones againts oral isolates of *C. pseudotropicalis*, *G. candidum*, *C. lipolytica*, and dentures isolates of *C. glabrata* and *C. lipolytica* (Table 2). Active ingredients of Toothpaste 1 were zinc citrate, triclosan and sodium fluoride (0,145%). Triclosan [5-chloro-2-(2,4-dichlorophenoxy) phenol] has been used for more than 30 years as a general antibacterial and antifungal agent, which is found in formulations toothpastes and it has recently been suggested that triclosan blocks lipid biosynthesis by specifically inhibiting the enzyme enoyl-acyl carrier protein reductase (ENR). Zinc too has been used in a number of products, having substantivity in the mouth and having known effects on bacterial enzymes and metabolism. The combination of the two antimicrobial agents has been shown to have the advantage that additive or synergistic interactions can occur resulting in increased clinical benefits [2,6,8,11,25—27,30].

Toothpaste 2 showed high-inhibition zone against *C. krusei* and *C. glabrata* 19,3 and 21 mm, respectively. Also, there was a good anticandidal activity detected from Toothpaste 3 and Toothpaste 6 against tested *Candida* spp., especially *C. krusei* with 30 and 22 mm inhibition zone, respectively.

Toothpaste 2, Toothpaste 3, and Toothpaste 6 contained sodium monofluorophosphate and sodium fluoride as active ingredients. Fluorides are essential in caries prevention. When formulated correctly and used as directed, fluoride toothpaste will help to safely and effectively prevent tooth decay. Numerous clinical studies have demonstrated fluoride's effectiveness in reducing cavities. Fluoride helps diminish demineralization of tooth enamel and even enhances the remineralization of potential-decay spots. However, based on a variety of mechanisms, fluorides also demonstrate some antibacterial and antifungal effects, such as metabolic interference and reduction of dental plaque acidogenicity [3,21,29,35].

The Toothpaste 4, containing herbal components, exhibited good antifungal activity, thereby, confirming the medicinal value of plant products. It is composed of sodium bicarbonate and herbal ingredients: *Chamomilla* (*Chamomilla recutita*) which is supposed to have anti-inflammatory properties and to decrease gingivial inflammation; *Echinacea* (*Echinaceae purpurea*) which is reputed to stimulate the immun response; sauge (*Salvia officinalis*) and rhatania (*Krameria triandra*) which have antihemmorhagic effect; myrrhe (*Commiphora myrrha*) claimed to be a natural antiseptic and peppermint oil which has analgesic, antiseptic and anti-inflammatory properties [28]. In addition to these effects, chamomille, *Echinacea*, peppermint oil, rhatany and sage are known to have antifungal activity [4,10,15,31]. Our data are in accordance with the literature knowledge above. Sodium bicarbonate (baking soda) is added for taste and mouth feels. It is a mild abrasive. It may reduce the numbers of acid loving bacteria in the mouth, although this effect lasts only as long as the mouth stays alkaline. Sodium saccharin and xanthan gum are other ingredients in Toothpaste 4 and sodium saccharin are added as sweeteners, xanthan gum is used to thicken the paste. These components have no antimicrobial activity [12,24].

The last one, Toothpaste 5, was least effective compared with the other test-toothpaste, which may be due to the ingredients in it. This paste contained only sodium monofl-

uorophosphate as an active indegrents. Caries reduction by sodium fluoride was 6.4% higher than by sodium monofluorophosphate [16,32].

Active compounds of toothpastes, besides reducing cariogenic microorganisms, along with other compounds in the paste formula (peroxides, silica, pyrophosphates and polymers, baking soda, chlorides and nitrates, detergens and surfactants), helps to strengthen the teeth by reducing demineralization and increasing remineralization of the teeth. Toothpastes detergents, such as sodium lauryl sulphate (SLS) have a variety of functions, including, almost by definition, the removal of organic material on the tooth surfaces. Additionally, SLS has antimicrobial effects, which extend over several hours in the mouth and a moderate plaque-inhibitory action. The aqueous and viscous vehicles, such as water, saline, anesthetic, glycerine and polyethyleneglycol have no antimicrobial action [12,24]. Pottasium nitrate provides effective desensitization [36]. Essential oils, such as eucalyptol, menthol and thymol, frequently used for flavoring in oral products, can also contribute to the antiseptic properties of these products [5]. However, detergents and abrasives may alter the substantivity or the antimicrobial activity of active ingredients.

Interest in alternative mouthrinses and toothpastes based on plant extracts has increased recently. Dentifrices labeled as ''natural'' typically do not include ingredients, such as synthetic sweeteners, artificial colors, preservatives, additives or synthetic flavors and fragrances. The products that herbal compounds make antibacterial often claim to do so under the guise of killing germs that cause breath or reducing gingivitis. To validate such claims, however, researchers must evaluate and determine the level of antimicrobial activity [18].

In conclusion, the herbal toothpaste presented significant-anticandidal activity over the conventional toothpaste with fluoride and triclosan and it can be used as an alternative to conventional formulations for individuals with an interest in naturally-based products.

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