A Comparison of the Antifungal Activity of Herbal Toothpastes against Other Brands of Toothpastes on Clinical Isolates of *Candida albicans* and *Candida dubliniensis*

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Key Words

Candida · Toothpaste · Antifungal effect

Abstract

Objectives: To evaluate the anti-*Candida* activity on *Candida albicans* and *Candida dubliniensis* species of 2 herbal and 7 other brands of toothpastes commonly used in Kuwait. **Materials and Methods:** Antifungal activity was determined by agar diffusion test on 65 isolates of *C. albicans* and 21 isolates of *C. dubliniensis* for each toothpaste. A uniform quantity of toothpaste was filled into wells punched into Sabouraud dextrose agar medium plates inoculated with the test isolates, incubated at 37°C; inhibition zone diameters were read after 24 h. **Results:** The mean inhibition zone diameters ranged between 12 and 23 mm for *C. albicans* and between 12 and 27 mm for *C. dubliniensis*. A herbal toothpaste brand manufactured in the Middle Eastern region (United Arab Emirates) consisting of many herbal ingredients compared to other brands was found to be the most active ($p < 0.001$) against both *Candida* species tested, which also demonstrated higher inhibitory activity against *C. dubliniensis* isolates compared to *C. albicans*. **Conclusions:** The herbal toothpaste brand presented significant anticandidal activity over conventional toothpastes and may be useful in reducing the pathogenic potential of *Candida* species.

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Introduction

The oral cavity is known to harbor a large number of microorganisms which coexist with one another as normal microbiota. Among *Candida* species, *Candida albicans* is the predominant species normally occurring in the oral cavity [1]. However, under compromised conditions, colonizing *C. albicans* may give rise to different pathologic conditions ranging from acute pseudomembranous thrush to more chronic forms which may persist for a longer period despite treatment [2]. A number of factors such as immunosuppression, antibiotic/radiation therapy, nutritional deficiencies, dental prostheses, high carbohydrate diet, diabetes mellitus, heavy cigarette smoking or poor oral hygiene, which tend to enhance *Candida* colonization or infection, have been identified [2]. *Candida dubliniensis* is closely related to *C. albicans* in evolutionary terms, sharing its properties of commensalism and opportunistic infection. A recent study has shown that there is genomic similarity between these two species as well [3]. In addition to *C. albicans*, there is current evidence to suggest that *C. dubliniensis* isolates are also involved in the pathogenesis of oral candidal infection in many clinical scenarios [4, 5]. Further evidence suggests that *C. dubliniensis* can disseminate to other sites as it has been isolated from urine and blood specimens [6, 7].

In recent years, many brands of toothpastes containing different formulations have become available claiming to improve oral hygiene, reduce plaque, gingival inflammation or dental caries [8]. Despite the wide spectrum of antibacterial activity of different toothpaste formulations [8], information on their antifungal properties is scarce [9, 10], and none of these studies have determined the anti-candidal activity of toothpastes on *C. dubliniensis* isolates. Hence, we investigated the anti-*Candida* activity on oral as well as nonoral *C. albicans* and *C. dubliniensis* isolates of 2 toothpastes consisting of herbal extracts and compared these 2 with 7 other brands of toothpastes commonly used in Kuwait.

Materials and Methods

Ethical clearance was granted by the Health Sciences Center Ethical Clearance Committee, Faculty of Medicine, Kuwait University. Like other *Candida* species, *C. dubliniensis* is part of microbiota with a preference to colonize the oral cavity, has a potential to spread to other anatomic sites and may even cause bloodstream or disseminated infection [6, 7]. For this reason, we also included *C. dubliniensis* and *C. albicans* isolates from sources other than oral cavities, namely blood and urine, in our study.

Isolation and Identification

A total of 86 isolates of *Candida* species recovered from various clinical specimens and identified by phenotypic and molecular characteristics were included in the study. The specimens used in the study included 49 oral isolates of *C. albicans* out of 65 isolates and 20 oral isolates of *C. dubliniensis* out of 21 isolates. The rest of the non-oral isolates were from blood and urine. All the yeast isolates were tested for germ tube formation. Thereafter, the colony characteristics were observed using CHROMagar *Candida* medium (Becton Dickinson and Company, Sparks, Md., USA) and were provisionally classified as *C. albicans*. All the isolates were further identified by the Vitek 2 yeast ID system (bioMérieux). The identity of *C. dubliniensis* was confirmed by production of rough colonies with hyphal fringes and chlamydo spores on simplified sunflower seed agar [11]. The species-specific identity was investigated by seminested PCR amplification of the internal transcribed spacer-2 region of rDNA and further confirmed by direct DNA sequencing of the internal transcribed spacer region of the rDNA as described previously [12].

Susceptibility Testing

Nine different brands of toothpaste (table 1) were purchased from the local market. The particulars of ingredients included in each toothpaste formulation by the manufacturers are provided in table 1. Each brand has been identified by serial number, brand name and country of production. The antifungal activity of the toothpastes was determined by agar diffusion test. Growth from freshly subcultured isolates was suspended in 5 ml of sterile saline to obtain a turbidity of 0.5 McFarland standard. Using a sterile swab, the Sabouraud dextrose agar plates (Difco TM, Becton Dickinson and Company), each containing 60 ml of medium,

Fig. 1. Inhibition zones of different toothpastes (numbered 1–9) against a *C. dubliniensis* isolate on a Sabouraud agar plate. Note the maximum zone of inhibition with toothpaste No. 8.

were evenly inoculated with the yeast suspension. A uniform quantity of each toothpaste preparation was filled into 6-mm diameter wells punched into the pre-inoculated plates. The plates were incubated at 37°C and readings for inhibition zone diameters were taken after 24 h. Measurement of the inhibition zone was read independently by 1 coauthor (R.C.) and 2 other laboratory microbiologists who were unaware of the type of coded toothpaste filled into the 6-mm diameter wells. Each *Candida* isolate was tested separately for each of the 9 toothpaste brands.

Statistical Analysis

Statistical analysis was done by one-way ANOVA and the Bonferroni multiple comparison test to compare the inhibitory effect of different brands of toothpaste on *Candida* species and to compare the inhibitory effect among different brands of toothpaste. A p value of <0.05 was considered significant. However, for illustration, the results are presented as means and standard deviations.

Results

The inhibition zones observed for one such *C. dubliniensis* isolate against all 9 toothpaste brands used in the study are shown in figure 1. Different toothpaste brands showed varying inhibitory activity against the two *Candida* species tested (table 2). The mean inhibition zone diameters of the 9 toothpaste brands ranged between 12

Table 1. Ingredient details of formulations of different brands of toothpaste tested for anti-*Candida* activity

Serial No.	Brand name and country of manufacture	Ingredients used in different brands of toothpaste
1	Signal, Saudi Arabia	sorbitol, water, hydrated silica, PEG-32, sodium lauryl sulfate, flavor, cellulose gum, sodium fluoride, sodium saccharin, PEG-40, hydrogenated castor oil, phenoxyethanol, glycerin, limonene, CI 19140, CI 42090, CI 74260
2	Elgydium, France	chlorohexidine digluconate 0.004 g, calcium carbonate 25 g, sodium lauryl sulfate, glycerol, colloidal silica, amorphous hydrated precipitated silica, carrageenate, sodium carboxymethyl cellulose, titanium oxide, methyl parahydroxybenzoate, propyl parahydroxybenzoate, saccharin sodium, artificial flavoring, water
3	Colgate, Saudi Arabia	sodium monofluorophosphate 0.76%, sodium fluoride 0.1%, dicalcium phosphate dihydrate, aqua, sorbitol, glycerin, PEG-12, sodium lauryl sulfate, aroma, cellulose gum, sodium monofluorophosphate, tetrasodium pyrophosphate, sodium saccharin, sodium fluoride, limonene; contains no sugar
4	Aquafresh, UK	sodium fluoride 0.306% w/w, aqua, hydrated silica, sorbitol, glycerine, PEG-6, sodium lauryl sulfate, aroma, xanthan gum, titanium dioxide, carrageenan, sodium fluoride, sodium saccharin, limonene, CI 73360, CI 74260, CI 74160
5	Sensodyne, UK	strontium chloride hexahydrate 10%
6	Close up, Saudi Arabia	sodium fluoride 0.32%, triclosan, sorbitol, silica, water, PEG-32, sodium lauryl sulfate, mix of flavors, cellulose gum, sodium saccharin, tocopheryl acetate, CI 47005, CI 42090
7	Miswak Herbal, United Arab Emirates	calcium carbonate, sorbitol, treated water, silica, sodium lauryl sulfate, flavor, miswak extract, sodium carboxymethyl cellulose and/or sodium carrageenate, sodium silicate, sodium benzoate, glycerine, sodium saccharin
8	Dabur Herbal, United Arab Emirates	sodium fluoride 0.32%, sorbitol, triclosan 0.20%, treated water, silica, polyethylene glycol 1500, sodium lauryl sulfate, miswak extract, neem extract, lemon extract and/or lemon oil, flavor containing mint, clove, basil and other natural oils, glycerine, titanium dioxide, sodium carrageenate and/or sodium carboxymethyl cellulose, trisodium orthophosphate, sodium saccharin, citric acid, FD&C Yellow No. 5, FD&C Blue No. 1
9	Salz F, Japan	sodium chloride, dipotassium glycyrrhizinate, sorbitol, propylene glycol, sodium lauryl sulfate, calcium carbonate, ethyl paraben, butyl paraben, sugar-free

and 23 mm for *C. albicans* ($p < 0.001$) and between 12 and 27 mm for *C. dubliniensis* ($p < 0.001$). Based on inhibition zone diameter, the toothpaste which contained as its ingredients many herbal extracts from the miswak tree (*Salvadora persica*/arak tree), neem tree (*Azadirachta indica*), cloves (*Syzygium aromaticum*), basil (*Ocimum basilicum*) and some other natural oils (No. 8) was the most active against the two *Candida* species. This brand showed a higher inhibitory activity against *C. dubli-*

niensis (27 ± 3.7 mm) than against *C. albicans* (23 ± 3.9 mm). A nonherbal toothpaste (No. 9) also showed a higher inhibitory activity against *C. albicans* (20 ± 4.1 mm). However, the inhibitory effect of this brand against *C. dubliniensis* was much lower when compared to the herbal brand (18 ± 3.6 vs. 27 ± 3.7 mm). The inhibitory effect of a nonherbal toothpaste containing only strontium chloride hexahydrate (No. 5) had the lowest inhibitory effect on both *Candida* species (table 2). The

Table 2. Antifungal activity of different brands of toothpaste tested against clinical isolates of *Candida* species

Serial No.	Mean zone of inhibition of <i>Candida</i> , mm	
	<i>C. albicans</i> (n = 65)	<i>C. dubliniensis</i> (n = 21)
1	16 ± 1.8	17 ± 2.1
2	17 ± 2.4	18 ± 2.9
3	18 ± 2.5	19 ± 3.5
4	14 ± 1.9	15 ± 3.0
5	12 ± 3.2	12 ± 2.8
6	17 ± 2.5	20 ± 2.9
7	17 ± 2.8	19 ± 3.2
8	23 ± 3.9	27 ± 3.7
9	20 ± 4.1	18 ± 3.6

Values denote means ± SD.

herbal toothpaste (No. 8) was the only brand which produced a significant inhibitory effect on both *Candida* species even when compared with all other brands of toothpastes used in the study ($p < 0.001$). The inhibitory activity of the nonherbal toothpaste No. 9 against *C. albicans* was also significantly higher when compared to the inhibitory effect of other toothpaste brands ($p < 0.001$). However, the inhibitory effect of this brand on *C. dubliniensis* was not significantly different when compared to the inhibitory effect of other toothpaste brands. The inhibitory effect of a toothpaste containing only strontium chloride hexahydrate (No. 5), which had the lowest inhibitory effect on both *Candida* species, was significantly lower ($p < 0.001$) when compared to the higher inhibitory activity of other toothpaste brands.

Discussion

Candida species occupy a predominant place in the etiology of oral fungal infections [2], and recent studies have confirmed the etiologic role of *Candida* species in endodontic infections [13]. Although the dorsum of the tongue is believed to be the primary oral habitat of *C. albicans*, other sites may also be colonized [2], including the buccal mucosa, palate, angle of the mouth and tooth structures such as the dentin and the root [2, 13, 14]. *Candida* species can also colonize subgingival pockets and may cause gingivitis particularly in those using dental prostheses [15, 16]. Besides *C. albicans*, other yeast species including *C. dubliniensis* have also been isolated from the oral cavity in both healthy and compromised individuals [4, 5].

In recent years, a number of toothpaste preparations containing herbal ingredients that may be beneficial by improving oral health have been developed. One such ingredient is an extract of aqueous miswak, which possesses antibacterial activity against *Streptococcus mutans* [17], *Actinobacillus actinomycetemcomitans* [18], *Bacteroides* species [19] and some other oral microorganisms associated with periodontitis and caries [20], although it did not show any inhibitory effect on *Staphylococcus aureus* and *Staphylococcus epidermidis* [17]. A few studies carried out to determine the anti-*Candida* activity of miswak extracts have yielded inconsistent results [17]. For instance, in one study it was found that miswak extract exhibited fungistatic activity against *C. albicans* in concentrations of 15% and above up to 48 h as determined by turbidity measurements [21]. In another study, however, no inhibitory activity against *C. albicans* was observed even at a concentration as high as 50% [17]. Hence, the information on the active ingredients possessing antibacterial or antifungal activity is inconsistent.

The other herbal ingredient known to demonstrate inhibitory activity against *Candida* species is neem. Neem and its products (seeds and oil) have been shown to possess antifungal properties against phytopathogenic filamentous (mould) fungi such as *Fusarium* species, *Drechslera* species, *Alternaria* species and dermatophytes [22, 23]. Some of the latter species are also opportunistic human pathogens. The chemical nature of some of the ingredients that exhibit antifungal activity in neem extracts against filamentous fungi have been characterized [22], but information on their anti-*Candida* activity is lacking. Other herbal preparations such as oils obtained from clove and basil have also shown antifungal activity against a number of fungi including *C. albicans* [24]. A contact time of about 1 min with clove oil is sufficient to eliminate an inoculum of 1×10^6 colony-forming units per milliliter of *C. albicans*. In addition, extracts of chamomile, *Echinacea*, peppermint and rhatany have also been reported to possess some antifungal properties [25].

In the current study, the herbal toothpaste brand (No. 8) commonly used in Kuwait showed a significantly higher activity against *C. dubliniensis* than against *C. albicans*, most probably due to a synergistic effect of the herbal constituents (at least 6) included in this formulation. The other toothpastes containing non-herbal ingredients (table 1) also elicited anti-*Candida* activity, most probably due to the antifungal properties documented in some of these ingredients. For instance, it has recently been suggested that triclosan blocks lipid biosynthesis by specifically inhibiting the enzyme enoyl-acyl carrier pro-

tein reductase [8, 10]. Chlorhexidine suppresses many pathogenic attributes of *Candida* species [26]. Fluorides have also demonstrated some antibacterial and antifungal effects such as metabolic interference and reduction of dental plaque acidogenicity [10]. Similar antifungal activity of toothpastes containing triclosan and sodium monofluorophosphate/sodium fluoride on *Candida* species has previously been reported [8–10]. The non-herbal toothpaste containing only strontium chloride hexahydrate (No. 5) had the lowest inhibitory effect on both *Candida* species, which may be due to the presence of a single ingredient in its formulation.

The inhibitory zone diameters observed in our study for the herbal toothpaste with the highest inhibitory effect range of 23–27 mm were within the range of a toothpaste containing monofluorophosphate (19–36 mm) [9], but not of another herbal toothpaste (35–50 mm) [10]. These differences in inhibitory effect may be attributed to the ingredients used in the toothpaste formulations as well as to the susceptibility of *Candida* species used in the aforementioned studies. However, none of the previous studies [9, 10] tested the anti-candidal effect of toothpastes on *C. dubliniensis* isolates. *C. dubliniensis* is now well recognized as an opportunistic pathogen associated with oral candidiasis. Invasive *C. dubliniensis* infections have also been described in immunocompromised HIV-seropositive individuals and in patients with gynecological malignancies, chronic myelogenous leukemia, liver disease or myelodysplastic syndromes [27, 28]. Resistance to fluconazole has also been observed in *C. dubliniensis* isolates obtained from AIDS patients, and stable fluconazole resistance can be readily induced in *C. dubliniensis* following exposure to the drug in vitro [29]. In addition, it was recently reported that breakthrough *C. dubliniensis* fungemia occurred in a patient during prolonged exposure to voriconazole [30]. In this context the antifungal effect of the herbal brand of toothpaste, especially on *C. dubliniensis* isolates as shown in our study, would be of interest as it may provide possible beneficial effects by reducing the pathogenic potential of this species in the oral cavity in susceptible individuals.

The currently available toothpaste formulations that are recommended by oral healthcare providers for their ability to control plaque, thereby preventing dental caries and periodontal disease, are mostly those active against bacterial pathogens. A number of antibacterial agents, such as bromochlorophene, phenolic compounds, tin and zinc salts, chlorhexidine and, more recently, triclosan have been used as therapeutic agents in toothpastes and

mouthwashes to reduce plaque formation, gingivitis and calculus [8]. In this context the current study is noteworthy as it contributes to the understanding of the effectiveness of different toothpaste preparations against commonly encountered oral *Candida* species. Although none of the toothpaste brands investigated was developed for use as an antifungal formulation, the information provided herein, especially the anti-*Candida* property of the toothpaste containing herbal extracts, could be valuable to oral healthcare providers in recommending a toothpaste to reduce the burden of *Candida* in the oral cavity in individuals susceptible to oral candidosis. Furthermore, this herbal toothpaste may be used as an alternative to conventional formulations by individuals who have an interest in naturally based products. Further clinical studies using a larger number of oral *Candida* isolates are warranted to confirm these findings in vitro and to allocate herbal toothpastes to be used as therapeutic agents in oral candidosis. In addition, as isolates from non-oral sources were also used in this study, it suggests that the anticandidal properties of ingredients in toothpastes may have a wider applicability and could be considered for the future development of anti-candidal drugs principally for topical application.

Conclusion

The herbal toothpaste presented significant anti-candidal activity against two important *Candida* species associated with oral candidosis compared to other conventional formulations. Hence, the herbal brand may be considered to be used as an alternative to conventional formulations in patients susceptible to oral candidosis.

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