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Assessment of antifungal activity of herbal and conventional toothpastes against clinical isolates of *Candida albicans*

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ABSTRACT

Objective: To detect the anticandidal activity of nine toothpastes containing sodium fluoride, sodium monofluorophosphate and herbal extracts as an active ingredients against 45 oral and non oral *Candida albicans* (*C. albicans*) isolates. **Methods:** The antifungal activity of these toothpaste formulations was determined using a standard agar well diffusion method. Statistical analysis was performed using a statistical package, SPSS windows version 15, by applying mean values using one-way ANOVA with post-hoc least square differences (LSD) method. A P value of less than 0.05 was considered significant. **Results:** All toothpastes studied in our experiments were effective in inhibiting the growth of all *C. albicans* isolates. The highest anticandidal activity was obtained from toothpaste that containing both herbal extracts and sodium fluoride as active ingredients, while the lowest activity was obtained from toothpaste containing sodium monofluorophosphate as an active ingredient. Antifungal activity of Parodontax toothpaste showed a significant difference ($P< 0.001$) against *C. albicans* isolates compared to toothpastes containing sodium fluoride or herbal products. **Conclusions:** In the present study, it has been demonstrated that toothpaste containing both herbal extracts and sodium fluoride as active ingredients are more effective in control of *C. albicans*, while toothpaste that containing monofluorophosphate as an active ingredient is less effective against *C. albicans*. Some herbal toothpaste formulations studied in our experiments, appear to be equally effective as the fluoride dental formulations and it can be used as an alternative to conventional formulations for individuals who have an interest in naturally-based products. Our results may provide invaluable information for dental professionals.

1. Introduction

The oral cavity is a habitat for a large number of microorganisms species which coexist with one another as normal microbiota. There are more than 20 species of *Candida*, the most common opportunistic oral fungus associated with oral candidial infection, both in medically compromised and otherwise healthy individuals is *Candida albicans* (*C. albicans*)[1,2]. Theses opportunistic human pathogens colonize at several anatomically distinct surfaces of human body, mainly in warm and moist areas such as oral cavity, skin, gastrointestinal tract and vagina. *C. albicans* is considered as the most common yeast isolated from the oral cavity[3,4]. However, under specific conditions, colonizing *C. albicans* may give rise to different pathological conditions ranging from acute pseudomembranous thrush to more chronic forms which may persist for a longer period despite treatment. Risk of candidal infection or colonization is markedly tend to increase due to a group of predisposing factors such as poor oral hygiene, immunosuppression, nutritional deficiencies, long term of use antibiotic/radiation therapy, dental prostheses, diabetes mellitus, high carbohydrate diet or heavy cigarette smoking[5,6].

Ingredients used in modern toothpaste formulations include abrasive agents, tensoactives, humectants, thickening agents, flavoring, coloring agents and antimicrobial agents. These antimicrobial agents include metal salts, phenols, herbal extracts, enzymes, essential oils, and bisbiguanides[2,7,8]. Toothpastes have been formulated to contain a chemotherapeutic agents to improve oral health, to produce inhibitory action on plaque formation and bacteria and *Candida* colonisation[2,4,9-11].

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Different types of toothpastes have their own composition and concentration of ingredients for their efficacy. Many toothpastes claim to have antimicrobial properties, but in fact more research is needed to evaluate these effectiveness claims. The success of any toothpaste, in part, is determined by its ability to eliminate pathogenic oral microorganisms and decrease the risk of infection in mouth. In this research, *C. albicans* has been chosen because it is one of the major pathogen that is implicated in oral diseases and the most common yeast isolated from oral cavity. The present *in vitro* study was carried out to determine the anticandidal activity of different toothpastes (herbal and conventional) commonly used in Palestine against oral as well as non oral *C. albicans* isolates using a standard agar well diffusion method.

2. Materials and methods

2.1. Yeast strains

A total of forty five clinical isolates of *C. albicans* were recovered from various clinical specimens during year 2008-2009. The specimens used in the study included 27 oral isolates while the rest are non-oral isolates. These isolates were identified by phenotypic characteristics. *C. albicans* were differentiated from other *Candida* and *Cryptococcus* species by their ability to grow on the Levine formula of EMB agar and to produce germ tubes within 3 hours, and pseudohyphae and budding cells at 18-24 hours when incubated at 35 ℃ in 5-10% CO_2. The addition of tetracycline to the Levine formulation helps in the selection of *C. albicans* from clinical sources that are contaminated with bacteria.

2.2. Antifungal assay

Nine different brands of herbal and conventional toothpaste were purchased from the local market. The particulars of ingredients included in each toothpaste formulation by the manufacturers are listed in Table 1. The antifungal activity of these toothpaste formulations was determined using a standard agar well diffusion method as described previously with a little modification[2]. Growth from freshly subcultured isolates was suspended in 10 mL of sterile saline to obtain a turbidity of 0.5 McFarland standard. Using a sterile swab, the Sabouraud dextrose agar plates each containing 25 mL, were evenly inoculated with the *C. albicans* suspension. The diluted toothpaste solutions (25% w/v) were prepared in sterile distilled water, then wells of 6 mm diameter, were punched on Sabouraud dextrose agar plates surface with equal distance from each other. Then each well was filled with 60 μL diluted toothpaste solutions, while the same amount of sterile distilled water was also added as a control. The plates were then incubated at 35 ℃ for 48 h. The antifungal activity was evaluated by measuring the diameter of zones of inhibition (mm). All the plates were made in duplicates and the experiments were repeated twice. A *C. albicans* ATCC 10231 was also included in this study as a reference strain.

2.3. Statistical analysis

Results were expressed as Mean ± SD for illustration. Statistical analysis was done using a statistical package,

Table 1
Ingredients of various toothpastes tested for antifungal potential.

Toothpaste	Ingredients as listed on packages
Perioteva (Israel)	Sodium fluoride, combination of medicinal plant extracts (sage, myrrh, echinacea, chamomile, rhatany) with a high concentration of sodium bicarbonate salt)
Parodontax (England)	Sodium bicarbonate, aqua, glycerin, cocamidopropyl betaine, alcohol, krameria triandra extract, *Echinacea purpurea* juice, xanthan gum, *Chamomilla Recutita* extract, *Commiphora myrrha* extract, sodium saccharin, sodium benzoate, salvia officinalis oil, *Mentha piperita* oil, *Mentha arvensis* oil, Limonene, Cl 77491
Crest (Germany)	Aqua, sorbitol, hydrated silica, sodium lauryl sulphate, cellulose gum, aroma, sodium fluoride, sodium saccharin, carbomer, Cl 77891, trisodium phosphate, limonene, citral, sodium fluoride
Dentiste′ plus white vitamin c & xylitol toothpaste (Thailand)	Xylitol, silicone dioxide, peppermint oil, clove oil, menthol, vitamin C, *Eucalyptus* oil, sage extract, *Chamomile* extract (1), fennel extract, *Glycyrrhiza* extract, *Cinnamon* park extract
Dabur Miswak herbal toothpaste (UAE)	Calcium carbonate, sorbitol, treated water, silica, sodium lauryl sulphate, flavour, miswak extract, sodium carboxy methyl cellulose and / or sodium carragreenate, sodium silicate, sodium benzoate, glycerine, sodium saccharin
el-ce med (Germany)	Aqua, sorbitol, pentapotassium triphosphate, propylene glycol, hydrated silica, silica, PFG-32, urea, sodium lauryl sulphate, aroma, titanium dioxide, xanthan gum, citric acid, sodium fluoride, sodium saccharin, *Ananas sativus* extract, potassium thiocyanate,sodium mehtylparaben
Signal (Egypt)	Sorbitol, aqua, hydrated silica, PEG-32, sodium lauryl sulphate, aroma, cellulose gum, sodium fluoride, sodium saccharin, PEG-40, hydrogenated castor oil, phenoxyethanol, glycerine, limonene, Cl 10140, Cl 42090, Cl 47260
Close up (Egypt)	Sorbitol, water, hydrated silica, sodium lauryl sulphate, PEG-32, flavour, cellulose gum, sodium fluoride, sodium saccharin, citral, limonene, Cl 42090, Cl 47005
Barbie (Israel)	Sorbitol, water (aqua), hydrated silica, glycerin, sodium lauryl sulphate, cellulose gum, flavor, sodium monofluorophosphate, sodium saccharin, sodium methylparaben, sodium propylparaben, citric acid, FD&C Red No. 40

SPSS windows virsion 15 by applying mean values using one-way ANOVA with post-hoc least square differences (LSD) test to determine if there was a significant difference among different brands of toothpastes against *C. albicans* isolates. A P value of less than 0.05 was considered significant.

3. Results

The antifungal activities of nine different dentifrices were investigated against 45 *C. albicans* isolates using a standard agar well diffusion method. Different toothpaste brands exhibited variations in their inhibitory activity against these test *C. albicans* isolates, but all toothpastes were effective in inhibiting the growth of all tested *C. albicans* isolates. Our results showed that, the herbal dental formulations studied appear to be equally effective as the fluoride dental formulations, but not superior to them. In addition to that, results showed that toothpaste with combination of sodium fluoride and herbal extracts (Perioteva) as an active ingredients exhibited higher antifungal activity against all *C. albicans* isolates than the formulations containing sodium fluoride or herbal extracts alone. Herbal toothpaste containing sodium monofluorophosphate (Barbie) as an active ingredient has exhibited the lowest inhibitory effect against *C. albicans*. Activity of test-toothpastes was in decreasing order: Perioteva, Parodontax, Crest, Dentiste′ plus white vitamin C & xylitol toothpaste, Dabur Miswak, El-ce med, Signal, Close up and the last one was Barbie (Table 2). The mean inhibition zone diameters, range of inhibition zone against *C. albicans* strains of 9 toothpaste formulations were ordered according to their descending potential activities against *C. albicans* isolates (Table 2). The mean inhibition zone diameters of these toothpastes against *C. albicans* ATCC 10231 were also included in this study as a reference. There was a statistically-significant difference ($P < 0.001$) among the anticandidal activities of toothpastes on *C. albicans* isolates (Table 2).

Table 2

Anti-microbial activity of different toothpaste formulations against 45 *C. albicans* isolates after 48 h of incubation (Mean ± SD).

Toothpaste	Mean zone of inhibition of 45 *C. albicans* isolates (mm)	Range of zone of inhibition (mm)	Mean zone of inhibition of *C. albicans* ATCC 10231 (mm)
1. Perioteva [a]	41.956 ± 4.000	33-50	35
2. Parodontax [a]	30.800 ± 3.116	25-38	27
3. Crest [c]	27.756 ± 2.656	21-35	23
4. Dentiste′ plus white vitamin c & xylitol toothpaste [d]	26.889 ± 3.076	20-37	22
5. Dabur Miswak [c]	26.379 ± 2.987	21-34	25
6. El-ce med [c]	25.889 ± 2.014	23-30	22
7. Signal [c]	24.882 ± 3.353	20-35	22
8. Close up [b]	23.489 ± 1.961	20-29	25
9. Barbie [a]	20.378 ± 3.025	15-29	18

a- Have a significant difference at $P < 0.001$ to all other toothpastes; b- Have a significant difference at $P < 0.001$ to some toothpastes and at $P < 0.05$ to others; c- Have a significant difference at $P < 0.001$ and $P < 0.05$ to some toothpastes and not significant to others; d- Have a significant difference at $P < 0.001$ to certain toothpastes and not significant to others.

4. Discussion

Plaque control is an important procedure involves the removal of microbial dental plaque biofilm and the prevention of its accumulation on the teeth and adjacent gingival surfaces to prevent tooth decay and periodontal disease. Most studies on antimicrobial activity of dentifrices have been focused on their potential to inhibit bacterial growth. In particular, little information was found in recent literature research concerning their antifungal potentials[2,12].

A number of toothpaste preparations containing herbal ingredients that may be beneficial by improving oral health have been developed in recent years. In our experiments, among all the investigated toothpastes, Perioteva toothpaste emerged as the most effective, based on the mean diameter of the zone of microbial inhibition produced by a standard agar well diffusion method, against all the 45 test *C. albicans* isolates. The highest anticandidal activity, most probably due to the synergistic effect between the active ingredients of this toothpaste formulation. Effective ingredients of Perioteva toothpaste are sodium fluoride, and combination of herbal extracts such as sage, myrrh, *Echinacea*, chamomile and rhatany. Fluorides are frequently used in oral health products including toothpastes. Many clinical studies have demonstrated the efficiency of sodium fluoride in reducing cavities, helping diminish demineralization of tooth enamel and even enhancing 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[13,14]. In addition to sodium fluoride, herbal components such as sage, myrrh, *Echinacea*, chamomile and rhatany exhibit antifungal activity[2,15-18]. Also these plant extracts are known to have positive effects, such as *Chamomilla* which is supposed to have anti-inflammatory properties; *Echinacea* has a reputed ability to stimulate the immune response; sage and rhatania have antihemmorhagic effect; myrrhe are claimed to have a natural antiseptic effect[2,15,16].

Crest, El-ce med, Signal, Close up are conventional toothpastes and all have one effective ingredient which is sodium fluoride. These toothpastes showed a good antifungal activity against *C. albicans* isolates with mean zones of inhibition (mm) 27.8, 25.9, 24.9 and 23.5, respectively.

The Dentiste′ plus white vitamin C and xylitol toothpaste, Dabur Miswak Herbal toothpaste and Parodontax are herbal toothpastes, which all are containing herbal components as effective ingredients, exhibited good antifungal property, thereby, confirming the medicinal value of plant products. Dentiste′ plus white vitamin C and xylitol toothpaste has many herbal components including peppermint oil, clove oil, menthol, *Eucalyptus* oil, sage extract, *Chamomile* extract, fennel extract, *Glycyrrhiza* extract, cinnamon park extract. Herbal preparations such as oils obtained from clove, basil and other plants have also shown antifungal activity against a number of fungi including *C. albicans* and act as a promising agents in the treatment of oral diseases and other infections[2,19,20]. In addition, extracts of *Chamomile*, *Echinacea*, peppermint and rhatany have also been reported to possess some antifungal properties. Dabur Miswak Herbal toothpaste has an aqueous extract of miswak, which possesses antibacterial activity against different types of bacteria including oral bacteria[21,22]. different studies were carried out to determine the anticandidal activity of miswak extracts. It was found that both aqueous and methanol extracts had equal antifungal activity against *C. albicans* based on the turbidity test[21]. The efficacy of natural toothbrush or miswak in the prevention of dental caries has been investigated and compared with the efficacy of ordinary toothbrush and toothpaste. The data collected at the end of the study showed that the risk of dental caries for each tooth in the control group was 9.35 times more than the case group[23]. Rinsing with miswak extract stimulated parotid gland secretion and raised the plaque pH, suggesting a potential role in caries prevention[24]. Recent new study showed that dried miswak has antifungal activity against several *Candida* strains better than fresh plants[25]. Our results were consistent with a recent report where miswak extract in Dabur Miswak Herbal toothpaste exhibited antifungal activity against *C. albicans*[12]. Parodontax toothpaste is composed of many herbal ingredients. Its antifungal activity is greater than others test toothpastes except Perioteva. The principle components of this toothpaste include *Krameria triandra* extract, *Echinacea purpurea* juice, *Chamomilla recutita* extract, *Commiphora myrrha* extract, *Salvia officinalis* oil, *Mentha piperita* oil, *Mentha arvensis* oil. Synergistic interactions between the principal components of these herbs are considered to be a vital part of their efficacy. This synergistic activity, however, needs to be established[26]. Some of these ingredients were previously demonstrated and known to have antifungal activity against *C. albicans*. Essential oils in these toothpastes are frequently used for flavoring in oral products and contribute to the antiseptic properties of these products, although antimicrobial properties depend on the type and the concentration of the oil, as well as the tested microbial strain[2,27,28].

The non-herbal toothpaste (Barbie) containing sodium monofluorophosphate as an active ingredient, which prepared for children. It has the lowest inhibitory effect against *C. albicans*, this may be due to the presence of a single active ingredient in its formulation. According to a large number of clinical trials sodium fluoride is approximately 6-7% more effective than sodium monofluorophosphate against decayed tooth[2,29].

In this study, the herbal dental formulations studied appear to be equally effective as the fluoride dental formulations, but not superior to them[4,26], this may be due to the ingredients present. The antifungal activity of the herbs is due to the presence of by-products called phytochemicals. The consumers who are using herbal products often view these products as being safer than products that have chemicals[30,31], although toothpaste allergy resulting from herbal and conventional toothpastes containing unspecified flavourings, cocamidopropyl betaine (CAPB), propylene glycol, triclosan or essential oils and biological additives have been reported[28,32]. Using the herbal extracts dentifrices in combination with sodium fluoride appears to improve the effectiveness of antifungal activity assessed by *in vitro* well diffusion method. This testing method functioned as a screening method, and may not have been able to detect the effects of a chemical agents that do not able to diffuse through the agar matrix[4,30,33], so other techniques may be used to detect non diffusible molecules such as broth microdilution method. It cannot be assumed that the results of our experiments could be translated into clinical effectiveness. Because the toothpaste used *in vivo* is likely to be diluted by saliva, the level to which antimicrobial properties are buffered or lost in dilution *in vitro* is of interest[33]. Results of this study may provide invaluable information for dental professionals. In certain cases, a physician may recommend a dentifrice that has good inhibition properties against *C. albicans* for a patient who is susceptible to oral fungal infections. It is known that there is a state of balance found in individual′s oral microbial population. If this state is damaged or lost, opportunistic microorganisms can proliferate and enabling the initiation of disease processes. Therefore, toothpaste formulation which was identified as having the largest fungal inhibition zone and thus, probably the strongest antifungal properties may not be necessarily superior to those found to have smaller diameter inhibition zones[30, 33], because dentifrices may diffuse at different rates.

In conclusion, the herbal toothpaste formulations studied in our experiments, appear to be equally effective as the fluoride dental formulations, but not superior to them and it can be used as an alternative to conventional formulations for individuals with an interest in naturally-based products. In addition to that, combination between sodium fluoride and herbal extracts increases the antifungal activity of these toothpastes against *C. albicans*.

Conflict of interest statement

We declare that we have no conflict of interest.

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