Assessment of antifungal activity of six popular toothpastes against clinical isolates of *Candida albicans*

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Abstract

Introduction

The oral cavity is a habitat for a large number of microorganisms which coexist with one another as normal microbiota. *Candida* species are ovoid budding yeast-like fungi. The organism is a normal commensal of humans found on skin and throughout the gastrointestinal tract. Poor oral hygiene, high carbohydrate diet, nutritional deficiencies, diabetes mellitus, dental prostheses, heavy cigarette smoking, immunosuppression and HIV infection are associated with increased incidence of oral thrush. Oral candidiasis may present as oral thrush, acute atrophic candidiasis, chronic atrophic candidiasis/denture sore mouth, angular cheilitis and *Candida* leukoplakia. Different toothpaste brands have their own composition and concentration of ingredients. Many toothpastes claim to have antimicrobial properties. More research is needed to evaluate these claims.

Objectives

The study aims to determine the antifungal activity of six different toothpastes commonly used in the locality.

Materials & Methods

Six brands of toothpastes were selected to determine the antifungal activity against 10 clinical isolates of *Candida albicans* by standard agar well diffusion method. Antifungal activity of toothpastes was determined in both undiluted and diluted forms on antimycotic sensitivity media by measuring the zone of inhibition.

Results

All toothpastes showed antifungal activity in undiluted forms. Brand-1 and brand-3 showed antifungal activity even in diluted solutions.

Conclusion

All six toothpastes have antifungal activity in undiluted forms, but brand-1 & 3 have activity in diluted forms also. There is a need to create a standardized method to evaluate antifungal activity of different brands of toothpastes.

Keywords: *Candida albicans*, Agar diffusion method, Tooth pastes

1. Introduction

The oral cavity is a habitat for a large number of microorganisms which coexist with one another as normal microbiota [1]. Written description of oral lesions that were probably thrush, date to the time of Hippocrates and Galen [1]. *Candida* organisms are yeast-like fungi that exist predominantly in unicellular form. They are small (4-6µm), thin walled, ovoid budding cells (blastospores) that reproduce by budding. There are more than 200 species of *Candida*, but only a small percentage is regarded as frequent pathogens for humans [2]. The organisms are normal commensals of humans and are commonly found on skin, throughout the gastrointestinal tract, in expectorated sputum, in the female genital tract and urine of patients with indwelling Foley’s catheters [3].

Risk of candidal infection or colonization of the oral cavity increases due to a group of predisposing factors such as poor oral hygiene, high carbohydrate diet, nutritional deficiencies, diabetes mellitus, dental prostheses, heavy cigarette smoking, long term use of antibiotics and/or steroids, radiation therapy, immunosuppression and HIV infection [4]. Introduction of inhaled steroids in the treatment of asthma has resulted in increased incidence of oral thrush in children. Incidence has ranged from 0 to 77% [1].

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Although vast majority of Candida infections are endogenous in origin, human-to-human transmission is possible. Example is thrush of the newborn, which may be acquired from the maternal vagina [1]. The term thrush is applied to a specific reaction at the corners of the mouth and – a chronic inflammatory reaction and epithelial thinning candidiasis, chronic atrophic candidiasis or denture sore mouth thought to be a sequela of acute pseudomembranous candidiasis – a nonspecific atrophy of the tongue that is obtained commercially from HiMedia laboratories is used for susceptibility test. Growth of freshy subcultured isolates were suspended in 10ml of sterile saline to obtain a turbidity of 0.5 McFarland standard. Using a sterile swab, the plates were inoculated with C. albicans suspension [6]. The diluted toothpaste solutions (50g/100ml) were prepared in sterile distilled water. This stock solution was serially diluted (25, 12.5, 6.25 and 3.12g/100ml). Wells of 6mm diameter were punched on media surface with equal distance from each other. Wells were filled with 60µl of diluted toothpaste solutions, while the same amount of sterile distilled water was added as a control. In one plate, wells were filled with 60µl of undiluted toothpaste. The plates were then incubated at 37°C for 24hr. The antifungal activity was evaluated by noting the zone of inhibition. The above procedure is repeated for the rest nine C. albicans isolates. C. albicans ATCC 90028 was used as control.

3. Results and Discussion
All six toothpastes showed antifungal activity when tested without dilution (Table 2). Out of six toothpastes, brand-1 and brand-3 showed highest zone of inhibition (22mm and 21mm respectively). On dilution, only brand-1 and brand-3 showed zone of inhibition. Brand-1 showed zone of inhibition in all the dilutions tested. Brand-3 showed zone of inhibition only at 50g/100ml, 25g/200ml and 12.5g/100ml. No zone of inhibition was observed at lower concentrations. No other brands showed zone of inhibition on dilution.

Table 1: Contents of various brands tested

<table>
<thead>
<tr>
<th>Brand</th>
<th>Contents</th>
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<tbody>
<tr>
<td>Brand-1</td>
<td>1000ppm fluoride, calcium carbonate, sorbitol, hydrated silica, sodium lauryl sulphate, sodium monofluorophosphate, cellulose gum, sodium silicate, benzyl alcohol, potassium nitrate, triclosan, sodium saccharin, CI45430, CI12490</td>
</tr>
<tr>
<td>Brand-2</td>
<td>Sodium fluoride, sorbitol, hydrated silica, sodium lauryl sulphate, PEG 32, Cocamidopropylbetaine, cellulose gum, sodium saccharin, zinc sulphate, Mica/Cl 77019, sodium hydroxide, Cl 16255, Cl17200, CL 77491, CL77891</td>
</tr>
<tr>
<td>Brand-3</td>
<td>Sodium fluoride, sorbitol, silica, sodium lauryl sulphate, PVM/MA copolymer (Gantrez) Carrogeenan gum, sodium hydroxide, titanium dioxide, sodium saccharin, triclosan, titanium dioxide coated mica, pigment green (CL 74260), lake quinoline yellow (CL 47005:1)</td>
</tr>
<tr>
<td>Brand-4</td>
<td>Calcium carbonate, sorbitol, silica, sodium lauryl sulphate, babul extract, cellulose gum, carrogeenan, sodium silicate, sodium saccharin, formaldehyde, foaming, non-fluorinated</td>
</tr>
<tr>
<td>Brand-5</td>
<td>1000ppm fluoride, precipitated calcium carbonate, sorbitol, glycine, hydrated silica, sodium lauryl sulphate, sodium silicate, sodium carboxymethyl cellulose, carrogeenan, sodium saccharin, methylparaben, propylparaben, neem extract, sodium monofluorophosphate, sodium dihydrogen phosphate</td>
</tr>
<tr>
<td>Brand-6</td>
<td>1000ppm fluoride, sorbitol, hydrated silica, glycerin, sodium lauryl sulphate, xanthan gum, titanium dioxide, sodium saccharin, menthol, sodium benzoate, Punica Granatum pericarp extract, potassium sorbate, calcium fluoride, Zanthoxylum alatum fruit extract, Acacia arabica stem bark extract, Terminalia chebula fruit extract, Terminalia bellirica fruit extract, Emblica Oficinalis fruit extract, Embelia ribes fruit extract, Azadirachta Indica bark extract, Vitex Negundo extract, thymol, citric acid, Salvadora persica stem extract, Acacia farnesiana flower/stem extract, Acacia catechu bark powder, Mimosops elengi flower extract</td>
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demineralization of tooth enamel and even enhancing the remineralization of potential decay spots [7]. Based on a variety of mechanisms, fluoride also demonstrates some antibacterial and antifungal effects such as metabolic interference and reduction of dental plaque acidogenicity [8]. Sodium monofluorophosphate also has activity against C. albicans but inhibitory effects are less and needs to be combined with other better ingredients [4].

Tooth pastes containing herbal components like, peppermint oil, clove oil, menthol, Eucalyptus oil, sage extract, Chamomile extract, fennel extract, Glycyrhiza extract, cinnamon bark extracts, as effective ingredients exhibited antifungal activity at undiluted concentrations [9]. Echinacea also has antifungal activity along with its reputed ability to stimulate the immune response. Extracts of Chamomile, Echinacea, peppermint and rhatany have also been reported to possess some antifungal properties [10]. The antifungal activity of the herbs is also due to the presence of by-products called phytochemicals [4].

In our experiments, brand-1 toothpaste emerged as the most effective against all the test C. albicans isolates and in all six concentrations used, followed by brand-3. The highest anticanidal activity is most probably due to the synergistic effect between the active ingredients of these toothpaste formulations. Other brands showed antifungal activity without dilution and no activity at lower concentrations. Using herbal extracts 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 chemical agents that do not diffuse through agar matrix. 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 not known. Results of this study may provide invaluable information for dental professionals. In situations as described, a physician may recommend a dentifrice that has good inhibition properties against C. albicans.

4. Conclusions
In conclusion, there is a need to create a standardized method to evaluate the claims made by various brands of toothpastes. All the toothpastes studied showed antifungal activity when used without dilution. Sodium Fluoride containing toothpastes showed higher antifungal activity than others.


