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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 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].

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 form of oral candidiasis characterized by creamy white, curd like patches on the tongue and on other oral mucosal surfaces; the patches are removable by scraping and leave a raw bleeding and painful surface. The patches are actually pseudomembranes consisting of *Candida*, desquamated epithelial cells, leukocytes, bacteria, keratin, necrotic tissue and food debris [1].

In addition to the classic lesions, which have been described by Lehner, other manifestations include, acute atrophic candidiasis – a nonspecific atrophy of the tongue that is thought to be a sequela of acute pseudomembranous candidiasis, chronic atrophic candidiasis or denture sore mouth – a chronic inflammatory reaction and epithelial thinning under the dental plates, angular cheilitis – an inflammatory reaction at the corners of the mouth and *Candida* leukoplakia – a firm, white plaque affecting the cheek, lips and tongue that has a protracted course and in rare instances, may be precancerous [1].

Modern toothpaste formulations include abrasive agents, tensioactives, humectants, thickening agents, flavoring, coloring agents and antimicrobial agents. These antimicrobial agents include metal salts, phenols, herbal extracts, enzymes, essential oils and bisbiguanides [4]. Chemotherapeutic agent containing toothpastes inhibit plaque formation and colonization with bacteria and *Candida*, by which it improves oral health.

Different toothpaste brands have their own composition and concentration of ingredients. Many toothpastes claim to have antimicrobial properties, but in fact more research is needed to evaluate these effectiveness claims. Good toothpaste is one that has the ability to eliminate pathogenic oral microorganisms and decreases the risk of infection in mouth [4].

The study aims to determine the antifungal activity of six different toothpastes commonly used in the locality against oral *Candida albicans* isolates using standard agar well diffusion method.

2. Materials and Methods

A total of 10 *C. albicans* recovered from various clinical specimens were obtained from Department of Microbiology, A. J. Institute of Medical Sciences, Mangalore. Out of 10 *C. albicans* isolates, nine were oral isolates and remaining one is non-oral isolate. The isolates were identified by germ tube test, chlamydospore formation on cornmeal agar and color formation on chromogenic media.

Six different brands of toothpastes (including three herbal toothpastes) that are commonly used in the region were purchased from the local market. The ingredients in the toothpastes are listed in Table 1. The antifungal activity of these toothpaste formulations was determined using standard agar well diffusion method [5]. Antimycotic sensitivity media obtained commercially from HiMedia laboratories is used for susceptibility test. Growths from freshly 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 37^0C 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/100ml 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

Brand-1	1000ppm fluoride, calcium carbonate, sorbitol, hydrated silica, sodium lauryl sulphate, sodium monofluorophosphate, cellulose gum, sodium silicate, benzyl alcohol, potassium nitrate, triclosan, sodium saccharin, Cl45430, Cl12490
Brand-2	Sodium fluoride, sorbitol, hydrated silica, sodium lauryl sulphate, PEG 32, Cocamidopropylbutein, cellulose gum, sodium saccharin, zinc sulphate, Mica/Cl 77019, sodium hydroxide, Cl 16255, Cl17200, CL 77491, CL77891
Brand-3	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)
Brand-4	Calcium carbonate, sorbitol, silica, sodium lauryl sulphate, babul extract, cellulose gum, carrogeenan, sodium silicate, sodium saccharin, formaldehyde, foaming, non-fluorinated
Brand-5	1000ppm fluoride, precipitated calcium carbonate, sorbitol, glycerine, hydrated silica, sodium lauryl sulphate, sodium silicate, sodium carboxymethyl cellulose, carrogeenan, sodium saccharin, methylparaben, propylparaben, neem extract, sodium monofluorophosphate, sodium dihydrogen phosphate
Brand-6	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 bellerica fruit extract, Emblica Officinalis fruit extract, Embelia ribes fruit extract, Azadirechta 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

Table 2: Zone of inhibition to various concentration of toothpaste

Isolates	Concentrations	Zone of inhibition (mm)					
		Brand-1	Brand-2	Brand-3	Brand-4	Brand-5	Brand-6
Candida albicans 1	Undiluted	22	18	21	15	15	17
	50g/100ml	20	6*	16	6	6	6
	25g/100ml	19	6	12	6	6	6
	12.5g/100ml	12	6	8	6	6	6
	3.25g/100ml	10	6	6	6	6	6
Candida albicans 2	Undiluted	24	20	22	16	17	19
	50g/100ml	20	6	18	6	6	6
	25g/100ml	16	6	12	6	6	6
	12.5g/100ml	10	6	8	6	6	6
	3.25g/100ml	8	6	6	6	6	6

Similar results were obtained for *Candida albicans* isolates 3, 4, 5, 6, 7, 8, 9 and 10.

* 6mm is the diameter of the well-used

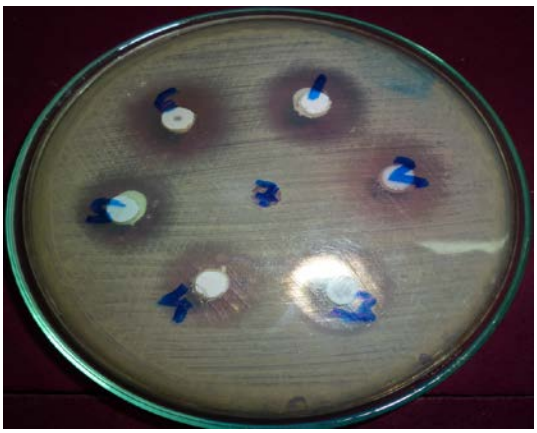

Fig 1: Susceptibility of undiluted samples

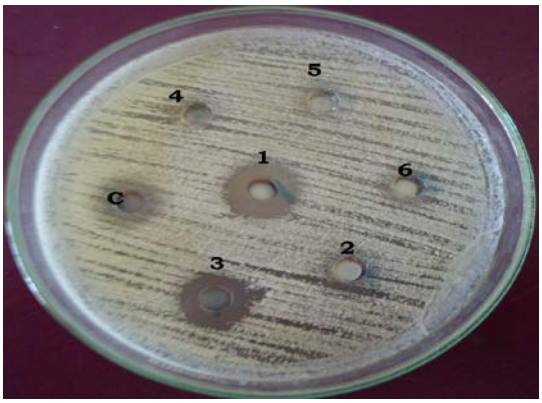

Fig 2: Susceptibility at 25g/100ml

3.1 Discussion

A number of toothpaste preparations containing chemical/herbal ingredients that may be beneficial by improving oral health have been developed in recent years. Plaque control is an important procedure that removes the microbial dental plaque biofilm and the prevention of its accumulation on the teeth and adjacent gingival surfaces. This in turn prevents tooth decay and periodontal disease [4]. Most studies on antimicrobial activity of dentifrices have focused on inhibition of bacterial growth, but are less concerned about antifungal activity [4].

Recently, there is an increasing incidence of diabetes mellitus and children who are on oral/inhaled steroids for asthma [1]. These patients are predisposed to oral candidiasis. These patients need toothpaste with antifungal activity or need to use mouthwash having antifungal activity following brushing [1].

Many clinical studies have demonstrated the efficacy of sodium fluoride in reducing cavities, helping diminish 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, *Glycyrrhiza* 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 anticandidal 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.

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