



Scholars Research Library

Der Pharma Chemica, 2012, 4(4):1742-1748  
(<http://derpharmachemica.com/archive.html>)



ISSN 0975-413X  
CODEN (USA): PCHHAX

## ***In vitro* Antimycotic Potentials of Consumers' Preferred Toothpastes and Toothgels on Human Oral *Candida* Species**

\*Adenike A.O. Ogunshe<sup>1</sup> Olayinka Ademiluka<sup>2</sup> and Mercy E. Okoedo<sup>2</sup>

<sup>1</sup>*Applied Microbiology and Infectious Diseases, Department of Microbiology, Faculty of Science, University of Ibadan, Nigeria*

<sup>2</sup>*Department of Botany & Microbiology, Faculty of Science, University of Ibadan, Nigeria*

### **ABSTRACT**

*Studies on antimycotic potentials of toothpastes on human oral yeast pathogens have been sparse, especially in developing countries, in spite of the increasing reports on problems with inadequate oral hygiene. Using a modification of agar well-diffusion method, in vitro antimycotic potentials of 10 most preferred Nigerian toothpastes and tooth-gels on 86 oral Candida species, C. albicans 43 (50.0%), C. glabrata 5 (5.8%), C. pseudotropicalis 10 (11.6%) and C. tropicalis 28 (32.6%) indicated overall low (20.0%) to high (100%) susceptibility rates among the Candida species. Varied susceptibility rates of 18.6-100%, 20.0-100%, 30.0-100% and 60.7-96.4% were exhibited by the oral C. albicans, C. glabrata, C. pseudotropicalis and C. tropicalis respectively towards the test toothpastes and tooth-gels, although their susceptibility patterns were not species-specific. Minimal overall in vitro inhibitory activities were however, displayed by some strains towards Close Up Red / Dabur Herbal Gel (20.0%), Macleans (18.6-30.0%) and MyMy (23.3%). In conclusion, high in vitro inhibitory potentials of some commonly available toothpastes and tooth-gels in Nigeria on oral Candida strains and can therefore, serve as active teeth-cleansing agents in oral hygiene.*

**Keywords:** oral *Candida*, oral health & hygiene, tooth-gels, toothpastes.

### **INTRODUCTION**

Oral candidiasis (oral thrush) is a common clinical manifestation of candidiasis, which is a common opportunistic infection of the oral cavity that can progress to oesophagitis [1], and which can interfere with adequate oral intake and in turn contributes to the general morbidity in patients. It is a frequent superficial infection in humans, caused by an overgrowth of *Candida* species, and it is associated with mechanical or traumatic factors or with immunocompromised states. *Candida* species are most frequently isolated from the oral cavity and have even been detected in approximately 31-55% of healthy individuals [2]. However, the incidence varies depending on age and certain predisposing factors [3-5]. The commonest aetiologic agent of oral candidiasis is *C. albicans* but over the last decade, reports of non-*albicans* *Candida* causing this condition have been increasing [6-8].

Oral candidiasis may present in a variety of clinical forms and there are a number of different types including acute pseudomembranous candidiasis, commonly known as thrush, erythematous and hyperplastic variants of candidiasis - acute atrophic, chronic hyperplastic, chronic atrophic, median rhomboid glossitis and angular cheilitis [9]. The most discrete lesion represents conversion from benign colonisation to pathological over-growth but when two or more of these variants appear in unison, the term *multifocal candidiasis* is used [10]. Other common lesions include *Candida*-associated denture stomatitis, angular cheilitis and median rhomboid glossitis; however, it is generally accepted that oral hygiene maintenance through regular removal of dental plaque and food deposits is an essential factor in the prevention of dental caries and periodontal diseases, although methods for oral hygiene vary from country to country

and from culture to culture.

There are several goals to personal oral hygiene, including preventing oral infection and disease but the purpose of oral hygiene using toothpaste is usually to reduce oral microbial flora [11]. Toothpaste has a history that stretched back nearly 4000 years, however, different abrasives, green lead and incense were used to clean stains from the teeth until mid 19<sup>th</sup> century but in the Middle Ages, fine sand and pumice were the primary ingredients in the tooth cleaning formulas used by Arabs. In 1950 A.D., Dr. Washington Wentworth Sheffield, a dental surgeon and chemist, invented the first toothpaste [12], and since then, the market of toothpaste has never been slowed down. In addition to teeth cleaning, the modern toothpaste was invented to aid in the removal of foreign particles and food substances, and during 1940-60 A.D., fluoride was added to toothpastes, which further aided in protection from tooth decay. Many innovations were later incorporated into toothpastes after the fluoride break-through, which involved the addition of ingredients with special potentials, as well as toothpaste packaging [13, 14].

Previous studies have shown that dental plaque can be controlled by physical removal of plaque, use of antimicrobial toothpastes and mouthwashes [15-16]. Toothpastes and formulation compositions however, differ among countries; therefore, the effectiveness of toothpastes will vary, and this has significant effect in oral health and hygiene. The aim of this study therefore, was to determine the *in vitro* inhibitory efficacy of commonly available toothpastes and tooth-gels in Nigerian markets.

## MATERIALS AND METHODS

### Collection of oral specimens:

*Candida* strains used in this study were obtained in form of three sets of early-morning oral swabs from oral cavities and saliva of 40 healthy volunteers, who were 19-28 years old students of various faculties of University of Ibadan, and who had not been on antifungal therapy at least six months prior to collection of oral specimens. Verbal informed consents were obtained, followed by instruction on how to collect the oral specimens. Each subject was instructed to separately swab the tongue and teeth areas before morning mouth brushing. The swabs were dropped into separately labeled McCartney bottles containing the unbuffered sterile peptone water (Lab M, Lab M Ltd., Lancashire, UK, Lot: 085018/065), immediately after swabbing. Saliva specimens were also collected from respective subjects. Samples were collected from subjects at each of two oral locations (teeth surfaces (Tt), dorsal surface of the tongue (Tg) and from saliva (Sv).

All the inoculated peptone water specimens were transported to the laboratory within 4 hours after collection and were incubated at 30-35<sup>o</sup>C for 24-48 hours, serially diluted and then pour-plated. Aliquots of each dilution (10<sup>-3</sup>) were separately plated on Sabouraud dextrose agar (SDA, Lab M) to which was added ofloxacin antibiotic. The culture plates were incubated at 25-32<sup>o</sup>C for 24-72 hrs.

### *Candida* isolates:

Representative colonies on the primary SDA plates were sub-cultured by repeated streaking and incubated at 30-35<sup>o</sup>C for 24-48 hrs until pure colonies were obtained.

Colonial morphology: Macroscopic examinations included (creamy moist circular colonies) and microscopic (yeast cells, pseudohyphae and blastospores).

Microscopic morphology: A smear of each pure *Candida* isolate was Gram-stained to determine the Gram's identity under the microscope. The germ tube test was also carried out in order to test for the ability of the *Candida* strains to produce germ tubes. Two milliliters of blood plasma in sterile test tubes were inoculated with the isolates and incubated at 37<sup>o</sup>C for 3 hours, after which the isolates were observed microscopically for the production of germ tubes.

Sugar assimilation test: Sugar assimilation patterns of the *Candia* strains were determined using arabinose, fructose, galactose, glucose, lactose, maltose, mannitol, sucrose, rhamnose and xylose in sterile peptone water [4.5gm yeast extract, 7.5gm peptone and 20gm sugar in 1 litre distilled water] as the basal medium and phenol red (1mg/ml) as indicator. The medium was adjusted to pH 7.0 before dispensing into test tubes containing inverted Durham tubes and sterilised by autoclaving at 121<sup>o</sup>C for 15 mins. Pure culture of each *Candida* strain was then inoculated into duplicates of the sugar medium and later incubated at 32<sup>o</sup>C for 24-96 hrs. The results were indicated by colour change from red to yellow [17] and production of gas was determined by displacement of the sugar medium by air in the inverted Durham tubes. Un-inoculated tubes served as control. The *Candida* strains were finally characterised using standard phenotypic taxonomic tools [18, 19].

**Presentation of Antifungal drugs:**

The antifungal drugs used in this study were ketoconazole oral caplets (batch no: 08-320701; NAFDAC reg. no: 04-4511), Flucamed capsules (batch no: 351001; NAFDAC reg. no: 04-4514), Mycoten cream (batch no: 0401; NAFDAC reg. no: 04-1468), manufactured by Drugfield Pharmaceuticals Ltd., Lynson Chemical Avenue, Km 38, Lagos Abeokuta Expressway, Sango-Otta, Nigeria; Candid mouth paint (batch no: N2067014; NAFDAC reg. no: 04-6761) manufactured by Glenmark Pharmaceuticals Ltd, Plot N<sub>0</sub> E-37, 39, D road, M.I.D.C Industrial area, Satpur, Nasik-422007, India and Mycogem cream (batch no: 2813U; NAFDAC reg. no: 04-3499) manufactured by Gemini Pharmaceuticals Nigeria Ltd, Plot 13, Block A, Industrial Estate, Amuwo-Odofin, Lagos, Nigeria. The active ingredients of the antifungal drugs were Ketofung oral caplets (ketoconazole 200mg), Flucamed capsules (fluconazole 50mg), Candid mouth paint (clotrimazole), Mycogem cream (bifonazole 1% w/w) and mycoten cream (1g of cream contains 10mg bis-phenyl-(2-chlorophenyl)-1-imidazole-methane).

**Determination of anti-candidal activities of antimycotic agents against oral *Candida* strains using modified agar well-diffusion method:**

Holes, measuring 6.0 mm in diameter were aseptically bored and punched out of sterile SDA agar plates, followed by surface flaming of the agar plates. Each SDA agar plate was then inoculated by streaking the entire surface of the culture plate with each oral *Candida* strain. The seeded plates were then incubated at 30<sup>0</sup>C for 24-48 hrs after dispensing 500 $\mu$ l of the antifungal drug solutions into the agar wells, using the modified method of Tagg *et al.* [20]. The modification was by incorporating the antifungal drug solutions into sterile semi-solid agar before dispensing them into the agar wells to prevent spreading of the drugs on the agar surface. Inhibitory activities depended on the release of diffusible inhibitory metabolites from the antifungal drug solutions into the assay medium during incubation. Inhibitory zones surrounding the agar wells were noted and recorded in mm diameter, while holes without zones of inhibition or inhibition zones less than 10.0 mm were recorded as negative. Results were recorded in triplicates.

**Presentation of toothpastes**

Toothpastes used in this study were manufactured by Unilever Nigeria Plc., RC 113, 1, Billing's way, Oregun, Lagos, Nigeria [Close Up Menthol Chil (NAFDAC reg. no: 02-4984), Close Up Red, New close up Herbal (NAFDAC reg. no: 02-5132)]; Shanghai White Cat, Shareholding. Co Ltd, 1829 Jin Sha Jiang Road, Shanghai, China [Maxam], PZ Nigeria, 45/47, Town planning way, Ilupeju, Lagos, Nigeria [Florish Gel (NAFDAC reg. no: 02-0479)], GlaxoSmithKline Consumer Nigeria Plc, RC 8726, Igbesa road, Agbara, Ogun state, Nigeria [Macleans (NAFDAC reg. no: 02-1989)], Daily Need Ind. Ltd. Plot 10, Oshodi Scheme Ind. layout, Isolo, Lagos [Daily Need family size fluoride toothpaste (NAFDAC reg. no. 02-0338)] African consumer care limited, Plot B, Olympic street, Amuwo Odofin industrial area, FESTAC Town, Lagos, Nigeria [Dabur herbal toothpaste with basil (NAFDAC reg. no: 02-1297)], African consumer care limited, 18 Burma Road, Apapa, Lagos, Nigeria [New Dabur gel, mint and lemon (NAFDAC reg. no: 02-4271)] and Daraju industrial Ltd, 159/161, Ladipo street, Mushin, Lagos, Nigeria [My My Dental fresh (NAFDAC reg. no: 02-3099)].

Active and other ingredients of the toothpastes were- Close Up Menthol Chil [sodium fluoride (1450 ppm fluoride), sorbitol, water, hydrated silica, sodium lauryl sulphate, PEG-32, flavour, cellulose gum, sodium saccharin and limonene], Close Up (Red) [sodium fluoride (1450 ppm fluoride), sorbitol, water, hydrated silica, sodium lauryl sulphate, PEG-32, flavour, cellulose gum, sodium saccharin, eugenol and C116035, C17200], Close Up herbal [sodium fluoride (1450 ppm fluoride), herbal extracts of *Eucalyptus*, peppermint, sage, clove, aloe vera, barbadensis leaf extract C173360, C174260, C177268, C177492, C177891, sorbitol, water, hydrated silica, sodium lauryl sulphate, PEG 32, flavour, cellulose gum, sodium saccharin, trisodium phosphate], MAXAM [F.G.N.C sorbitol, silica, sodium lauryl sulphate flavour, SCMC and sodium monofluorophosphate], Florish [0.76% sodium monofluorophosphate, sorbitol, water, silica, PEG, sodium saccharin, trisodium phosphate and methyl parabin menthol], MyMy [sodium monofluorophosphate, sorbitol, precipitated silica, distilled water, sodium lauryl sulphate, flavour, sodium saccharin, binder PEG, food grade colour], Macleans [sodium fluoride (0.306%w/w), aqua, hydrated silica, sorbitol, glycerin, PEG-6, sodium lauryl sulphate, flavour, xanthan gum, sodium saccharin, C173360, C174160], Dabur herbal with basil [basil oil (0.01%), 5% herbal extracts obtained from bullet wood (*Acacia arabica*), lotus bark, pellitory root, bark of blackberry, chalk (calcium carbonate), sodium lauryl sulphate, blend of peppermint, spearmint, coriander, ginger, *Eucalyptus* and lemon oils, sodium silicate, glycerin, purified water, gum carrageenan, chlorophyllin and sodium saccharin], New Dabur gel mint and lemon [natural lemon extract, flavour containing natural blend of mint, *Eucalyptus*, Rosemary, chamomile, sage, myrrh and other natural oils, sorbitol, silica, treated water, polyethylene, glycol 1500, sodium lauryl sulphate, sodium carboxy methyl cellulose, sodium saccharin, trisodium orthophosphate, citric acid FD and C Blue #1, FD and C yellow #5].

Determination of anti-candidal activities of toothpastes against oral *Candida* strains using modified agar well-diffusion method:

Determination of *in vitro* inhibitory activities of aqueous suspensions of nine toothpastes used in this study was the modification of Tagg *et al.* [20] method. Holes, measuring 6.0 mm in diameter were aseptically bored and removed from sterile SDA agar plates, followed by surface flaming of the agar plates. Each SDA agar plate was then inoculated by streaking the entire surface of the culture plate with each oral *Candida* strain. 500µl of aqueous suspension of each toothpaste was dispensed into each agar well, followed by incubation at 30°C for 24-48 hrs. The modification method was by incorporating the chewing stick extracts into sterile semi-solid agar before dispensing into the holes to prevent spreading of the extracts on the agar surface. Inhibitory activities depended on the release of diffusible inhibitory metabolites from the toothpastes into the assay medium during incubation. Inhibitory zones surrounding the agar wells were noted and recorded in mm diameter. Zones of inhibition and the diameter of the zones in mm were noted and recorded in millimetres, while holes without inhibition zones or zones less than 10.0 mm in diameter were recorded as resistant. Results were recorded in triplicates.

## RESULTS

The total recovery rates of the 86 oral *Candida* strains from healthy human subjects were *C. albicans* 43 (50.0%), *C. glabrata* 5 (5.8%), *C. pseudotropicalis* 10 (11.6%) and *C. tropicalis* 28 (32.6%) as shown in Table 1.

Mycogen cream were inhibited by 30.2% of the oral *C. albicans* strains, while 53.3%, 53.5%, 65.1% and 97.7% of the strains were inhibited by Ketofung oral caplets, Flucamed capsules, Mycoten cream and Candid mouth paint respectively. Higher susceptibility rates of 60.0%, 80.0%, 80.0%, 100% and 100% were displayed by the oral *C. glabrata* towards Mycogen cream, Ketofung oral caplets, Flucamed capsules, Mycoten cream and Candid mouth paint respectively. Lower susceptibility rates of 40.0% was displayed by the oral *C. pseudotropicalis* strains towards mycogen cream, while higher rates of 80.0% was recorded in Ketofung oral caplets, Flucamed capsules, Mycoten cream and Candid mouth paint. Apart from mycogen cream which was inhibited by 32.1% of the *C. tropicalis* strains, moderate to higher susceptibility rates were recorded in other antimycotic agents - flucamed (57.1%), ketofung (60.7%), mycoten cream (71.4%) and Candid mouth paint (89.3%) (Table 2).

The 43 strains of oral *C. albicans* isolated in this study were mostly susceptible *in vitro* to Dabur herbal with basil (100%), Close Up Red (97.7%), Dabur herbal Gel (95.3%), Maxam (93.0%), Close-Up menthol chill (90.7%), Florish gel (88.4%) and Close-Up herbal (76.7%). Minimal inhibitory activities were however, displayed towards MyMy (20.9%) and Macleans (23.3%), while the multiple susceptibility profiles of the *C. albicans* strains were between 44.4 and 100% (Table 3).

Only five oral *C. glabrata* strains were identified in this study, out of which four had 60.0% susceptibility towards Close-Up Red, Florish gel, MyMy and Maxam toothpastes; three had 80.0% susceptibility towards Close-Up Menthol chill, Dabur Herbal Gel and Macleans; while two had 100% susceptibility towards Close-Up Herbal and Dabur Herbal with basil toothpastes (Table 3).

Table 3 shows that 10 oral *C. pseudotropicalis* strains exhibited varying *in vitro* inhibitory activities against the test toothpastes. 7 (70.0%) were susceptible to Close-up Menthol chill and MyMy; 8 (80.0%) were susceptible to Florish Gel; 9 (90.0%) were susceptible to Close-up Red and Dabur Herbal Gel, while 10 (100%) were susceptible to Maxam and Dabur Herbal with basil. Moderate susceptibility 5 (50.0%) were recorded towards Close-up Herbal but low susceptibility of 3 (30.0%) was recorded towards Macleans. Multiple inhibitory susceptibility of between 33.3 and 100% were recorded among the *C. pseudotropicalis* strains.

The *in vitro* susceptibility results of the 28 oral *C. tropicalis* strains indicated varying susceptibility rates- Close-Up Menthol chill, 23 (82.1%), Close-Up Herbal, 18 (64.3%), Close-Up Red, 26 (92.9%), Dabur Herbal Gel, 26 (92.9%), Florish Gel, 21 (75.0%), Macleans, 17 (60.7%), MyMy, 23 (82.1%), Maxam, 26 (92.0%) and Dabur Herbal with basil, 27 (96.4%) (Table 3).

## DISCUSSION

Recurrent oral candidosis is a common problem, especially in immunocompromised patients, and it is frequently triggered by resistance induced by antifungal treatment [21]. The oral *Candida* spp., *C. albicans*, *C. glabrata*, *C. pseudotropicalis* and *C. tropicalis* identified in this study were not species-specific as regards their sources of isolation because they were randomly recovered from tongue, teeth and saliva specimens. They are however, similar to those reportedly isolated from human oral sources in cases of oral candidosis by previous workers [6, 8, 9, 22]. Since there could be varied human oral bacterial species among different nations, it is necessary that differentiation

in oral fungal flora associated with dental carries be put into consideration when preparing antifungal and teeth-cleansing agents.

Pathogenic species of *Candida* are of serious clinical importance because of their severity of their infections, probably due to their resistance to antifungals. Although many antimycotics are available for the treatment of oral candidosis, it was reported that the diluent effect of saliva and the cleansing action of the oral musculature often tend to reduce the availability of the agents below that of the effective therapeutic concentration. Therefore, the yeasts undergo only a limited exposure to the antifungals during therapy [23]; i.e., antifungal agents may effectively treat mucosal candidiasis but their repeated use can lead to colonisation with less susceptible species, and to resistance among normally susceptible strains [23, 24]. Candidal adherence to mucosal surfaces is usually considered as the first step in the pathogenesis of oral candidiasis [4, 25]; however, increased fungal adherence to buccal epithelial cells during antibiotic therapy may explain in part, the increased incidence of *Candida* colonisation in patients receiving antibiotics; i.e., involvement of candidal adherence in mucosal colonisation [26].

Proper oral hygiene will suggestively reduce oral infections, thus, the oral importance of toothpastes in oral health and hygiene. Toothpaste that efficiently reduces oral bacterial flora should therefore, contribute to dental health [11]. Toothpaste is classified as drugs because drugs should contain an ingredient to achieve the effect the consumer desires. The main purpose of toothpaste is may be to reduce oral bacterial flora but it is intended to deliver fluoride to the teeth because fluoride has been proven to protect teeth against attack from bacteria. Root caries can also be successfully treated non-invasively with fluoride toothpastes, as well as the use of antimicrobial toothpastes and mouthwashes [15, 17]. In this study, except in few cases, very high *in vitro* susceptibility rates of 50.0-100% were exhibited by the oral *Candida* species towards the test toothpastes (Dabur Herbal with basil, Close Up Red, Dabur Herbal Gel, Maxam, Close-Up Menthol Chil, Florish Gel and Close-Up Herbal), although minimal *in vitro* inhibitory activities were displayed by some strains towards MyMy (20.9%) and Macleans (23.3-30.0%). However, the inhibitory activities were not species-dependent or strain-dependent, neither were they dependent on type of toothpaste assayed for.

Presently in almost all countries, including the US, there is no available toothpaste with significant anti-fungal properties. Patients who present with candidiasis are therefore placed on an appropriate antifungal medication; with the stress for importune of good oral hygiene, as it has been shown that *Candida* species can live in the biofilm surrounding teeth and on the surface of the tongue. A large number of studies have been performed, which were targeted at the bacterial biofilms [27-29]; meanwhile, little attention has been paid to medically relevant fungal biofilms [30], in spite of the fact that it has been shown that *Candida* species can survive in the biofilm of the mouth [1]. The recorded higher *in vitro* susceptibility profiles displayed by most of the toothpastes in this study may not be replicated *in vivo*, since there is the likelihood that obtainable results *in vivo* will be different. Formation of biofilms by the oral *Candida* strains, as an example would have caused increased adherence to oral surfaces, and thereby, lower susceptibility rates are likely to be recorded.

*Candida* biofilms have been shown to be resistant to the action of clinically important antifungal agents [31-33] and since biofilms contribute to the pathogenesis of oral candidiasis, some of which may be due to dual *Candida* species [34-37]; high prevalence of oral *Candida* may therefore, serve as a likely indication of oral candidiasis (oral thrush). Reduction of oral *Candida* population with potent toothpastes is therefore, of much needed oral health and hygiene significance. Of all the chemical compositions of the types of toothpastes used in this study, the major active ingredients were sodium fluoride (1450 ppm fluoride / 0.306 % w/w) and sodium monofluorophosphate. Fluoride has been a vital agent in caries prevention since the last century; while since the 1940s, researchers have understood the positive effect of fluoride on anti-caries activity. The continuous presence of high-fluoride concentrations was found to have more significant protective effect than enamel fluoride [38].

Topical fluoride therapy (TFT) in the form of toothpastes, mouth-rinses, varnishes and gels are effective caries preventive measures [39, 40]. However, certain alternative chemical agents like sodium lauryl sulfate (SLS), triclosan, monofluorophosphate and glycerophosphate have been found to be more active than fluoride in toothpastes [41-43]. SLS in toothpastes was found to significantly increase the incidence of desquamation of the oral mucosa compared with toothpastes containing the detergent cocoamidopropyl-betaine (CAPB), indicating that sensitive patients may contract mucosal irritation through SLS in toothpastes [44], while another significant consumer health importance is adulteration of toothpastes in developing countries like Nigeria.

As earlier suggested, based on mouth feel in an oral hygiene study [8], which involved checking the teeth with the tongue, at the inner teeth surfaces for any feeling of teeth coating, especially on the molars and premolars; breath smell; presence or absence of saliva foam at the lip sides or minimal spitting while talking for about 15 minutes or more, and lightness of the tongue, it is hereby further advocated that teeth cleansing, at least twice a day is the best

mode of good oral hygiene, most especially since continuous spitting and or accumulation of foamy or thick whitish saliva deposits at the corners of the lips when speaking were prevented or very minimal, due to multiple brushing of the teeth/mouth per day.

Ability to perform regular and effective self-care is important to the long-term success of therapeutic and restorative treatment and overall well-being [45] but the study of Ogunshe and Odumesi [8] further suggested the need for adjunct mouth-cleansing agents for oral hygiene, with regards to oral fungi, since *Candida* species are members of mixed oral biofilms and subject to various antagonistic and synergistic interactions, which are beginning to be explored. It is believed that new insights will allow for more efficacious treatments of fungal oral infections and offer a wide range of potential targets for therapeutic intervention [37] and for improved oral health. However, comparative *in vitro* antimycotic potentials of toothpastes obtained from foreign countries are currently being studied in our laboratories.

**Table 1: Percentage recovery rates of *Candida* species from human oral specimens**

Recovery rates (%)			
	Tongue	Teeth	Saliva
<i>C. albicans</i> (43)	25.6	17.4	9.30
<i>C. glabrata</i> (5)	1.20	1.20	3.50
<i>C. pseudotropicalis</i> (10)	3.50	3.50	4.70
<i>C. tropicalis</i> (28)	9.30	12.7	8.10

**Table 2: *In vitro* mean percentage susceptibility rates of *Candida* species (antifungal agents)**

Lab codes of antimycotics	Mean percentage susceptibility rates of <i>Candida</i> species			
	<i>C. albicans</i> (43)	<i>C. glabrata</i> (5)	<i>C. tropicalis</i> (28)	<i>C. pseudotropicalis</i> (10)
KETO	53.3	80.0	60.7	80.0
FLU	53.3	80.0	57.1	80.0
MYCOT	65.1	80.0	71.4	80.0
MYCOG	30.2	60.0	32.1	40.0
CAND	97.7	89.3	75.0	80.0
	[40.0-100]*	[60.0-100]	[40.0-100]*	[80.0-100]*/**

**Keys:** KETO = Ketofung oral caplets; FLU = Flucamed capsules; MYCOT = Mycoten cream  
MYCOG = Mycogem cream; CAND = Candid mouth paint  
Values in parenthesis are percentage multiple susceptibility rates

**Table 3: *In vitro* mean percentage susceptibility rates of *Candida* species (toothpastes)**

Lab codes of toothpastes	Mean percentage susceptibility rates of <i>Candida</i> species			
	<i>C. albicans</i> (43)	<i>C. glabrata</i> (5)	<i>C. tropicalis</i> (28)	<i>C. pseudotropicalis</i> (10)
CMC	90.7	80.0	82.1	70.0
CH*	76.7	100	64.3	50.0
CR	97.7	20.0^	92.9	90.0
DHG*	95.3	20.0^	92.9	90.0
FG	88.4	60.0	75.0	80.0
MC	18.6^	80.0	60.7	30.0^
MM	23.3^	60.0	82.1	70.0
MX	93.0	60.0	92.0	100
DHB*	100	100	96.4	100
DN	100	92.9	96.4	90.7
	[44.4-100]	[22.2-100]	[33.3-100]	[33.3-100]

**Keys:** CMC = Close-up Menthol Chil      CH= Close-up Herbal CR = Close-up Red  
DHG = Dabur Herbal Gel                      FG= Florish Gel                      MC= Macleans  
MM= MyMy    MX= Maxam                              DHB = Dabur Herbal with basil  
DN = Daily Need  
^ = susceptibility rates less than 50.0%      \* = herbal toothpastes /gel  
Values in parenthesis are percentage multiple susceptibility rates

## REFERENCES

- [1] CM Abraham, *The Open Pathol J*, **2011**, 5: 8-12.
- [2] FC Odds, *Candida and candidosis: a review and bibliography*. 2nd edition London: Bailliere Tindall, **1988**, p.67.
- [3] SB Wey, M Mori, MA Pfaller, RF Woolson, RP Wenzel *Archv Intern Med*, 1988, **148**, 2642-2645.
- [34] RD Cannon, AR Holmes, AB Mason, BC Monk *J Dent Res*. **1995**, 74, 1152-1161.
- [5] A Akpan, R Morgan *Postgrad Med J*, **2002**, 78, 455-459.
- [6] MA Shaheen, M Taha, *Egypt Dermatol Online J*, **2006**, 2 (1), 14.
- [7] Y Zadik, S Burnstein, E Derazne, V Sandler, C Ianculovici, T Halperin, *Oral Dis*, **2010**, 16 (2), 172-175.
- [8] AAO Ogunshe, OG Odumesi, *Afr J Clin Experiment Microbiol*, **2010**, 11 (3), 182-191.
- [9] T Axell, LP Samaranayake, PA Reichart, I Olsen, *Oral Surg Oral Med Oral Pathol*, **1997**, 84, 111-112.
- [10] LP Samaranayake, HB Yaacob, Classification of oral candidosis. In: Samaranayake L P, MacFarlane T W, editors. Oral candidosis. London, United Kingdom: Wright. **1990**, pp. 124-132.
- [11] J Okpalugo, K Ibrahim, US Inyang, *Trop J Pharm Res*, **2009**, 8 (1), 71-77.
- [12] SS Lee, W Zhang, Y Li, *J Am Dent Ass*, **2004**, 135, 1135-1141.
- [13] JK Clarke, *Br J Exp Path*, **1942**, 5: 141-147.
- [14] R Hawkins, D Locker, J Noble, EJ Kay, *Br Dent J*, **2003**, 6: 313-317.
- [15] WJ Collins, TF Walsh, Handbook for dental hygienists, **1998**, pp. 272-273.
- [16] British Dental Health Foundation, FAQ, Caring for my teeth. <http://www.dentalhealth.org>. Accessed on 21 February **2008**.
- [17] B Shrestha, AP Sharma, Manual on practical pharmaceutical microbiology. 1st Ed., 1995; pp. 83-84.
- [18] J Lodder General classification of yeasts. In J Lodder (ed.), The yeasts. 3rd ed., North-Holland Publishing Co., Amsterdam, **1984**.
- [19] JA Barnett, D Yarrow, RW Payne, The yeasts: classification and identification. Cambridge University Press, London. 2nd Ed., **1990**, pp. 50-77.
- [20] JR Tagg, AS Dajani, LW Wannamaker, *Bacteriol Revs*, **1976**, 40, 722-756.
- [21] F Gallè, M Sanguinetti, G Colella, V Di Onofrio, R Torelli, F Rossano, G Liguori, *New Microbiol*, **2011**, 34, 379-389.
- [22] R Latha, R Sasikala, N Muruganandam, RB Venkatesh, *J Microbiol Biotechnol*. **2011**, 1, 113-119.
- [23] AN Ellepola, LP Samaranayake, *J Oral Pathol Med*, **1998**, 27(7), 325-332.
- [24] JD Sobel, SE Ohmit, P Schuman, RS Klein, K Mayer, A Duerr, JA Vazquez, A Rampalo, *J Infect Dis*, **2001**, 183 (2), 286-293.
- [25] RD Cannon, WL Chaffin, *Crit Rev Oral Biol Med*, **1999**, 10, 359-83.
- [26] MA Al-Fattani, LJ Douglas, *J Med Microbiol*, **2006**, 55, 999.
- [27] GA O'Toole, LA Pratt, PI Watnick, DK Newman, VB Weaver, R Kolter, *Methods Enzymol*, **1999**, 310, 91-109.
- [28] GA O'Toole, HB Kaplan, R Kolter *Ann Revs Microbiol*, **2000**, 54, 49-79.
- [29] P Watnick, R Kolter *J Bacteriol*, **2000**, 182, 2675-2679.
- [30] (<http://www.medicalmycology.org/biofilms.htm>, 2009)
- [31] GS Baillie, LJ Douglas, *Antimicrob Agents Chemother*, **1998**, 42, 1900-1905.
- [32] GS Baillie, LJ Douglas, *Enzymol*, **1999**, 310, 644-656.
- [33] JD Chandra, M Kuhn, PK Mukherjee, LL Hoyer, T McCormick, MA Ghannoum, *J Bacteriol*, **2001**, 180, 5385-5394.
- [34] E Budtz-Jorgensen, *Acta Odontol Scandinav*, **1990**, 48, 61-69.
- [35] G Ramage , K Tomsett , BL Wickes, JL Lopez-Ribot , SW Redding, *Oral Surg Oral Med Oral Pathol Oral Radiol Endodon*, **2004**, 98 (1), 53-59.
- [36] ZM Thein, YH Samaranayake, LP Samaranayake, *Arch Oral Biol*, **2007**, 52 (12), 1200-1208.
- [37] JM ten Cate, FM Klis, T Pereira-Cenci, W Crielaard, PWJ de Groot, *J Dent Res*, **2007**, 88 (2), 105-115.
- [38] JR Mellberg *Compend Contin Educ Dentist*, **1997**, 18 (2 Spec No), 37-43.
- [39] LC Martens, RM Verbeeck, *Revs of Belge Med Dentist*, **1998**, 53(1), 295-308.
- [40] VC Marinho, JP Higgins, A Sheiham, S Logan, *Cochrane Database Syst Revs*, **2004**, (1):CD002781.
- [41] G Bruhn, L Netuschil, S Richter, M Brex, T Hoffmann, *Clin Oral Investgv*, **2002**, 6 (2): 124-127.
- [42] S Peter, DG Nayak, P Philip, NS Bijlani *Int Dent J*, **2004**, 54(5 Suppl 1): 299-303.
- [43] HP Müller, KM Barrieshi-Nusair, E Könönen, M Yang, *J Clin Periodontol*, **2006**, 33 (11), 811-818.
- [44] BB Herlofson, P Barkvoll, *Eur J Oral Sci*, **1996**, 104(1): 21-26.
- [45] DM Lyle, Use of a water flosser for interdental cleaning. *Compend Continuing Education in Dentstr*, **2011**, 32, (9): <http://www.dentalaegis.com/cced/2011/12/use-of-a-water-flosser-for-interdental-cleaning> Accessed 28/12/11.