Microbial Biosystems 3(1): 25–33 (2018) ISSN 2357-0334
http://fungiofegypt.com/Journal/index.html Microbial Biosystems
Copyright © 2018 Attia Online Edition
ARTICLE

Antifungal activity of some toothpastes and antiseptic solutions against yeasts isolated from the oral cavity of cancer radiated patients

Attia EA

Departmet of Botany, Faculty of Science, University of Suez Canal, Ismailia 45122, Egypt

Attia EA 2018 – Antifungal activity of some toothpastes and antiseptic solutions against yeasts isolated from the oral cavity of cancer radiated patients. Microbial Biosystems 3(1), 25-33.

Abstract

This study aimed to investigate the antifungal efficacy of different types of toothpaste and some antiseptic solutions with varying concentrations against yeast isolated from the oral cavity of radiated patients suffering from head and neck cancer in radiotherapy unit in Suez Canal University teaching's hospital, Egypt. Eight different species of yeast were isolated taxonomically, assigned to 1 phylum, two classes, one order, and three families and identified phenotypically. The antifungal activity of five kinds of toothpaste and three antiseptic solutions were investigated by agar well diffusion method against the eight isolated yeasts namely: *Candida glabrata*, *C. albicans*, *C. tropicalis*, *C. kefyr*, *C. guilliermondii*, *C. parapsilosis*, *Dipodascus geotrichum* and *Geotrichum beigelii* and expressed as the value of the clear zone diameter, however, Their activity was expressed by minimum inhibitory concentration (MIC). Results showed that toothpaste no 1, 3, 4 and 5 were effective against all tested taxa by variable inhibitory degrees. However, no. 2 showed efficacy only against *C. glabrata*, *C. guilliermondii*, and *C. parapsilosis*, Antiseptic solution no 1 showed the highest efficacy against all tested species except *C. kefyr* while no. 2 was effective against all the tested species except two taxa and finally no. 3 exhibited good antifungal activity against all the tested taxa. In conclusion, we recommended the use of toothpaste contained sodium monofluorophosphate, or sodium fluoride, as an active component followed by a mouthwash which contained Chlorohexidine or Cetylpridinium or miswak as active components to prevent colonization and development of oral candidiasis, especially in cancer patients.

Key words– *Candida*, efficacy, *Dipodascus geotrichum*, *Geotrichum beigelii*, Suez Canal University.

Introduction

The oral cavity is a habitat for a large number of microbiota which coexists with another as a normal microbiome. However, *Candida* species are ovoid budding yeast-like fungi found as a normal commensal in the oral cavity, vagina, and skin and throughout the gastrointestinal tract of humans. Some factors leading to increased incidence of oral thrush and candidiasis as poor oral hygiene, nutritional deficiencies, high carbohydrate diet, diabetes mellitus, heavy cigarette smoking, dental prostheses, treatment by radiotherapy, immunosuppression, and HIV infection (Meghana *et al*. 2015). Thus the changes occur in mechanisms of host defense, and the buccal

Submitted 19 July 2018, Accepted 14 August 2018, Published online 15 August 2018

Corresponding Author: Attia EA – e-mail – emananwarattia@hotmail.com

environment may cause mucosal and periodontal opportunistic infections (Farah *et al*. 2010). Dental caries and related oral diseases like gingivitis and periodontitis are most common oral diseases throughout the world including both developed and developing countries and are continuously increasing with the change in eating custom between peoples of different age group and increased utilization of sugar (Saini *et al*. 2003). Yeasts are among opportunistic pathogenic fungi that able to infect immunocompetent patients and healthy individuals under certain conditions. *Candida albicans* is the most cause of oral thrush, endocarditis, vaginitis, and infection of nails, skin, and lungs and it is also the fungal species which most commonly isolated from infected root canals (Oztan *et al*. 2006). Beside *C. albicans* which is the major pathogen in oral candidosis infections other pathogenic taxa e.g. *C. krusei*, *C. glabrata*, and *C. dubliniensis* have been reported in both compromised and non-compromised hosts and has been isolated from oral plaque and saliva (He *et al.* 2006; Maijala *et al*. 2007; Zhu *et al*. 2008).

Toothpaste has a history that may be dated back to 4000 years ago (Tiwari *et al*. 2008) recently various studies have been carried out by several investigators on the antimicrobial efficacy of different dentifrices on oral microbes (Davies *et al*. 2004; Alsaimary 2008; Okpalugo and Ibrahim 2009; Manupati 2011; Andiara *et al.* 2014; Gibraiel *et al.* 2014; Nwakanma *et al.* 2014) and their effectiveness against the test microorganisms *in vitro*. Many types of toothpaste and mouthwash have been formulated over recent years to contain antibacterials compounds with the aim of preventing or reducing plaque, calculus, gingival inflammation or dental caries without specific antifungal compounds. Despite great improvements in the global oral health status, dental caries remains one of the most prevalent diseases (Abirami and Venugopal 2005; Marsh and Percival 2006; Hatti *et al*. 2007; Inetianbor *et al.* 2014; Glick *et al*. 2017).

This study aimed to evaluate the antifungal efficacy of different types of dentifrices toothpaste and some mouthwash solutions against recovered yeast from the oral cavity of cancer patients irradiated for head and neck cancer in Suez Canal University teaching's hospital, Egypt *in vitro*.

Materials and Methods

Study population

A total number of 80 patients (40 females and 40 males) from the radiotherapy unit in Suez Canal University teaching's hospital were screened for oral yeast species load. Patients were classified as 40 elders (20 females and 20 males) aged between 50 to 78 years and 40 adults (20 females and 20 males) aged between 40 to 50 years old. All selected cancer patients were subjected to radiotherapy treatment of head and neck with a total dose of 44 grays. From each patient, successive samples were taken along the period of his/her treatment. The collected samples were routinely processed for the isolation of yeast. Ethical approval from patients was obtained for this study. Also, about 40 oral samples were collected from healthy volunteers (haven't any sign of oral disease) to compare between cancer patients and healthy peoples.

Sampling, isolation and identification of yeast species

The clinical specimens including saliva, palate mucosa, and tongue dorsum were collected with a sterile swab, immersed in 1 ml sterile saline tube 25 mM) and agitated for 30 seconds. Aliquot of 100 µl from each specimen was directly diluted into 10-fold and 100-fold in sterile saline. From each dilution, aliquots of 100 µl in triplicate were inoculated in the Sabouraud dextrose agar (SDA- Merc/Germany) supplemented with 0.1 mg/ml chloramphenicol and incubated at 37 ºC for 48 hours (Samaranayake *et al*. 2012). Isolates were phenotypically identified by standard-taxonomic yeasts criteria e.g. the production of germ-tube, typical microscopic appearances on cornmeal agar with Tween-80, chlamydospores production, colony morphology and pigment production on chromogenic medium. Yeast species were isolated and identified using appropriate keys according to Kurtzman *et al*. (2011). The identification of all

recovered taxa was confirmed by the API 32 C AUX (bioMerieux, Marcy-l'Etoile, France) identification system for yeasts. Isolates were stored and maintained in Sabouraud dextrose broth accordingly at 4˚C to be used later. Recovered yeasts were examined by using bright field microscopy (Leitz Laborlux S, Germany).

The names of authors of fungal taxa are abbreviated according to Kirk and Ansell (1992). The systematic arrangement in the present list follows the latest system of classification appearing in the 10th edition of Ainsworth and Bisby's Dictionary of the Fungi (Kirk *et al*. 2008) Name corrections, authorities, and taxonomic assignments of all taxa reported in this work were checked against the Index Fungorum database (www. indexfungorum.org).

Toothpaste and oral disinfectant solutions

Five different kinds of commercial toothpaste and three oral washes were selected as they the most used in our daily life then evaluates them as antifungal against recovered oral yeast, which was purchased from local pharmacies in Ismailia, Egypt. The different kinds of toothpaste ingredients and oral disinfectant solutions were listed in Table 1 and 2.

Table 1 Ingredient details of toothpaste formulations tested for antifungal potential.

Toothpaste	Ingredients as listed on packages
Toothpaste No. 1	Calcium carbonate, sorbitol, hydrated silica, sodium lauryl sulfate, sodium monofluorophosphate, aroma, cellulose gum, potassium citrate, trisodium phosphate, sodium bsaccharin,calcium glycerophosphate,, phenylcarbinol, glycennin
Toothpaste No. 2	Potassium nitrate5% w/w, Sodium fluoride 0.32% (1450ppmf).
Toothpaste No. 3	Sorbitol, water, hydrated silica, sodium laryl sulfate, falvour,cellulose gum, sodium fluoride (1450ppmf), sodium saccharin, eugenol.
Toothpaste No. 4	Aqua, hydrated silica, sodium laryl sulfate, cellulose gum,aroma, sodium saccharin, sodium fluoride,carbomer,C177891 ,trisodiumphosphate,limonene,C174160
Toothpaste No. 5	Calcium carbonate, sorbitol, treated water, silica, sodium laryl sulfate, falvour, miswak extract, sodium carboxy methyl cellulose, and or sodium carrageenate, sodium silicate, sodium benzoate, glycerine, sodium saccharin.

Table 2 Ingredient details of three antiseptic solutions tested for antimicrobial potential.

Mouthwash solution	Ingredients as listed on packages
Mouthwash No. 1	Chlorohexidine 0.1%, Propolis 1%, clove oil 1%
Mouthwash No. 2	Each 1 ml contains Nystatin 100,000 IU
Mouthwash No. 3	Each 100 g contains:- lidocaine HCl 2.0g, Cetylpridinium chloride 0.1 g.

Antifungal activity against isolated yeasts

A culture of each taxon (48 hr old) was suspended in 5 ml of sterile saline solution. A volume of 0.1ml of the microbe suspension was adjusted to 0.5 McFarland standards (approximately 1 X $l0^6$ to 5 X $l0^6$ CFU for yeast was seeded in the SDA plates. For estimation of toothpaste efficacy, five wells (6mm in diameter and 4 mm in depth) at equal distance in each seeded agar plate were created by sterile cork borer. Each well was filled with 1.0 g (0.8 ml) of toothpaste which withdrawn by a sterile syringe under sterile conditions, then plates were

incubated at 37 ºC for 48 h. For assessing of oral disinfectants, three wells of each seeded agar plate were created by the same volume by cork borer. Mouthwash under investigation was added to each well (0.8 ml) and incubated for 48 h at 37 °C. The effect of toothpaste and disinfectant solution on yeast growth was determined by measuring the diameter of inhibition zone against 25 mg fluconazole discs® (Oxoid) as a positive control (Lai *et al*. 2001; Takahashi *et al*. 2006; and Laina *et al* 2016).

Determination of minimum inhibitory concentration (MIC)

The MIC of the different toothpaste and the mouthwash against the tested organisms were determined. The minimum inhibitory concentration (MIC) was the lowest concentration of the toothpaste and the Mouthwash that inhibit the growth of yeast cells, as indicated by the absence of growth (Houshmand *et al*. 2013).

Statistical analysis:

Statistical analysis was performed using Analytical Software SPSS® 13.0 (2005). The values were compared by one way ANOVA followed by Duncan's test and the result considered significant when $p<0.05$. All the experiments were held in triplicates.

Results and Discussion

A total of 668, 616 yeast colony-forming units (CFU) from elder patients female and male and 514 (CFU) and 490 (CFU) from adult patients female and male respectively were recovered during the entire study. Only a total number of 100 CFU were recovered from the healthy volunteers. All identified taxa were deposited in Suez Canal University Fungarium (SCUF) at Arab Society for Fungal Conservation. From the cancer patients eight species namely: *Candida glabrata, C. albicans, C. tropicalis, Candida kefyr, C. guilliermondii, C. parapsilosis, Dipodascus geotrichum* and *Geotrichum beigelii* were recovered while from the healthy volunteers only *Candida tropicalis* and *Saccharomyces cerevisiae* were obtained (Fig. 1, Table 3). Taxonomically, isolated species were assigned to 1 phylum, two classes, one order, and three families. Order Saccharomycetales accommodates the most significant range of species (7 species) followed by Trichosporonales (1 species). Taxa with uncertain position were distributed among subclasses, orders and families.

On the higher taxanomic level, Ascomycota was represented only by seven species (87.5 % of the total species number) and Basidiomycota (1 species, 12.5 %). Isolated species belonged to three genera. The dominant geneus was *Candida* (6 species; 75% of the total isolates), and the remaining genera were represented only by one species each. The species genus ratio (S/G) per family, however, shows that family with uncertain position (Incertae sedis) in Saccharomycetales was the most diverse taxonomical rank by recording a ratio of (6) followed by Dipodascaceae (2).

Table 3 Presence/absence of recovered yeast species during the study from cancer patients.

Recovered yeast	Adult	Elder
Candida albicans (C.P. Robin) Berkhout	+	+
C. tropicalis (Castell.) Berkhout	+	+
C. glabrata (H.W. Anderson) S.A. Mey. & Yarrow	_	+
C. guilliermondii (Castell.) Langeron & Guerra	+	+
C. kefyr (Castell.) Basgal	+	+
C. parapsilosis (Ashford) Langeron & Talice	+	
Dipodascus geotrichum (E.E. Butler & L.J. Petersen) Arx	+	+
Geotrichum beigelii (Küchenm. & Rabenh.) Coudert	+	+

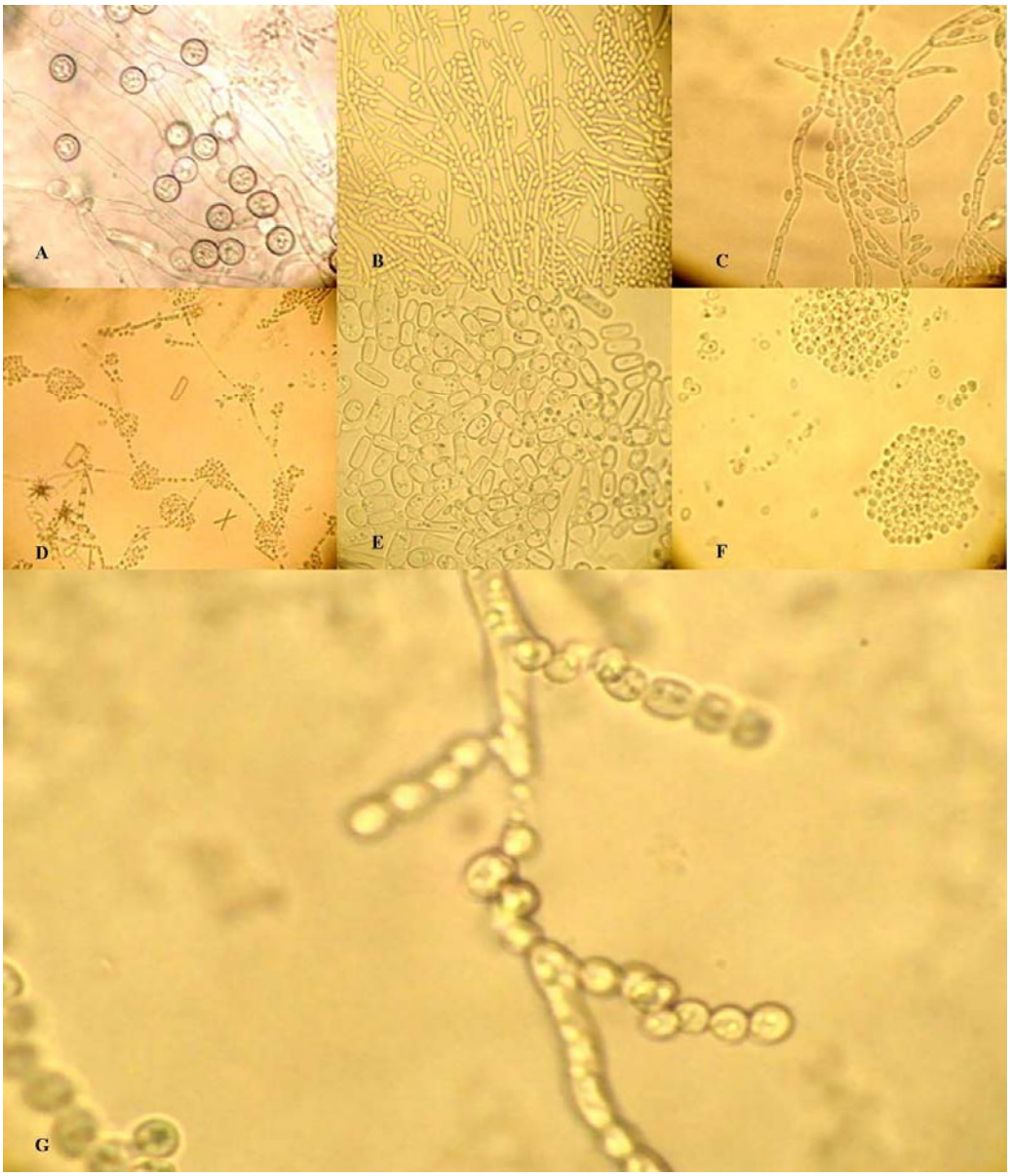

Fig. 1 - Isolated yeast, A- *Candida albicans*, B- *C. tropicalis*, C- *C. kefyr*, D- *C. guilliermondii*, E- *Dipodascus geotrichum*, F- *Candida glabrata*, and G- *Geotrichum beigelii* (X400).

Head and neck cancer patients treated with radiotherapy were suffering from oral mucositis, fungal infection, salivary gland dysfunction (xerostomia), dental caries and taste dysfunction, however, these due to dysfunction in the salivary gland which leads to a decrease in saliva secretion (defense protein) which protect the mouth from microbes (Khan *et al*. 2010).

Most studies on the antimicrobial activity of toothpaste and antimicrobial compounds have focused on inhibition of bacterial growth, but are less focused on the antifungal activity (Adwan *et al*. 2012). There is an increasing incidence of neck cancer in adult as in children, which by far leads to increase the oral fungal infection. These types of patients need toothpaste and mouthwash with antifungal activity following brushing. So, this study was designed to evaluate the antifungal efficacy of different dentifrices toothpaste and some antiseptic solutions against yeast isolated from the oral cavity of cancer patients.

Our data revealed that all the five different toothpaste brands showed varying inhibitory activity degree against the oral tested isolates without dilution as shown in Table (4). Among the five toothpastes, brand-1 showed the highest zone of inhibition on *C. albicans* (2.43 ±0.03). However, brand-3 showed the highest zone of inhibition on *C. parapsilosis* (3.06 ±0.07); brand-4 and brand-5 showed the highest zone of inhibition on *Dipodascus geotrichum* (2.60 ±0.0, 3.20 ±0.10) respectively. Brand 2 showed the least effect on yeast species. However, it had no effects on *C. albicans; C. tropicalis, C. kefyr, D. geotrichum* and *G. beigelii.*

Table 4 Inhibition zone diameter in (mm) of the 5 tested toothpastes against isolated yeast species.

Species	Toothpaste No				
	1	**2**	**3**	**4**	**5**
Candida albicans	2.43 ±0.03 ^{d}	Nil	1.73 ±0.033^{b}	2.55 ±0.05 ^{d}	2.10 ±0.10 ^{c}
C. tropicalis	2.06 ±0.07^{c}	Nil	1.50 ±0.057^{b}	2.55 ±0.05 ^{d}	1.95 ±0.05 ^{c}
C. glabrata	2.03 ±0.03^{b}	1.56 ±0.06 ^{a}	2.43 ±0.066^{c}	2.30 ±0.10 ^{c}	1.60 ±0..a
C guilliermondii	2.16 ±0.088 ^{d}	0.3 ±0.03 ^{a}	1.30 ±0.06 ^{b}	1.55 ±0.05 ^{c}	1.55 ±0.05 ^{c}
C. kefyr	1.63 ±0.09 ^{b}	Nil	2.03 ±0.033 ^{c}	1.45 ±0.05 ^{b}	1.55 ±0.05 ^{b}
C. parapsilosis	1.53 ±0.09^{b}	0.6 ±0.06 ^{a}	3.06 ±0.07 ^{d}	2.00 ±.0.0 ^{c}	3.15 ±0.050^{d}
Dipodascus geotrichum	2.26 ±0.07^{b}	Nil	2.60 ±0.06 ^{c}	2.60 ±.0.0 c	3.20 ±.0.10 ^{d}
Geotrichum beigelii	1.60 ±0.06 ^{c}	Nil	1.60±0.06 ^{c}	1.35 ±.05 ^{b}	2.50 ±0..0 ^{d}
MIC (%w/v)	0.50	1.0	0.55	0.60	0.7

- Data represented in means of triplicates ± standard deviation.
- $^{A,b,c,..}$ Means values in the same column marked with unlike letters are significantly different (p<0.05).

Toothpaste 1 contained the active component sodium monofluorophosphate which give an antifungal effect against all the tested species. However, it gives a high zone of inhibition on *Candida albicans* (2.43 ±0.030), this result was confirmed previously by Flisfisch *et al*. (2008) who reported that sodium monofluorophosphate has un activity against *C. albicans*.

Toothpaste 2 contained the active component sodium fluoride, which had no effects on *C. albicans; C. tropicalis, Candida kefyr, Dipodascus geotrichum* and *Geotrichum beigelii*. These data were in contrast with Satari (1990) who reported that fluoride demonstrates some antibacterial and antifungal effects. Fluoride inhibited glycolysis and prevented the transfer of glucose into cells (Ellepol and Amaranayake 2001).

Toothpaste 3 and 4 contained the same active component sodium fluoride, but they had a potent fungal inhibition effect on the all tested oral yeast species. They had a high zone of inhibition on *Candida parapsilosis* (3.06 ± 0.66) and *Dipodascus geotrichum* (2.60 ±.0.0) respectively. Although the toothpaste 2 contained the same active components sodium fluoride, toothpastes 3 and 4 were more active against the most isolated oral yeast species because they contained other several components that may make a synergistic effect with the active components to become more active. Toothpaste 3 contained sorbitol, water, hydrated silica, sodium lauryl sulfate, flavour, cellulose gum, sodium saccharin, eugenol, while the toothpaste 4 contained aqua, hydrated silica, sodium lauryl sulfate, cellulose gum, aroma, sodium saccharin, carbomer, C177891, trisodium phosphate, limonene, C174160 as shown in table 1.the different toothpastes activity was expressed by minimum inhibitory concentration (MIC), The MIC of tested toothpastes ranged between 0.50 to 1.2 % (w/v). It could be due to the different type and concentration of the active ingredients incorporated in each type as shown in table 4.

The three tested antifungal oral wash solutions showed varying inhibitory effect against the tested isolates without dilution.The three antifungal types of mouthwash 1, 2 and 3 showed the highest zone of inhibition against *C. glabrata* as 4.60±0.05, 4.40± 0.05 and 3.33±.33 respectively. However, mouthwash (1) showed zero effects on *C.kefyr*, mouthwash (2) showed no effect on *C. albicans* and *C. guilliermondii* (Table 5).

Table 5 Inhibition zone diameter in (mm) of the 3 antifungal compounds against isolated yeast species.

Species	Antifungal compounds		
	Compound (1)	**Compound (2)**	**Compound (3)**
Candida albicans	2.5333±0.03 [b]	Nil	2.333±0.33 [b]
C. tropicalis	2.600±0.03 [a]	3.50±1.50 [b]	3.200±0.91 [b]
C. glabrata	4.60±0.05 [a]	4.40±0.05 [a]	3.33±0.33 [b]
C. guilliermondii	01.10±0.05 [c]	Nil	0.533±0.03 [b]
C. kefyr	Nil	2.03±1.01 [a]	0.500±.0.0[a]
C. parapsilosis	0.366±0.18 [a]	0.600±0.06 [a]	0.533±0.03 [a]
Dipodascus geotrichum	0.500±.0.0[a]	2.00±0.58 [b]	2.00±0.58[b]
MIC (%w/v)	0.56	0.88	0.60

- Data represented in means of triplicates ± standard deviation.
- [A,b,c,..]Means values in the same column marked with unlike letters are significantly different (p<0.05).

The different antifungal compounds activity was expressed by minimum inhibitory concentration (MIC), which ranged between 0.56 to 8.8 % (w/v). These could be due to the different type and concentration of the active ingredients incorporated in each type as shown in table 5.

The use of antifungal mouthwash after brushing is necessary for complete removal of fungi from the oral cavity of cancer patients which treated with radiotherapy, however, in our work, the antiseptic wash no 1 contains chlorohexidine as an active antifungal component which may be the main factor for the inhibition against all the isolated oral yeast species except *C. kefyr*.

These results in agreement with previous work carried out by Shrestha *et al*. (2011) that chlorhexidine molecule is a highly cationic chlorophenyl bisbiguanide and keenly binds to negatively charged surfaces including epithelial cells. Besides, it was shown to adsorb onto enamel and salivary proteins. Also, about 30% of the total chlorhexidine dose may be retained in the mouth for 24 hours after a 1-minute rinse, although most of the agent is removed from the oral cavity within the first hour. Besides all of these, it can be used as antifungal therapy for *candida* infection, as it induces coagulation of nucleoproteins, inhibits budding, and makes changes in the cell wall that lead to cytoplasm component escape and yeast death.

Nystatin based antiseptic wash no 2 inhibited most of the isolates but showed no effect on both *C. albicans* and *C. guilliermondii*. These results proved previously by Ebelle *et al*. (2017) which showed that the potency of nystatin is both fungistatic and fungicidal *in vitro* against a great number of yeast fungi. Nystatin acts by binding to sterols in the cell membrane of susceptible *Candida* species with a resultant change in membrane permeability allowing leakage of intracellular components. Nystatin exhibits no significant activity against bacteria, virus or protozoa.

Antiseptic wash no 3 contained acetyl pyridinium chloride as an active component, it inhibited all the tested oral isolates. McDonnell and Russell (1999) reported that the cetylpyridinium chloride (CPC) is a cationic quaternary ammonium compound that is usually used in mouthwashes to avoid or treats candidiasis and bacterial infections. CPC alters the surface tension of the cell wall structure which may lead to cell wall leakage.

Conclusions

Oral cavity fungi always make oral complications in immunocompromised patients, especially in cancer radiated patients. So it is urgently required a specific toothpaste and antiseptic oral wash that have a potent antifungal activity to decrease the oral candidiosis. From this study, we recommended the use of toothpaste contained sodium monofluorophosphate, or sodium fluoride, as an active components and mouthwash which contained Chlorohexidine,

Cetylpridinium or miswak as active components to prevent colonization and development of oral candidiasis, especially in cancer patients.

Conflict of interest

None to declare.

References

Abirami C.P., and Venugopal P.V.V. 2005. In *vitro* evaluation of antifungal activity of toothpastes. J Mycol Med, 15:247-9 .

Adwan G., Salameh Y., Adwan K. and Barakat A. 2012. Assessment of antifungal activity of herbal and conventional toothpastes against clinical isolates of *Candida albicans.* Asian Pac J Trop Biomed, 2: 375-379.

Alsaimary I. 2008. Efficacy of some antibacterial agents against *Streptococcus* mutans associated with tooth decay. The internet J. Microbiology, 7: 1-5.

Andiara D.R., Danielly C.A., Raquel A.B., Alexandra M.Q., and Paulo N.F. 2014. Antimicrobial activity of toothpastes containing natural extracts, chlorhexidine or triclosan. Brazilian Dental Journal, 25:186-190.

Davies R.M., Ellwood R.P. and Davies G.M. 2004. The effectiveness of toothpaste containing triclosan and polyvinyl-methyl ether maleic acid copolymer in improving plaque control and gingival health: a systematic review. J. Clin. Periodontol., 31:1029-33.

Ebelle R.E., Mouokeu R.S., Assam J.P.A., Hopogap M.L., Masoohe A.M., Hiol M.C., Tientcheu R., Ngono N.R.A. and Etoa F.X. 2017. Potentiating antifungal activity of Fluconazole or Nystatin with methanol bark extract of *Harungana madagascariensis* stem bark. International Journal of Pharmacognosy and Phytochemical Research, 9: 674 -679.

Ellepol A.N.B.and Amaranayake L.P. 2001. Adjunctive use of chlorhexidine in oral candidoses: a review. Oral Dis, 7:11-17.

Farah C.S., Lynch N. and McCullough M.J. 2010. Oral fungal infections: An update for the general practitioner. Aust Dent J, 55(Suppl 1):48-54.

Flisfisch S., Meyer J., Meurman, J.H. and Waltimo T. 2008. Effects of fluorides on *Candida albicans*. Oral Dis., 14: 296- 301.

Gibraiel F., Rajput M., Rajput M.S., Manisha S., Vishal N.S.A. and Jha K. 2014. In *vitro* study to investigate the antimicrobial efficacy of different toothpastes and mouth rinses. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5: 245-257.

Glick M., Williams D.M., and Kleinman D.V., et al. 2017. A new definition for oral health developed by the FDI World Dental Federation opens the door to a universal definition of oral health. J Public Health Dent, 77: 3–5

Hatti S., Ravindra S., Satpathy A., Kulkarni R.D. and Parande M.V. 2007. Biofilm inhibition and antimicrobial activity of a dentifrice containing salivary substitutes. Int J Dent Hyg, 5: 218-24.

He X.Y., Meurman, J.H., Kari K.,Rautemaa R. and Samaranayake L.P. 2006. In *vitro* adhesion of Candida species to denture base materials. Mycoses, 49: 80-4.

Houshmand B., Mahjour F., and Dianat O. 2013. Antibacterial effect of different concentrations of garlic (*Allium sativum*) extract on dental plaque bacteria. Indian Journal of Dental Research, 24: 71–75.

Index Fungorum database. Avaliable from: http://www.indexfungorum.org/names/Names.asp (accessed 7 June 2018)

Inetianbor J.E., Ehiowemwenguan. G., Yakubu J.M. and Ogodo A.C. 2014. In-vitro antibacterial activity of commonly used toothpastes in Nigeria against dental pathogens. J. Adv Scires, 5:40-45.

Khan M.S.A., Ahmad I., Aqil F., Owais M., Shahid M. and Musarrat J. 2010.Virulence and pathogenicity of fungal pathogens with special reference to *Candida albicans*. Compating

fungal infection, 539: 21- 45.
Kirk P.M.,and Ansell A.E. 1992. Authors of Fungal Names Index of Fungi Supplement. International Mycological Institute, Institute of CAB, Kew. Surry, UK, pp: 95.
Kirk P.M., Cannon P.F., Minter D.W., and Stalpers J.A. 2008. Ainsworth & Bisby's Dictionary of the Fungi, 10th Edition. CABI Europe - UK: [i]-xi, 1 -771.
Kurtzman C.P.,Fell J. W., Boekhout T., and Robert V. 2011. Methods for isolation, phenotypic characterization and maintenance of Yeasts. The Yeasts (Fifth Edition) Chapter 7 - (pp. 87-110). London: Elsevier.
Lai C.C., Huang F.M., Chan H.W.Y.Y ., Huang M.S, and Chang M.Y.C.C. 2001. Antimicrobial activity of four root canal sealers against endodontic pathogens. Clin Oral Invest., 5: 236-9.
Marsh P.D. and Percival R.S. 2006. The oral microflora friend or foe? Can we decide? Int Dent J 56: 233-9.
Laina B., Metawee M., Benja N., Suwanna C. and Anek. P. 2016. Inhibitory activities of herbal based toothpaste on germ tube and adhesion of *Candida albicans*. journal of pure and applied microbiology, 10: 2551-2556.
Maijala M.,Rautema R., Jarvensivu.A., Richardson. M., Salo.T. and Jaderhane L.T. 2007. *Candida albicans* does not invade carious human dentine. Oral Dis,13: 279-84
Manupati. P. 2011. Antimicrobial Efficacy of mouth rinses. Dental Res Journal, 8: 85- 94.
McDonnell G., and Russell D. 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev., 12: 147-79.
Meghana S.K., Bhaskar U.A. and Sowmya B. 2015. Assessment of antifungal activity of six popular toothpastes against clinical isolates of *Candida albicans*. International Journal of Applied Dental Sciences, 5: 1-4.
Nwakanma C., Ejim C., and Unachukwu.2014The effects of selected toothpaste on the microbial flora of the mouth of GOU student. Int. J. Current Microbiology and Applied Sciences, 3: 785-792.
Okpalugo K. and Ibrahim U. 2009. Toothpaste formulation efficacy in reducing oral flora. Tropical J. Pharmaceutical Research, 8: 71 -77.
Oztan M.,Kiyan M. and Gerceker D. 2006. In-vitro antimicrobial effect of gutta-percha points containing root canal medications against yeast and *Enterococcus faecalis*. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics, 102: 410-416.
Satari H.M. 1990. Pengaruh larutan Natrium Florida terhadap *Streptococcus* mutans dalam upaya pencegahan karies [Tesis]. Bandung: Fakultas Kedokteran Gigi, Universitas Padjadjaran. pp 79-99.
Saini S., Aparn A., Gupta N., Mahajan A. and Arora D.R. 2003. Microbial flora in orodental infections. Indian J Med Microbiol, 21: 111-114 .
Samaranayake L. 2012, Diagnostic microbiology and laboratory methods in Essential Microbiology for Dentistry; 4 th edition. Churchill, Livingstone. Elsevier, p: 49-65.
Shrestha A., Rimal J., Rao A., Sequeira P.S., Doshi D . and Bhat G.K. 2011. In *vitro* antifungal effect of a mouthwash containing chlorhexidine and thymol, J Dent Sci., 6: 1-5.
Takahashi Y., ImazatoKaneshiro S., Ebisu S., Frencken J.E, and Tay F.R. 2006. Antibacterial effects and physical properties of glass-ionomer cement containing chlorhexidine for the ART approach. Dent Mater., 22: 647-52.
Tiwari K. B., Shrestha U.T., Acharya. A., Subedi B., Paudyal B.J., Nawal M ., Shakya P. and Agrawal P. 2008. Antibacterial activities of locally used toothpaste against dental pathogens. Journal of Institute of Medicine, 30:15-18.
Zhu H.W., McMillan A.S., McGrath, C., Li. L.S.W and Samaranayake L.P. 2008. Oral carriage of yeasts and coliforms in stroke sufferers: a prospective longitudinal study. Oral Dis, 14: 60-66.