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Research Article

COMPARATIVE ANALYSIS OF THE ANTIFUNGAL POTENTIALS OF SOME BRANDS OF TOOTHPASTES COMMONLY SOLD WITHIN EKPOMA METROPOLIS IN EDO STATE, NIGERIA

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ABSTRACT

Objectives: To evaluate the antifungal activity of some brands of toothpastes commonly sold to University students, within Ekpoma Metropolis of Edo State, Nigeria. **Materials and Methods:** Antifungal activity was determined by agar well diffusion method, were known weights of toothpastes were dissolved in 5ml of sterile pyrogen-free distilled water, to get concentrations varying from 12.50 to 200.00mg/ml respectively. These were filled into wells punched into sabouraud dextrose agar medium plates inoculated with the test isolates, incubated at 35^{o}C. Inhibition zone diameters were read after 48 hr. **Result:** Maclean's and Dabur Herbal toothpastes had antifungal activity against *Microsporumcanis*, *Aspergillusniger* and *Candida albicans*, with zones of inhibition which ranged from 12 to 39mm. There was no activity against *Aspergillusflavus*. Close-Up toothpaste had antifungal activity against *Microsporumcanis*, *Aspergillusflavus* and *Candida albicans*, with zones of inhibition which ranged from 13 to 42mm and no activity against *Aspergillusniger*. Close-Up Herbal toothpaste had antifungal activity against *Microsporumcanis* and *Candida albicans*, with no activity against *Aspergillus species* used in this study. It produced zones of inhibition which ranged from 24 to 45mm.
Conclusion: The various toothpastes evaluated had antifungal activities which varied and they may be useful in reducing the pathogenic potentials of the various fungi.

Keywords: Antifungal potentials, toothpaste, comparative analysis, non oral fungi.

1.0 INTRODUCTION

Fungal infections/diseases are infections/diseases caused by a fungus, a type of micro-organism. They can be broadly classified on the basis of causative agents as: (a) dermatophytosis (an example of causative agent is *Microsporumcanis*), (b) histoplasmosis (e.g. *H. capsulatum*), (c) blastomycosis (e.g. *B. dermatididis*), (d) coccidiomycosis (e.g. *C. immitis*), (e) Candidiasis (e.g. *C. albicans*), (f) cryptococcosis (e.g. *C. neoformans*), (g) aspergillosis (e.g. *Aspergillus species*), (h) hyalohyphomycosis (e.g. *Fusarium species*) and (i) zygomycosis (e.g. *Rhizopus species*) (Sullivan *et al*., 2005). Transmission maybe via soil, inhalation of spore, contact with contaminated animals and humans or secretions from infected persons, plant debris, ingestion of toxin contaminated plant parts amongst others (Weitzman and Summerbell, 1995; Hogan *et al*., 1996; Pommerville, 2004; Rappleye and Goldman, 2006; Willey *et al*., 2008). People at risk of fungal infections include those taking strong antibiotics (especially for a long period of time), people with weakened immune systems (such as people with HIV/AIDS or those taking steroid medications or chemotherapy), diabetic patients, the very young and the very old. Antibiotics kill bacteria (including healthy bacteria) which can alter the balance of micro-organisms in the mouth, vagina, intestines and other places in the body. This can result in an overgrowth of fungus. HIV/AIDS, steroid medications or chemotherapy all suppress the

immune system while the elevated level of sugar in diabetic patients provide food for some fungi and encourage its overgrowth. Fungal infections/diseases can cause varied symptoms, depending on the type of infection and the area of the body affected. Complications from such infections/diseases can be serious or even life-threatening.

Toothpastes are the most common vehicles for delivery of drugs to the oral cavity. They have been formulated to contain chemotherapeutic agents to improve oral health, provide inhibitory action on plaque formation and bacteria and Candida colonisation (Adejumo*et al*., 2008; Sadeghi and Assar, 2009; Prasanth, 2011). Different types of toothpastes have their own composition and concentration of ingredients for their efficacy, with the success of any toothpaste, in part, being determined by its ability to eliminate pathogenic oral micro-organisms and decrease the risk of infection in the mouth (Ghaleb*et al*., 2012).

In a healthy host, opportunistic fungal pathogens are commensal fungi commonly colonizing human mucosal surfaces. The oral cavity is known to harbour a large number of micro-organisms which coexist with one another as normal micro biota and sometimes, some non oral micro-organisms may find its way into the oral cavity. However, under compromised conditions, these opportunistic fungal pathogens or micro-organisms may cause infections/diseases in the skin, oral cavity, hair, nails, feet, e.t.c. Thus, since the antifungal activities reported in literature for different toothpastes were on *Candida albicans*, which is considered as the most common yeast isolated from the oral cavity (Yigit*et al*., 2008; Ellepola*et al*., 2011; Ghaleb*et al*., 2012), we choose to investigate the antifungal activities of different toothpastes on non oral fungi such as *Aspergillusflavus*, *Aspergillusniger*, *Microsporumcanis*and *Candida albicans* which maybe opportunistic fungal pathogens colonizing at several anatomically distinct surfaces of the human body, mainly in warm and moist areas such as the oral cavity, vaginal and the skin.

2.0 MATERIALS AND METHODS

2.1 Test Micro-organisms

Four (4) test organisms were used in this study. Pure cultures of *Aspergillusflavus*and *Aspergillusniger*isolated from food (yam) were obtained from the Department of Pharmaceutical Microbiology, University of Benin, Benin City, Edo State, Nigeria; while pure cultures of *Microsporumcanis*and *Candida albicans*isolated from infected human skin and pubic region respectively were obtained from the University of Benin Teaching Hospital (UBTH), Benin City. Sub-culturing was carried out aseptically by transferring a loopful of each of the organism into sabouraud dextrose agar slants by streaking the surface of the agar.

2.2 Evaluation of toothpastes

Four (4) brands of toothpastes (Close-Up Herbal, Close-Up, Maclean's and Dabur Herbal) commonly sold to and bought by University students at Ekpoma metropolis were purchased and used for this study. The composition of these toothpastes is given in Table 1. Each of the toothpaste was weighed into six clean and sterilized bottles that were properly labelled, using an electronic balance at various weights of 0.0625, 0.1250, 0.2500, 0.5000, 0.7500 and 1.0000g respectively. They were dissolved in 5ml of sterile pyrogen-free distilled water to get concentrations of 12.50, 25.00, 50.00, 100.00, 150.00 and 200.00 mg/ml respectively.

2.3 Antifungal assay

The antifungal activity of the toothpastes was determined by agar diffusion method. Growth from a freshly sub-cultured isolate was suspended in 10ml of sterile saline to obtain a turbidity of 0.5 McFarland standard. Using a sterile swab, the sabouraud dextrose agar plates, each containing 60ml of medium were evenly inoculated with the fungus suspension. A uniform quantity (1ml) of each toothpaste concentration was filled into 9mm diameter wells punched into the pre-inoculated plates. The plates were incubated at 35^{o}C and readings for inhibition zone diameters (mm) were taken after 48hr. All the plates were made in duplicates and the experiments were repeated twice (Ghaleb*et al*., 2012). The final results were recorded and values expressed as mean diameters.

3.0 RESULTS AND DISCUSSION

The antifungal activities of four different brands of toothpastes were investigated against *Aspergillusflavus*, *Aspergillusniger*, *Microsporumcanis*and *Candida albicans*using a standard agar well diffusion method. The composition of each of the toothpaste used in this study is as shown in Table 1.

The different brands of toothpastes exhibited variations in their inhibitory effect against the test organisms. The results showed that, Close-Up herbal toothpaste formulation had the best activity against *Microsporumcanis*and *Candida albicans*based on the mean diameter zone of inhibition values. Hence, it is superior in activity compared to the other three brands of

toothpastes tested. It produced zones of inhibition which ranged from 24 to 45mm while it had no activity against the *Aspergillus species* used in this study. Close-Up toothpaste had antifungal activity against *Microsporumcanis, Aspergillusflavus* and *Candida albicans*; with zones of inhibition which ranged from 13 to 42mm. It had no activity against *Aspergillusniger* at the tested concentrations. Maclean's and Dabur herbal toothpastes had antifungal activities against *Microsporumcanis, Aspergillusniger* and *Candida albicans*, with no activity against *Aspergillusflavus*. It produced zones of inhibition which ranged from 12 to 39mm. From results gotten, while Close-Up herbal had the best antifungal activities against *Microsporumcanis*and *Candida albicans*; Close-Up, Maclean's and Dabur herbal toothpastes could be said to have a broader spectrum of activity against the tested micro-organisms (fungi) than Close-Up herbal. These are seen in Table 2.

The herbal dental formulations studied appeared to be more effective than the fluoride dental formulations against *M. canis*and *C. albicans*. Close-Up (a fluoride dental formulation) had superior activity against *A. flavus*than the herbal dental formulations while maclean's appeared to be more effective than the herbal dental formulations against *A. niger*. These are seen in Table 3. Any agent that is able to kill susceptible fungi or inhibit their growth is considered antifungal. If the agent destroys pathogenic micro-organisms or inhibit their growth at concentrations low enough to avoid undesirable damage to the host, it is said to be a chemotherapeutic agent. Antifungal agents could be highly effective against fungi while showing little or no effects on others (Murray, 1995). Most studies on the antifungal activity of dentifrices have been focused on their potentials to inhibit *Candida albicans*(Yigit*et al*., 2008; Ellepola*et al*., 2011). In particular, no information was found in recent literature research concerning their antifungal potentials against other fungi, which may be opportunistic in nature.

The results obtained from the antifungal effect of the various brands of toothpastes showed that the toothpastes possessed some inhibitory effect that varied against the different species of fungi. This is similar to the findings of Ellepola*et al*., 2011 and Ghaleb*et al*., 2012 who showed that different toothpaste brands possessed some inhibitory effect that varied against different *Candida species*. The antifungal activity of the herbal dental formulations observed to be more effective than that of the fluoride dental formulations against *M. canis*and *C. albicans* ,may most probably be due to the synergistic effect between the active ingredients of these toothpastes, which are a combination of sodium fluoride or sodium monofluorophosphate and herbal extracts. The antifungal activity of the herbs is usually due to the presence of by-products called secondary plant metabolites or phytochemicals. Consumers who use herbal products often view these products as being safer than products that have chemicals (Lee *et al*., 2004; Minejade*t al*., 2011; Ghaleb*et al*., 2012), although toothpaste allergy resulting from herbal and conventional toothpastes have been reported (Robertshaw and Leppard, 2007; Zirwas and Otto, 2010; Ghaleb*et al*., 2012).

*M. canis*was used as a test organism for this study (though it is not implicated in oral infections) because it causes tineacapitis in humans and ring worm in pets and humans (Ginter-Hanselmayer*et al*., 2004) . The fungus secretes keratinolytic protease which invades keratinised structures of hair, skin and nails in humans. *A. flavus*, a common mold in the environment, invades arteries of the lungs or brain and cause infarction. Neutropenia predisposes to Aspergillus infections. *A. flavus*also produces a toxin, aflatoxin, which often contaminants foods such as nuts and is one of the aetiological agents for hepatocellular carcinoma (Crawford, 2005). After *A. fumigatus*, *A. flavus*is the second most common Aspergillusmold to infect humans. *A. niger*was used because it causes a disease known as black mold on food and also produce mycotoxins called orchratoxins (Abarca, 1994). Of the Aspergillus species, *A. niger*infects humans the third most often. *C. albicans*,a commensal and a constituent of normal gut flora comprising micro-organisms that live in the mouth and gastrointestinal tract, was used in this study because it is a causal agent of opportunistic oral and genital infections in humans as well as candidalonychomycosis (an infection of the nail plate) (Ryan and Ray, 2004).

Although of the different toothpaste brands investigated, none was developed for use as an antifungal formulation, the results of this study provided herein could be useful to oral healthcare providers in recommending a toothpaste in reducing the burden of *Candida* in individuals susceptible to oral candidosis as well as provide a base for the use of herbal toothpastes as alternatives to conventional toothpastes by individuals who have interest in the use of natural products. Also, results from this study suggests that, the antifungal properties of ingredients in toothpastes may have a wider applicability and could be considered for the future development of

antifungal drugs principally for topical application, against susceptible fungi used in this study.

4.0 CONCLUSION

The various toothpastes evaluated had antifungal activities which varied and they may be useful in reducing the pathogenic potentials of the various fungi.

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Table 1: Ingredients of various toothpastes tested for antifungal activity

S.NO	Toothpastes	Ingredients as listed on packages
1	Close-Up Herbal	Eucalyptus, Peppermint, Sage, Thyme, Aloe-vera leaf extracts,Sorbitol, Aqua, Hydrated silica, Sodium lauryl sulphate, PEG-32, Aroma, Cellulose, Gum, Sodium fluoride, Sodium saccharin, Aloe barbadensis leaf extracts, Limonene, C173360, C174260, C1772681, C177492.
2	Close-Up	Sodium fluoride(1450ppm fluoride), Sorbitol, Aqua, Hydrated silica, Sodium lauryl sulphate, PEG-32, Flavour, Cellulose, Eugenol, C116035, C117200.
3	Maclean's	Sodium fluoride 0.306%$^{w}/_{w}$, Aqua, Hydrated silica, Sorbitol, Glycerine, PEG-6, Sodium lauryl sulphate, Flavour, Xanthan gum, Sodium saccharin, C173360, C174160.
4	Dabur Herbal	Basil oil 0.01%, 5% herbal extract (obtained from bullet wood, Acacia arabica, laturbark,pelitory 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, sodium monofluorophosphate, chlorophyllin, tetrasodiumpyrophosphate, sodium saccharin and preservative.

Table 2: Antifungal activity of four different toothpaste formulations against four different fungi

Toothpastes	Conc. mg/ml	Zone of Inhibition (mm) Micro-organisms (Fungi)			
		M. canis	*A. flavus*	*A. niger*	*C. albicans*
Close Up Herbal	12.50	24	-	-	27
	25.00	29	-	-	30
	50.00	35	-	-	34
	100.00	38	-	-	38
	150.00	40	-	-	40
	200.00	42	-	-	45
Close Up	12.50	19	-	-	-
	25.00	21	13	-	-
	50.00	32	16	-	20
	100.00	34	20	-	25
	150.00	37	27	-	27
	200.00	40	30	-	42
Macleans	12.50	17	-	-	-
	25.00	20	-	14	-
	50.00	23	-	15	-
	100.00	24	-	18	20
	150.00	26	-	22	27
	200.00	31	-	24	30
Dabur Herbal	12.50	20	-	--	-
	25.00	23	-	-	17
	50.00	27	-	12	21
	100.00	32	-	15	24
	150.00	34	-	21	28
	200.00	39	-	22	31

Key: *M. canis* =*Microsporumcanis*; *A. flavus* =*Aspergillusflavus;*
A. niger =*Aspergillusniger* and
C. albicans= *Candida albicans*.– means no zone of inhibition.

Table 3: Minimum Inhibitory Concentrations of the various toothpaste formulations for the different fungi

Toothpastes	Fungi	Minimum Inhibitory Concentration (M.I.C) (mg/ml)
Close Up Herbal	*Microsporumcanis* *Candida albicans*	12.50 12.50
Close Up	*Microsporumcanis* *Aspergillusflavus* *Candida albicans*	12.50 25.00 50.00
Macleans	*Microsporumcanis* *Aspergillusniger* *Candida albicans*	12.50 25.00 100.00
Dabur Herbal	*Microsporumcanis* *Aspergillusniger* *Candida albicans*	12.50 50.00 25.00

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