Original Article

DOI: 10.7860/JCDR/2022/55875.16752

Dentistry Section

Evaluation of Antifungal Effectiveness of a Novel Probiotic Toothpaste: An In-vitro Study

PAVITA KATARIA[1], HARPREET SINGH[2]

ABSTRACT

Introduction: There is increasing public interest in probiotic healthcare products since their discovery by Élie Metchnikoff in the early 20th century. This trend is not only visible in supermarkets and dental practices, but also in the scientific world. An increasing number of clinical trials and laboratory investigations are being conducted to validate the claims made about these products with regards to their antifungal properties.

Aim: To evaluate the antifungal properties of commercially available probiotic, anticaries toothpaste, Purexa, against *Candida albicans* in-vitro.

Materials and Methods: An in-vitro study was carried out for a period of three months from December 2021 to February 2022 in the Department of Microbiology, Baba Jaswant Singh Dental College and Research Institute, Patiala, Punjab, India. Two wells were prepared in each blood agar plate containing lawn cultures of *Candida albicans*. The plates were incubated at 37°C for 24 hours. Purexa toothpaste was poured into one well as test material. The second well was filled with distilled water to act as control. A total of 10 sample plates were made. Antifungal activity was assessed by measuring the Zone of Inhibition (ZOI) in millimeters (mm) using a digital vernier caliper after 24, 48 and 72 hours, and then after one week. Increasing width of zones of inhibition indicated definite antifungal activity of Purexa toothpaste. A p-value <0.05 was considered to be statistically significant.

Results: All the sample plates showed definite zones of inhibition at 24 hours, 48 hours, 72 hours and one week intervals around the wells containing the test material, which indicated antifungal efficacy of Purexa probiotic toothpaste. No inhibition zones were seen around the wells containing distilled water. The mean width, if ZOI was the highest at day 7 (28.71 mm), followed by day 2 (26.66 mm), day 3 (26.36 mm) and least on day 1 (25.25 mm).

Conclusion: The preliminary data obtained from the present study indicates the prospective use of Purexa probiotic toothpaste in cases, where antifungal activity of toothpaste is desirable.

Keywords: *Candida albicans*, Dentrifices, Purexa

INTRODUCTION

The oral cavity provides a habitat to a large number of microbial species which co-exist with one another as normal microbiota [1]. Candida, one of the most commonly found opportunistic oral pathogens has more than 20 species [2]. *Candida albicans* (*C. albicans*) is most commonly associated with oral candidal infection, in both medically compromised as well as otherwise healthy individuals [2,3]. Different pathological conditions like acute pseudomembranous thrush to more chronic forms which may persist for a longer period despite treatment can be caused by *C. albicans* [1]. The risk of candidal infection is increased markedly due to various factors such as poor oral hygiene, diabetes mellitus, prolonged use of antibiotics, and use of dental prostheses, immunosuppression, high carbohydrates diet, or heavy smoking [4,5].

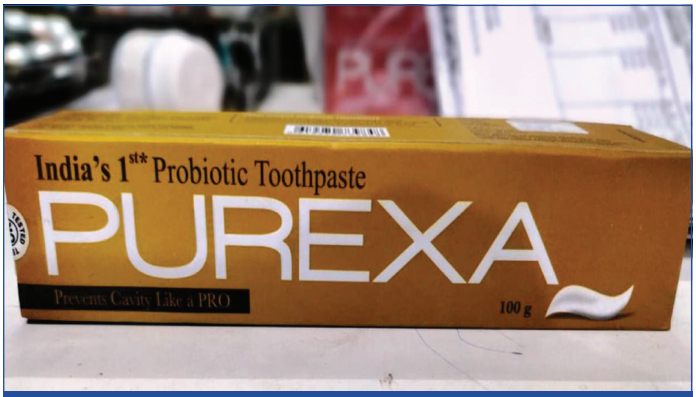
Many antifungal medicines in the market today have several side-effects and they can also lead to development of drug resistance, causing profound effects on human health [6]. These factors have led to the need to develop newer antifungal drugs with lesser side-effects. World Health Organisation (WHO) in 2001 defined probiotics as live microorganisms which when administered in adequate amounts confer health benefits on the host [7]. There is increasing public interest in probiotic healthcare products since their discovery by Elie Metchnikoff in the early 20th century [8]. Probiotics are known to perform several beneficial actions in the gastrointestinal tract. In relation to prevention of oral diseases, they help maintain and restore the natural saprophytic microflora against pathogen invasion, which plays the main role in development of major oral diseases [9]. A large number of clinical trials are being conducted to validate the claims made about various probiotic healthcare products with regards to claims of their antimicrobial properties [10-13].

Toothpastes are the most common vehicles for drug delivery in oral cavity [5]. They have been used since almost 4000 years [3]. Various types of abrasives were used to clean teeth till the mid 19th century. In middle-ages, the Arabs used fine sand and pumice as tooth cleaning agents. The first toothpaste was invented in 1950, by Dr. Washington Wentworth Sheffield, a dental surgeon and chemist [14]. Modern dentifrices contain abrasive agents, thickening agents, humectants, flavouring agents, and antimicrobial agents. They have been formulated to contain chemotherapeutic agents to improve oral health, and to produce inhibitory action on plaque formation and on colonisation of bacteria and Candida [3,15-18]. With newer toothpastes being introduced to the market regularly, there is a need for research to determine their antimicrobial and antifungal activities as well. Purexa probiotic toothpaste is novel toothpaste with probiotic activity, recently launched in Indian market. The effective ingredients of the toothpaste are bacillus coagulum, sodium monofluorophosphate, xylitol, calcium carbonate, hydrated silica, sodium citrate, and sorbitol [19]. Till date, no research is available to validate the antifungal activity of the toothpaste. Thus, the present study was conducted to determine the antifungal activity of a newly introduced commercially available probiotic, anticaries toothpaste, Purexa; against *C. albicans* isolates using agar well-diffusion method.

MATERIALS AND METHODS

This in-vitro study was conducted in Department of Microbiology at the Baba Jaswant Singh Dental College and Research Institute, Patiala, Punjab, India, over a total period of three months (December 2021-February 2022). Ethical clearance for the same was obtained from Ethical Committee of Baba Jaswant Singh Research Institute (BJSRI/21/140). Purexa Probiotic toothpaste was procured and used as a test material for the present study [Table/Fig-1]. Total

10 sample plates were made. Wells were prepared in blood agar plates containing lawn cultures of *C. albicans*. The plates were incubated at 37°C for 24 hours. Undiluted Purexa toothpaste was poured into one well as test material. The second well in each plate was filled with distilled water to act as control. Antifungal activity was assessed by measuring the ZOI in millimeters (mm) using a digital vernier caliper after 24, 48 and 72 hours, and then after one week.



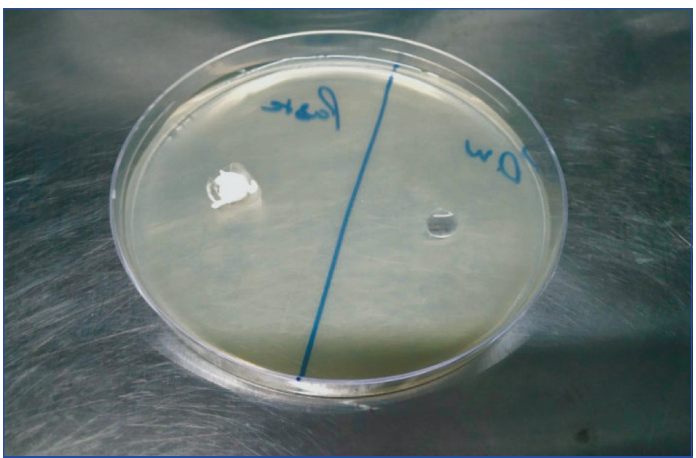
[Table/Fig-1]: Purexa probiotic toothpaste.

Reviving the Microbial Species

Freeze-dried microbial cultures were acquired from "Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh". Cultures of *Candida albicans* (MTCC-3018) were obtained in the form of freeze dried ampoules. Ampoules were cut with a diamond cutter and broken by placing them in cotton gauze. Culture of 0.5 mL was extracted from culture bottles (BACT/ALERT FA Plus Culture bottles, bioMerieux, USA) and injected into the ampoules and agitated for thorough mixing of bacterial cultures. After a resting period of 15 minutes, the microbe and culture solution was injected back into culture bottles and incubated for 48-72 hours [20]. Once the incubation period was over, pure microbial growth was obtained by streaking the culture from incubated bottles onto blood agar plates (Himedia) and incubated for 24 hours. Microbial colonies were verified by gross examination of colonies and observing under microscope.

Agar Diffusion Test

Agar plates were freshly made containing Himedia Blood Agar. Fungal suspension was made by adding colonies to 0.5 mL saline using a sterile inoculating loop and adjusting the turbidity to 0.5 McFarland standards [20]. A sterile swab was dipped in this suspension and used to make the lawn culture of fungi. Then, two wells, each in 4 mm depth and 5 mm diameter, were punched on the agar surface with equal distance from each other. One well was filled with 0.1 mg Purexa toothpaste and the second well was filled with distilled water, to act as control [Table/Fig-2]. Agar plates were incubated at 37°C for 48 hours and the diameter of the ZOI was measured and recorded after 24, 48 and 72 hours, and then after one week.



[Table/Fig-2]: Wells prepared in agar plate, filled with Purexa probiotic toothpaste and distilled water.

STATISTICAL ANALYSIS

One way analysis of variance (F-test) was used to compare the mean value of antifungal activity using a digital vernier caliper observed at four different days i.e. at day 1, day 2, and day 3 and after one week. The Bonferroni post-hoc test was also applied for multiple inter group comparisons. Statistical Package for the Social Sciences (SPSS) version 21.0 was used for calculations. A p-value <0.05 was considered to be statistically significant.

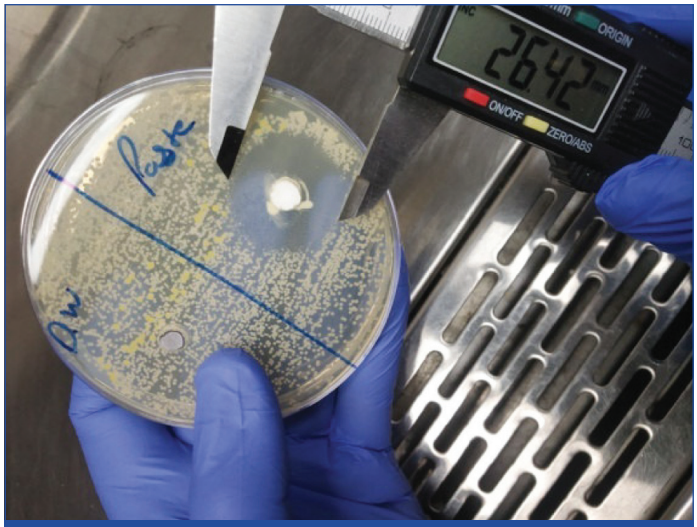
RESULTS

Definite ZOI was observed around the wells containing the test material (Purexa probiotic toothpaste, while no ZOI was observed around the wells containing the control material. [Table/Fig-3-7]. The zones of inhibition observed at 24 hours, 48 hours, 72 hours and one week intervals are depicted in [Table/Fig-8].

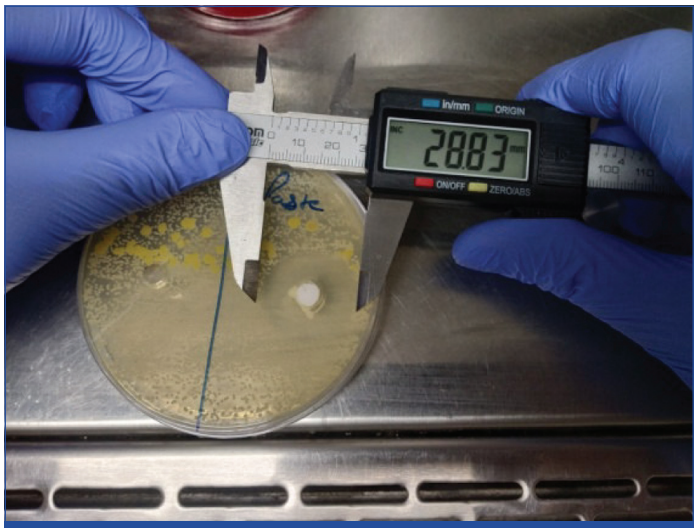
[Table/Fig-3]: Zone of inhibition for Purexa probiotic toothpaste recorded after 24 hours.

[Table/Fig-4]: Zone of Inhibition of distilled water recorded after 24 hours.

[Table/Fig-5]: Zone of inhibition for Purexa probiotic toothpaste recorded after 48 hours.



[Table/Fig-6]: Zone of inhibition for Purexa probiotic toothpaste recorded after 72 hours.



[Table/Fig-7]: Zone of inhibition for Purexa probiotic toothpaste recorded after one week.

Sample no.	24 hours	48 hours	72 hours	1 week
1.	25.50	26.94	26.42	28.83
2.	24.43	25.89	25.94	28.44
3.	25.86	27.02	26.86	28.93
4.	25.23	26.55	26.17	29.06
5.	25.12	26.83	26.28	28.51
6.	25.87	26.90	27.08	29.61
7.	24.99	26.37	25.97	27.40
8.	25.67	27.14	26.74	28.88
9.	25.08	26.81	26.25	28.85
10.	24.78	26.16	25.84	28.57

[Table/Fig-8]: Width of zone of inhibition (in mm).

The mean was observed to vary with time duration. The mean value came out to the highest at day 7 (28.71 mm), followed by day 2 (26.66 mm), day 3 (26.36 mm) and least on day 1 (25.25 mm). When the mean values were compared to each other using F-test, it was observed that the antifungal activity differed significantly from each other at day 1, day 2, day 3 and one week, respectively (p<0.001) [Table/Fig-9].

Intragroup multiple comparisons-Bonferroni post-hoc test: Further, the multiple comparisons between the days i.e. the groups each other has revealed that the mean width of ZOI of day 1 was statistically significant with day 2, day 3 and day 7 (p<0.001), whereas, ZOI of day 2 was significant with day 1 and day 7, while it was non-significant with day 3. Like-wise, the mean width of ZOI of day 3 was significant with day 1 and day 7, while it was non significant with day 2. The mean width of ZOI of day 7 was significant with day 1, day 2 and day 3 [Table/Fig-10].

Days	Mean	±SD	p-value
Day 1	25.25	0.472	p<0.001*
Day 2	26.66	0.406	
Day 3	26.36	0.418	
Day 7	28.71	0.567	

[Table/Fig-9]: Antifungal activity using a digital vernier caliper at day 1, day 2, day 3 and after one week.
One-way analysis of variance (F-test)
p<0.05* significant

Main group (I)	Compared between the groups (J)	Mean difference (I-J)	Std. Error	p-value	95% Confidence interval	
					Lower bound	Upper bound
Day 1	Day 2	-1.40800*	0.2102	<0.001*	-1.99	-0.82
	Day 3	-1.10200*	0.2102	<0.001*	-1.69	-0.52
	Day 7	-3.45500*	0.2102	<0.001*	-4.04	-2.87
Day 2	Day 1	1.40800*	0.2102	<0.001*	0.82	1.99
	Day 3	0.306	0.2102	0.92NS	-0.28	0.89
	Day 7	-2.04700*	0.2102	<0.001*	-2.63	-1.46
Day 3	Day 1	1.10200*	0.2102	<0.001*	0.52	1.69
	Day 2	-0.306	0.2102	0.92 NS	-0.89	0.28
	Day 7	-2.35300*	0.2102	<0.001*	-2.94	-1.77
Day 7	Day 1	3.45500*	0.2102	<0.001*	2.87	4.04
	Day 2	2.04700*	0.2102	<0.001*	1.46	2.63
	Day 3	2.35300*	0.2102	<0.001*	1.77	2.94

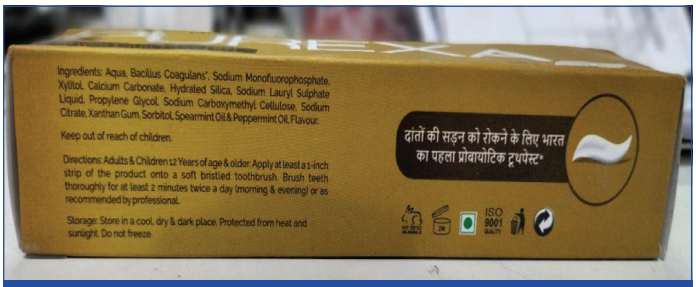
[Table/Fig-10]: Intragroup multiple comparisons using Bonferroni post-hoc test.
*Significant (p<0.001)
NS: Non-significant (p>0.05)

DISCUSSION

The present in-vitro study was performed to evaluate antifungal activity of newly introduced probiotic toothpaste, Purexa. Results indicate definite antifungal activity of Purexa probiotic toothpaste, as was evident from zones of inhibition observed during the given time period. The incidence of *Candida albicans* isolated from the oral cavity has been reported to be 45% in neonates, 45%-65% of healthy children, 30%-45% of healthy adults, 50%-65% of people who wear removable dentures, 65%-88% in those living in acute and long term care facilities, 90% of patients with acute leukemia undergoing chemotherapy, and 95% of patients with HIV [21-28]. Plaque control, a procedure that involves removal of microbial plaque biofilms and its accumulation on various hard surfaces and gingival surfaces in oral cavity, is an important step in preventing tooth decay and periodontal diseases [29]. Most of the research work related to determination of antimicrobial activity of toothpastes focus on their potential to inhibit bacterial growth. There is insufficient data concerning their antifungal activity [3,30].

A number of toothpastes containing probiotics that may be beneficial in improving oral health have been developed recently. Studies done by Chandhru TP et al., Amizic IP et al., and Adwan G et al., concluded that probiotic toothpastes can contribute in prevention of periodontal diseases [20,31,32]. The present study was designed to evaluate antifungal activity of new commercially introduced probiotic, anticaries, Purexa. The effective ingredients of the toothpaste are 350 million CFU/gm helpful bacteria bacillus coagulum (0.02%), sodium monofluorophosphate (0.8%), xylitol (2%), calcium carbonate (42.4%), hydrated silica (3%), sodium citrate (0.25%), and sorbitol (28%) [Table/Fig-11] [19]. While bacillus

coagulum is known to inhibit the growth of cavity causing *S. mutans* [33], various clinical studies indicate that fluorides, xylitol, and probiotics demonstrate antifungal effects [1,34,35]. Probiotics have always been considered an excellent solution for gastrointestinal issues. Their use in oral healthcare products has been investigated in recent years and is steadily gaining popularity. A large number of these investigations are conducted on dairy products that contain Probiotic strains: *Lactobacillus rhamnosus, Lactobacillus router, and Bifidobacterium* spp. were the most regularly used strains [33]. There was a need for a probiotic strain vehicle that was stable, had a long shelf life, and could be made available and used on a regular basis by a greater segment of society [36]. Purexa probiotic toothpaste, which is a therapeutic innovation, can be beneficial against *C. albicans* and subsequently against various dental diseases by restoration of healthy microflora. Results of the present study may provide invaluable information for dental professionals. They can recommend a dentifrice that has good inhibition properties against *C. albicans* for a patient, who is prone to oral candidal infections.



[Table/Fig-11]: Ingredients of Purexa probiotic toothpaste.

Limitation(s)

This testing method was more of a screening test, and may not have been able to detect the effects of chemical agents that did not diffuse through agar matrix. The findings of the present study cannot be used for evaluating its clinical effectiveness. This is because it was an in-vitro experiment and inside the oral cavity the toothpaste is likely to be diluted by saliva, to an extent that its antifungal properties may be buffered or lost due to dilution. Also, it is well understood that there is a state of balance in the oral microbiota of a healthy individual. If this balance is disturbed, the opportunistic microbes can proliferate and lead to initiation of disease processes. Therefore, a dentifrice that shows the largest inhibition zone against *C. albicans* may not necessarily be better than that giving a smaller inhibition zone.

CONCLUSION(S)

Probiotic toothpastes are viable microbes that are known for their various health benefits when administered in adequate quantities. Probiotic toothpaste is a relatively new concept that can be useful in prevention of periodontal diseases. The present study was aimed at evaluating antifungal activity of Purexa probiotic toothpaste, which contains fluorides, xylitol, and probiotics. The paste showed substantial antifungal activity in the laboratory analysis and may be beneficial to individuals prone to oral candidiasis. However, more research needs to be done to better understand its effectiveness in the oral cavity, and whether it can be used as an alternative to conventional toothpastes in case of oral candida infection.

Acknowledgement

The authors would like to thank Dr. Aditya Khichy and Dr. Vishwas Narang for their clinical assistance; and The Baba Jaswant Singh Trust.

REFERENCES

[1] Adwan G, Salameh Y, Adwan K, Barakat A. Assessment of antifungal activity of herbal and conventional toothpastes against clinical isolates of Candida albicans. Asian Pac J Trop Biomed. 2012;2(5):375-79.
[2] Furletti VF, de Cássia Mardegan R, Obando-Pereda GA, Aníbal PC, Duarte MCT, Gonçalves RB, et al. Susceptibility of Candida spp. Oral isolates for azolic antifungals and amphotericin B. Braz J Oral Sci. 2008;7(25):1543-49.
[3] Yigit N, Aktas E, Ayyildiz A. Antifungal activity of toothpastes against oral Candida isolates. J Mycol Med. 2008;18:141-46.
[4] Patel M, Shackleton JT, Coogan MM. Effect of antifungal treatment on the prevalence of yeasts in HIV-infected subjects. J Med Microbiol. 2006;55(Pt 9):1279-84.
[5] Nayak A, Nayak RN, Bhat K. Antifungal activity of a toothpaste containing Ganoderma lucidum against Candida albicans- An in vitro study. J Int Oral Health. 2010;2(2):51-57.
[6] Yun-Liang Yang. Virulence factors of Candida species. J Microbiol Immunol Infect. 2003;36:223-28.
[7] Food and Agricultural Organization of the United Nations and World Health Organization. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. World Health Organization [online], (2001); was accessed on April 28, 2022.
[8] Metchnikoff E. Optimistic studies. New York: Putmanys Sons; 1908:161-83.
[9] White DJ, Cox ER, Liang N, Macksood D, Bacca L. A new Plaque Glycolysis and Regrowth Method (PGRM) for the in vivo determination of antimicrobial dentifrice/rinse efficacy towards the inhibition of plaque growth and metabolism– Method development, validation and initial activity screens. J Clin Dent. 1995;6:59-70.
[10] Shudong P, Guo C, Wu S, Cui H, Suo H, Duan Z. Bioactivity and metabolomics changes of plant-based drink fermented by Bacillus coagulans VHProbi C08. LWT. 2022;156:113030.
[11] Nasrollahzadeh A, Mokhtari S, Khomeiri M, Saris PEJ. Antifungal preservation of food by lactic acid bacteria. Foods. 2022;11:395.
[12] Abd Ellatif SA, Bouqellah NA, Abu-Serie MM, Razik ES, Al-Surhanee AA, Askary AE, et al. Assessment of probiotic efficacy and anticancer activities of Lactiplantibacillus plantarum ESSG1 (MZ683194. 1) and Lactiplantibacillus pentosus ESSG2 (MZ683195. 1) isolated from dairy products. Environmental Science and Pollution Research. 2022:01-08.
[13] Safadi S, Maan H, Kolodkin-Gal I, Tsesis I, Rosen E. The products of probiotic bacteria effectively treat persistent enterococcus faecalis biofilms. Pharmaceutics. 2022;14:751.
[14] Tiwari KB, Shrestha UT, Acharya A, Subedi B, Paudyal B, KC U, et al. Antibacterial activities of locally used toothpastes against dental pathogens. Journal of Institute of Medicine [Internet]. 2008;30(2):15-18.
[15] Prasanth M. Antimicrobial efficacy of different toothpastes and mouthrinses: An in vitro study. Dent Res J. 2011;8:85-94.
[16] Gardiner J, Freeman S, Leach M, Green A, Alcock J, D'Emanuele A. PAMAM dendrimers for the delivery of the antibacterial triclosan. J Enzyme Inhib Med Chem. 2008;23:623-28.
[17] Sadeghi M, Assar S. An in vitro antimicrobial activity of ten Iranian-made toothpastes. Dent Res J (Isfahan). 2009;6(2):87-92.
[18] Adejumo OE, Olubamiwa AO, Ogundeji BA, Kolapo AL. Assessment of in vitro and in vivo antimicrobial activities of selected Nigerian tootpastes and mouth washes on some oral pathogens. Adv Med Dent Sci. 2008;2(3):61-65.
[19] Kaur J, Paul R, Gupta D, Manchanda A, Arora G. Clinical efficacy of a probiotic fluoride dentifrice: A comparitive clinical and microbiologic study. Int J Oral Health Dent. 2020;6(2):131-36.
[20] Chandhru TP, Anusha VR, Peedikayil FC, Ahmed MG, Kottayi S, Narasimhan D. Evaluation of antifungal activity of six children's toothpaste on Candida albicans isolated from early childhood caries patients. J Indian Soc Pedod Prev Dent. 2020;38(2):152.
[21] Manning DJ, Coughlin RP, Poskit EM. Candida in mouth or on dummy? Arch Dis Child. 1985;60:381-82.
[22] Berdicevsky I, Ben-Aryeh H, Sazargel R, Gutman D. Oral Candida in children. Oral Surg Oral Med Oral Pathol. 1980;57(1):37-40.
[23] Lucas VS. Association of psychotropic drugs, prevalence of denture-related stomatitis and oral candidosis. Community Dent Oral Epidemiol. 1993;21:313-16.
[24] Arendorf TM, Walker DM. The prevalence and intra-oral distribution of Candida albicans in man. Arch Oral Biol. 1980;25:01-10.
[25] Cumming CG, Wight C, Blackwell CL. Denture stomatitis in the elderly. Oral Microbiol Immunol. 1990;5:82-85.
[26] Holbrook WP, Hjorleifsdottir DV. Occurrence of oral Candida albicans and other yeast-like fungi in edentulous patients in geriatric units in Iceland. Gerodontics. 1986;2:153-56.
[27] Rodu B, Carpenter JT, Jones MR. The pathogenesis and clinical significance of cytologically detectable oral candida in acute leukaemia. Cancer. 1988;62:2042-46.
[28] Dupont B, Graybill JR, Armstrong D, Laroche R, Touzé JE, Wheat LJ. Fungal infections in AIDS patients. J Med Vet Mycol. 1992;30(suppl 1):19-28.
[29] Figuero E, Nobrega DF, Garcıa-Gargallo M, Tenuta LMA, Herrera D, Carvalho JC. Mechanical and chemical plaque control in the simultaneous management of gingivitis and caries: A systematic review. J Clin Periodontol. 2017;44(Suppl.18):S116-S134.
[30] Ellepola AN, Khan ZU, Chandy R, Philip L. A comparison of the antifungal activity of herbal toothpastes against other brands of toothpastes on clinical isolates of Candida albicans and Candida dubliniensis. Med PrinPract. 2011;20:112-17.
[31] Amižić IP, Cigić L, Gavić L, Radić M, Lukenda DB, Tonkiæ M, Barišiæ IG. Antimicrobial efficacy of probiotic-containing toothpastes: An in vitro evaluation. Med Glas. 2017;14(1):139-44.
[32] Adwan G, Salameh Y, Adwan K, Barakat A. Assessment of antifungal activity of herbal and conventional toothpastes against clinical isolates of Candida albicans. Asian Pac J Trop Biomed. 2012;2(5):375-79.

[33] Zirwas MJ, Otto S. Toothpaste allergy diagnosis and management. J Clin Aesthet Dermatol. 2010;3(5):42-47.
[34] Talatoff Z, Azad A, Zahed M, Shahradnia N. Antifungal activity of xylitol against candida albicans: An in vitro study. J Contemp Dent Pract. 2018;19(2):125-29. Doi: 10.5005/jp-journals-10024-25.
[35] Czaczyk K. Antifungal activity of Bacillus coagulans against Fusarium sp. Acta Microbiologica Polonica. 2002;51(3):275-83.
[36] Robertshaw H, Leppard B. Contact dermatitis to triclosan in toothpaste. Contact Dermatitis. 2007;57(6):383-84.

PARTICULARS OF CONTRIBUTORS:
1. Senior Lecturer, Department of Periodontics, Baba Jaswant Singh Dental College, Ludhiana, Punjab, India.
2. Dean, Department of Endodontics, Baba Jaswant Singh Research Institute, Patiala, Punjab, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Pavita Kataria,
B-24/623, Street No. 4, Harcharan Nagar, Ludhiana, Punjab, India.
E-mail: pavita_kataria@yahoo.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Mar 04, 2022
- Manual Googling: Apr 05, 2022
- iThenticate Software: May 20, 2022 (19%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Feb 28, 2022**
Date of Peer Review: **Apr 05, 2022**
Date of Acceptance: **May 21, 2022**
Date of Publishing: **Aug 01, 2022**