

Antibacterial activities of locally used toothpastes against dental pathogens

K. B. Tiwari, U. T. Shrestha, A. Acharya, B. Subedi, B. Paudyal, M. Jnawali, P. Shakya, U. K.C., V. P. Agrawal

Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal, Research Laboratory for Agricultural Biotechnology and Biochemistry (RLABB), Maitidevi, Kathmandu, Nepal

Correspondence to: Kiran Babu Tiwari, Research Laboratory for Biotechnology and Biochemistry (RLABB), e-mail: kiranbabu.babukiran@gmail.com

Background: Toothpastes need to contain various antimicrobial agents in order to reduce, control and prevent different kinds of dental diseases. Different brands have their own composition and concentration of ingredients for their efficacy. The consumers should aware about the facts associated with their health.

Methods: The bacterial pathogens were isolated and identified from various dental samples. Antibacterial activities of 11 different toothpastes available locally in markets were assessed against the isolates by standard agar well diffusion method.

Result: Monomicrobial infections were observed in all cases. The bacterial pathogens were found to be *Streptococcus mutans*, *S. salivarius*, *S. sanguis*, *S. sobrinus* and *S. mitis*. Of the assayed toothpastes- Colgate Total, Colgate, Anchor White and Pepsodent were found to be highly effective against the pathogens.

Conclusion: The result showed that the toothpastes containing Triclosan as a major chemical ingredient posses significant antibacterial activities.

Keywords: Streptococcus, Triclosan, Zone-of-inhibition

Introduction

Toothpaste has a history that stretches back nearly 4000 years. Different abrasives, green lead, incense were used to clean stain from teeth until mid nineteenth century. In middle ages, fine sand and pumice were the primary ingredients in the tooth cleaning formulas used by Arabs. In 1950 AD, Dr. Washington Wentworth Sheffield, a dental surgeon and chemist, invented the first toothpaste.¹ Then, the market of the toothpaste has never been slowed down. Modern toothpaste was invented to aid in the removal of foreign particle and food substance in addition to cleaning of tooth. During 1940-60 AD, fluoride was added which aided in protection from tooth decay. Many of the innovations were

made in toothpaste after the fluoride break through which involved the addition of ingredients with special abilities to toothpaste and toothpaste packaging.^{2,3}

Dental problem is the most common health problem in the human communities.⁴ Dental infections are mainly of three types, viz.: formation of dental plaques, dental caries and periodontal diseases.³ Dental plaque is material adhering to teeth, which consists of bacterial cell (60-70% of the volume of plaque) salivary polymers and bacterial extracellular products. Plaque is a naturally constructed biofilm of bacteria, which may reach thickness of 300-500 cells on the teeth. The very normal flora of the oral cavity, *S. mutans* and *S. sanguis*, are the most dominant bacterial

species in dental plaque. After initial weak attachment of streptococcal cells to salivary glycoprotein, stronger attachment takes place by polymer of glucose (glucan) synthesized by bacteria. Dental caries is the destruction of enamel, dentin or cement of teeth due to bacterial activities. Caries are initiated by demineralization of the enamel of teeth due to Lactic-acid bacteria. *Actinomyces* spp. and various proteolytic bacteria are commonly found in human caries as secondary invaders, contributing to the progression of the lesion. Periodontal diseases are bacterial infections that affect the supporting structure of the teeth (gingival, cementum, periodontal membrane and alveolar bone). The endotoxins, hydrolytic enzymes and toxic bacterial metabolites are involved in this disease. Gingivitis, an inflammatory condition of gum, is the most common form of periodontal disease. Serious forms of periodontal disease that affect the periodontal membrane and alveolar bone may result in tooth loss. Streptococci, actinomycetes, spirochetes and bacteroids are the possible bacteria responsible for the disease.

Materials and Methods

Collection of Sample: Thirty-four different samples were collected from 34 patients in Samaj Dental Clinic and People's Dental Hospital, Kathmandu, Nepal.^{5,6} Collected samples were transferred in nutrient broth and immediately transported to RLABB (Research Laboratory for Biotechnology and Biochemistry, Kathmandu) where the study was carried out.

Isolation of organisms: The samples were enriched in nutrient broth at 37°C for 4 hours and streaked on nutrient agar plate. Corresponding pure culture was obtained by streak plate method.^{5,6}

Identification: The organism were identified by standard microbiological techniques including colonial characteristics, morphological characteristics and biochemical characteristics.⁷

Assessment of Toothpastes (Antibacterial Activity):^{1,2,5,6-10} The toothpaste solutions were made by mixing the calculated amount of the toothpaste in measured volume of the solvent followed by continuous stirring for half an hour. In order to investigate antimicrobial activity of different toothpastes, toothpastes were diluted in two different diluents *viz:* distilled water and Tween-80. Five different dilutions of 1:5, 1:10, 1:20, 1:50 and 1:100 were made in each of the diluents. Muller-Hinton Agar (MHA) agar plate were prepared to assess antimicrobial activity of the toothpastes against the pathogens.

Results

Isolation and Identification: Of the total 34 dental subjects, 14 (41.2%) had plaques, 12 (35.2%) had Dental caries and 8 (23.6%) had Gingivitis. Altogether 15 isolates were recovered and identified as given in Table- 1. Higher percentage of isolates was found from plaque samples (7/14, 50.0%).

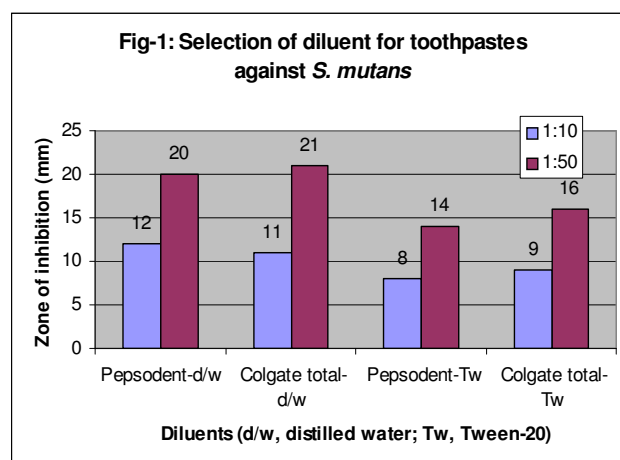
Table 1: Dental pathogens isolated from different dental samples

Type of samples	No. of samples	No. of isolates	Organisms*
Plaques (Pla)	14 (41.2%)	7 (50.0%)	<i>S. sobrinus</i> (2), <i>S. sanguis</i> (1), <i>S. mutans</i> (2), <i>S. mitis</i> (2)
Dental caries (Dc)	12 (35.2%)	5 (41.7%)	<i>S. sobrinus</i> (1), <i>S. sanguis</i> (1), <i>S. mitis</i> (3)
Gingivitis (Gg)	8 (23.6%)	3 (37.5%)	<i>S. sanguis</i> (1), <i>S. salivarius</i> (2)

Note: *= No. of corresponding isolates in parentheses

Assessment of antimicrobial activity

The distilled water extracts of the toothpaste were found to have marked antimicrobial properties compared to that of 2% Tween-20 (Fig-2). The optimum dilution of the toothpaste for antimicrobial activity assessment against *S. mutans*, *S. sobrinus* and *S. mitis* was determined to be 1:50 (Fig-2). The zones of inhibition (ZOI) against the pathogens offered by the toothpastes are shown in Table-2. Statistical analysis showed that the zones-of-inhibition of toothpastes against the test organism were not differed significantly on repeated attempts ($P > 0.05$).



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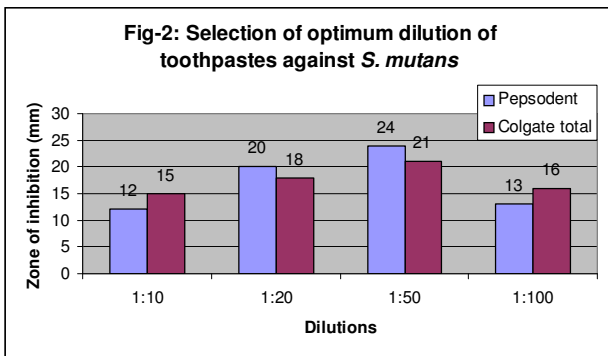


Table- 2: Mean Zone of inhibition shown by different toothpastes (mm) against the dental pathogens

Toothpastes	Dental pathogens				
	<i>S. salivarius</i>	<i>S. mutans</i>	<i>S. sobrinus</i>	<i>S. mitis</i>	<i>S. sanguis</i>
Colgate total	10	23	21	16	18
Colgate	13	22	20	16	18
Anchor white	12	20	18	16	22
Pepsodent	11	24	17	17	18
Close Up	9	13	8	5	6
Kidoos	0	10	0	0	0
Dabur powder	0	9	0	0	0

Note: Absolutely no ZOI was observed for Dabur paste, Neem, Babool and Brighter against the tested bacteria.

Discussion

The viridian streptococci- *S. mutans*, *S. sanguis*, *S. sobrinus* and *S. mitis* are the major pathogens while *S. salivarius* is an initiator of the dental infection. These oral streptococci poses the significant health risks if they enter into bloodstream via. Wounds, oral infection, dental procedures and can cause endocarditis. Followed by primary invaders, oral cavities are vulnerable for secondary invaders like *Candida albicans* and species of *Actinomyces*, *Bacteroids*, *Spirochetes* and *Lactobacillus* etc. inviting severe conditions.⁵⁻⁷

Dental problems are the most frequent cases in the general population associated mainly with dental hygiene practices.¹¹ Further, the efficacies of the toothpastes regarding their chemical composition is not less important especially in developing countries like Nepal where low grade products can be found in local markets and consumers are forced unknowingly to choose the products.

Plaque formation is the primary process of dental infections and hence the numbers of the cases are likely to be high among the common populations. Further, dental caries and gingivitis in Nepalese communities were found to be

frequent cases too. The results clearly indicate that the people should aware about their dental hygiene.

The aqueous diluent (water) was better than Tween-20 and the assessment was done in aqueous fractions, which, excludes non-polar components that might be present in the toothpastes/powder. It is self evident that the tooth-brushing is aqueous based procedure. Figure- 1 shows, however, non-polar components extracted in Tween-20 may be important and second major fractions. The optimum dilution of 1:50 (Figures 1& 2) of the pastes was selected as the more concentrated dilutions showed weaker antimicrobial activities, possibly, because of diffusion kinetics of the active ingredients in higher concentrations than optimum one that is achieved during tooth-brushing.

Only few of the locally available toothpastes were found to posses' efficient antimicrobial properties, especially, those that have triclosan as a major ingredient (Colgate total, Colgate, Anchor white and Pepsodont). Triclosan, a chlorophenol derivative, kills germs by interfering with the enzymes required for fatty acid synthesis. Next to triclosan, fluorinated products, e.g., Close-up and Kedoos, were found to posses marked antibacterial activities. These active compounds, besides reducing cariogenic microorganisms, along with other compounds in the paste/powder formula (Perooxides, silica, pyrophosphates and polymers, baking soda, chlorides and nitrates, detergents and surfactants as well as various plant extracts) helps to strengthen the teeth by reducing demineralization and increasing remineralization of the teeth.

Most commonly used and recommend by the WHO, ADA, FDI is the fluoride and triclosan. But the excess use of the fluoride can cause the dental fluorosis so the recommended amount of the fluoride should be used as the ingredients in the toothpaste. And the regular evaluation of the efficacy of the fluoridated toothpaste by the private laboratory have been recommended by the WHO.¹¹

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