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Evaluation of Antimicrobial Activity of Different Pepsodent and Colgate Toothpastes on Oral Flora of '*Lactobacillus acidophilus*'

Mr. S. V. Mamdapure
Maharashtra Udayagiri Mahavidyalaya,
Udgir

Abstract:

The objective of this in vitro study is clinical and laboratory evaluation of different Pepsodent and Colgate toothpaste on antimicrobial activity against Lactobacillus acidophilus. The initially antimicrobial activity evaluation was performed by using well diffusion method. Water was used as a control. Well impregnated with the Pepsodent and Colgate toothpaste were placed in Petri dishes containing culture media inoculated suspension of Lactobacillus acidophilus by the pour plate method. The diameter of the zone of inhibition were measured in centimetre and recorded after 24 hours incubation at 37^{o}C for each Pepsodent and Colgate toothpaste and result were obtained. The antimicrobial activity of Pepsodent triple protection, Pepsodent expert protection, Colgate maximum cavity protection, Colgate triple action, Colgate total whitening were given better result.

Keywords: *Oral, Lactobacillus acidophillus, Pepsodent and Colgate toothpaste, Antimicrobial activity*

Introduction:

Oral cavity of human supports life for many different species of microorganisms but bacterial infection happened when these microorganisms permeate into the tissues or in case of lower host immunity. The display of bacterial infection of dental decent is permanently changing and is a measurable reflexing of new evolution of oral flora. Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable Global infectious diseases. Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. The association between oral diseases and the oral microbiota is well established. Dental caries is one of the most prevalent oral diseases. It is highly prevalent among children and persists to be an important public health problem worldwide (Abdullah et.al, 2008).Practically there is no Geographic area in the world whose in inhabitants do not exhibit any evidence of dental caries. It is observed in both sexes, all races, all socioeconomic classes and all age groups (Prakash et.al., 1999). In this era of globalization, the distribution of dental caries present a varied picture, most of the countries with low caries prevalence are experiencing an unprecedented increase in caries prevalence and severity of dental caries (Dash et al.,2002). On the other hand, a reduction of dental caries incidence and improvement of gingival health care have been observed in several industrialized countries (Marthaler, 1990 and Kalsbeek and Virrips, 1960). This decline in dental carries was mainly due to the appropriate use of fluorides and preventive oral health measures (W. H. O., World Health No. 1, 1994 and oral health, ICMR Bulletin, 1994). The scenario in India is no different from other developing nations. According to the available literature of 1940 to 1960, the prevalence of dental caries in India showed a varied picture i.e. caries being very high in some areas and low in some areas (Damle and Patel, 1994; Tewari and chawla, 1977; Antia, 1962 and shourie, 1941). In spite of these conflicting reports it has been observed that during 1940 the prevalence of dental caries in India was 55.5% and during 1960 it was reported to be 68% (Simratvir et al., 2009; Mahejabeenet.al., 2006 and oral Health, ICMR Bulletin, 1994).

Proper oral hygiene aims to control the harmful effects and prevent disease transmission. Biofilms are communities of bacteria, or microorganisms, attached to surface in the body.Oral biofilms are more commonly referred to as plaque. Biofilms form almost everywhere bacteria are in the mouth, they naturally form on any stationary surface, namely teeth, gums, and tongue. Oral bacteria work with our immune system to keep our bodies diseases free by fighting disease-producing germs that try to come in through the mouth. Numerous kinds of bacteria grow in the mouth. "They are competing for

food and places to grow" and the many hard surfaces in the mouth provide an ideal place for bacteria and biofilms to grow and reproduce. Unlike most other places in or on the body, the mouth does not desquamate (shed). A tooth's distinct surface and stagnant nature makes it optimal for populations and growth of microorganisms and bacteria resulting in dental plaque. To grow and flourish in the mouth, biofilms go through four main stages of growth; first bacteria must be deposited from saliva onto a surface where it can attach. Then the bacteria, or microorganism, grow and replicate. Each day numerous bacteria grow in a person's mouth. Many diseases are related to oral bacteria. Proper oral care and habits often protect against and reduce the effects of some harmful bacteria because of the amount of bacteria in each person's mouth; there is a transfer of bacteria through saliva when lip balm, drinks, toothbrushes, or anything else is shared. Said bacteria transfer can lead to human illness. Some of these diseases that result are relative inconsequential while others could potentially have a serious impact on one's life. Although disease is possible through oral interactions, there are many ways to prevent or reduce the risk of infections and disease. Brushing and flossing teeth regularly are the mouth basic ways to reduce these risks. These two hygiene tips help to get rid of bacteria that try to stick to surfaces in the mouth to form plaque (oral biofilms). Brushing and flossing the teeth disrupts biofilms and their ability to permanently damage the teeth or gums. The less one brushes their teeth, the better chance that bacteria will form plaque, which may potentially causes serious oral health issues. The success of Pepsodent and Colgate toothpaste depends on its ability to remove oral microflora which causes the dental diseases. Pepsodent toothpastes containing sodiummonofluorophosphate, have been widely used in all over the world. The Colgate toothpastes containing sodium fluoride have been widely used in all over the world. So many researchers are working on the efficiency of different toothpastes containing chemicals that are working as antimicrobial agents functions as inhibitory effect against plaque formations (Itthagarum and wei, 1996; fine et. al. 2006). The recent investigation tries to fulfil gap on study of efficiency of various Pepsodent and Colgate toothpastes against oral Flora using standard well diffusion method.

Table: 1. Pepsodent and Colgate toothpastes evaluated in the study, their respective compositions

Sr. No.	Toothpaste	Composition listed on Packages
1	Pepsodent whitening	Calcium carbonate, water, sorbitol, hydrated silica, sodium lauryl sulphate, potassium nitrate, flavour, cellulose gum, sodium silicate, Benzyl alcohol, sodium saccharin, perlite, sodium monofluorophosphate.
2	Pepsodent Germi check+	Calcium carbonate, water, sorbitol, hydrated silica, sodium lauryl sulphate, flavour, sodium monofluorophosphate, cellulose gum, potassium nitrite, Benzyl alcohol, sodium silicate, Triclosan, Sodium saccharin, Cl 45 430, Limonene, Linalool.
3	Pepsodent clove salt	Calcium carbonate, water, sorbitol, sodium lauryl sulphate, hydrated silicakaolin, flavour, sodium monofluorophosphate, Potassium Nitrate, Benzyl alcohol, sodium chloride, sodium saccharin, Eugenia caryophyllusleaf oil, Cetylpyridinium chloride, Limonene, Cl 74160, Cl 12490, Cl77891.
4	Pepsodent super salt	Calcium carbonate, water, sorbitol, hydrated silica, sodium lauryl sulphate, Sodium silicate, sodium monofluorophosphate, cellulose gum, Benzyl alcohol, potassium nitrate, sodium saccharin, sodium chloride, CI 74260.
5	Pepsodent complete care	Sodium chloride (0.2 4%), sorbitol, water, hydrated silica, polyethylene glycol 32, sodium lauryl sulphate, SD alcohol 38- B, flavour, cellulose gum, sodium saccharin, Titanium dioxide.
6	Pepsodent expert protection	Water, sorbitol, hydrated silica, sodium lauryl sulphate, polyethylene glycol 32, zinc citrate, flavour, cellulose gum, Triclosan, sodium saccharin, sodium fluoride,Cl 74260, Cl 74160.

7	Pepsodent charcoal white	Aqua, sorbitol, hydrated silica, sodium lauryl sulphate, poly ethylene glycol 32, flavour, cellulose gum, Trisodium phosphate, methyl paraben, propyl paraben, sodium saccharin, zinc citrate, sodium chloride, CI 77891.
8	Pepsodent gum care	Aqua, sorbitol, hydrated silica, sodium lauryl sulphate, poly ethylene glycol 32,Flavour, cellulose gum, Trisodium phosphate, methyl paraben, Propyl paraben, sodium saccharin, zinc citrate, triclosan, sodium Fluoride, CI 77891.
9	Pepsodent triple protection	Calcium carbonate, water, sorbitol, hydrated silica, sodium lauryl sulphate, sodium monofluorophosphate, flavour, perlite, cellulose gum, potassium citrate, sodium silicate, sodium saccharin, Benzyl alcohol, CI 74160, CI 74260, glycerol, Limonene
10	Colgate MaxFresh	Sodium chloride 0.24 %, Sorbitol, Water, Hydrated silica, Polyethylene glycol, Sodium Lauryl sulphate, Flavour, Cellulose gum, Tetrasodium pyrophosphate, Cocamidopropyl Betaine, Sodium saccharin, Methyl cellulose
11	Colgate cibaca	Sodium Monofluorophosphate, Sodium carboxymethyl cellulose, White film, Sorbitol, Silica, Sodium Beurylsulphate, Flavour
12	Colgate triple action	Sorbitol, Water, Hydrated silica, Sodium lauryl sulphate, Flavour, Polyethylene glycol, Tetrasodium pyrophosphate, Cocamidopropyl Betaine, Cellulose gum, Sodium saccharin, Sodium fluoride, Xanthan gum, Titanium dioxide, Pigment green, Pigment blue
13	Colgate total whitening	Sodium fluoride 0.24%, Water, Hydrated silica, Glycerine, Sorbitol, Sodium lauryl sulphate, Flavour, Cellulose gum, Sodium hydroxide, Propylene glycol, Carrageenan, Sodium saccharin, Titanium dioxide, PVM/ MA copolymer
14	Colgate Visible white toothpaste	Silica, Sorbitol, Glycerine, Polyethylene glycol, Sodium triphosphate, Tetra potassium, Pyrophosphate, Sodium lauryl sulphate, Flavour, Cocamido propyl betadine, Sodium carboxymethyl cellulose, Sodium saccharin, Sodium fluoride, Xanthan, Sodium hydroxide, Blue poly 50, Titanium dioxide in aqueous base
15	Colgate maximum cavity protection	Sodium fluoride 0.24%, Sorbitol, Water, hydrated silica, Polyethylene glycol, Cellulose gum, Sodium lauryl sulphate, Flavour, Sodium saccharin, Mica, Titanium dioxide
16	Colgate optic white	Calcium pyrophosphate, Propylene glycol, Polyethylene glycol, Copolymer, Polyethylene glycol, Glycerine, Flavour, PVR, Sodium lauryl sulphate, Tetrasodium pyrophosphate, Silica, Hydrogen peroxide, Sodium saccharin, Phosphoric acid, Sucralose, Butylated, Hydroxytoluene, Water
17	Colgate Max white	Sorbitol, Hydrated silica, Sodium lauryl sulphate, Aroma, Polyethylene glycol, Tetrasodium pyrophosphate, Cellulose gum, Cocamidopropyl Betaine, Sodium saccharin, Sodium fluoride, Hydroxypropyl methylcellulose, Limonene
18	Colgate cavity protection	Sodium monofluorophosphate 0.76%, Calcium phosphate, Dehydrate, Water, Glycerine, Sodium lauryl sulphate, Cellulose gum, Favour, Tetrasodium, Pyrophosphate, Sodium saccharin
19	Colgate total advanced health	Water, Hydrated silica, Glycerine, Sorbitol, PVM/MA copolymer, Sodium lauryl sulphate, Flavour, Cellulose gum, Sodium hydroxide, Propylene glycol, Carrageenan, Sodium saccharin, Titanium

Materials and Methods:

Collection of samples: Samples were collected 1 hour after breakfast from the individual by swapping over the teeth and gum region and were inoculated by streaking the swab on Nutrient agar plates.

Isolation and identification of bacterial species:

The samples that were collected were streak it on the surface of nutrient agar plate using four quadrant methods and incubated at 37°C for 24 to 48 hours.

Colonies with visually distinguishable morphologies were randomly selected and isolated by directly streaking on nutrient Agar plates and incubated for another 24 hours.These isolated samples were spread onto a number of freshly prepared agar plates and incubated at allow cells to form microbial colony. The above agar plate inoculated by streak method and incubated at 37°C for 24 to 48 hours. After incubation period of 48 hours the colonies were identified by morphology, Gram staining and biochemical reaction.

Morphological identification:

Lactobacillus acidophilus species are creamy white colour,Gram positive, rod shaped, fermentative, organotrophs, non motile, grow readily at low pH values i.e. below pH 5.0 and it has optimum growth temperature is 37°C.Its size range between 0.5 to 0.8 Micron metre.

Biochemical characteristics identification:

Main biochemical index of isolated *lactobacillus acidophilus* were determined by via the biochemical identifications by Indole production test, Methyl red (MR) test, Voges-Proskaeur (VP) test, Citrate utilisation test, Catalase test, Oxidase test, Urea hydrolysis, Nitrate reduction and triple sugar iron test. Strains cultured overnight at 37°C were inoculated into the biochemical identification tube via the sterile inoculating loop.Every strain inoculating was triplicated. Negative control tubes were void of bacteria results were read within 24 to 48 hours.

Table: 2. Biochemical Characterization of the isolated organism

Sr. No.	Test	Result
1.	Indol production	-
2.	Methyl red	-
3.	Voges-Proskaeur	-
4.	Citrate Utilization	-
5.	Catalase	-
6.	Oxidase	-
7.	Nitrate Reduction	-
8.	Urea hydrolysis	-
9.	Triple Sugar ion	-

Result and discussion:

The antimicrobial activity of the Pepsodent and Colgate toothpaste was determined by the well diffusion method using standard diffusion techniques.The results of the present investigation showed that the bio efficiency of Pepsodent triple action, Pepsodent expert protection, Colgate maximum cavity protection, Colgate triple action, Colgate total whitening are highest among all the toothpaste against the test organism.The zone of inhibition were average range in Pepsodent whitening, Pepsodent complete care, Pepsodent charcoal white, Pepsodent Germi check +. The zone of inhibition were less in Pepsodent clove salt, Pepsodent super salt, Pepsodent gum care, Colgate optic white, Colgate Max fresh, Colgate cibaca.

The main objective of the study was to evaluate the antimicrobial efficiency of commercially available toothpaste. Lactobacillus acidophilus was chosen as the test organism on the basis that in the oral cavity, they are almost the predominant colonizers of the oral cavity. Oral diseases seem appear after and imbalance among the indigenous microbiota, leading to the emergence of potentially pathogenic bacteria.

The results obtained in this study suggest differences among the tested dentifrices regarding antimicrobial properties. Each and every test comparing other toothpaste which gives zone of inhibition amongst the oral bacteria especially called *lactobacillus acidophilus*. The reason for this could be attributed to the differences in interactions between the bacteria and different Colgate.

When compared to water and conventional toothpaste, all Pepsodent and Colgate toothpaste containing antimicrobial agents showed antimicrobial activity with significant differences. The addition of antimicrobial agents to conventional toothpaste to increase effectiveness in the control or elimination of microorganisms involved in a wide variety of microbial infections in the human mouth, such as lactobacillus acidophilus the primary etiological agents of dental caries. Thus, the objective of the study was evaluated commercially available Pepsodent and Colgate toothpaste that include the most widely used and most studied antimicrobial agents in the composition. Water was

used as a control like in other studies to confirm the microbial growth around the well.

Table: 3. Zone of Inhibition in different Pepsodent and Colgate toothpaste

Sr. No.	Name of Colgate toothpaste	Zone of Inhibition (in Cm)
1.	Pepsodent whitening	1.6
2.	Pepsodent Germi check+	1.5
3.	Pepsodent clove salt	1.4
4.	Pepsodent super salt	1.4
5.	Pepsodent complete care	1.6
6.	Pepsodent expert protection	1.8
7.	Pepsodent charcoal white	1.5
8.	Pepsodent gum care	1.4
9.	Pepsodent triple protection	2.0
10.	Colgate MaxFresh	2.1
11.	Colgate cibaca	2.0
12.	Colgate triple action	2.8
13.	Colgate total whitening	2.7
14.	Colgate Visible white toothpaste	2.5
15.	Colgate maximum cavity protection	2.9
16.	Colgate optic white	2.2
17.	Colgate Max white	2.4
18.	Colgate cavity protection	2.6
19.	Colgate total advanced health	2.6

Oral hygiene is the practice of keeping one's mouth clean and free of disease and other problems by regular brushing of the teeth and cleaning between the teeth. It is important that oral hygiene be carried out on a regular basis to enable prevention of dental disease and bad breath. The most common types of dental disease are tooth decay and gum diseases including gingivitis and periodontitis. Toothpaste (dentifrice) with fluoride is an important tool to readily used when tooth brushing. The fluoride in the dentifrice is an important protective factor against caries, and an important supplement needed to remineralize already affected enamel. However, in terms of preventing gum disease, the use of toothpaste does not increase the effectiveness of the activity with respect to the amount of plaque removed.

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