Comparative Biological Studies on the Three Toothpaste Samples Available in the Market

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Abstract: In the present study, three tooth paste samples Colgate, Sensodyne and Himalaya have been taken**.** This experiment show sensodyne have good antimicrobial activity against *S.aureus* than in Colgate and Himalaya. Colgate total contains which kills a wide range of bacteria. From these studies we conclude Himalaya is showing good DPPH radical scavenging activity at 90 min. This may be due to high phenolic content in Himalaya. Based on the polyphenol estimation, the phenolic content in Himalaya is high. Superoxide scavenging assay shows that Sensodyne it is 95.38%, for Himalaya, 63.59% and for Colgate it is 69.78%. All the paste samples are having no effect on squamous epithelial cells.

Index Terms: Toothpastes, antioxidant, antimicrobial

1. INTRODUCTION:

Toothpaste is a dentifrice used to clean and maintain the aesthetics and health of teeth. Toothpaste is used to promote oral hygiene: it serve as an abrasive that aids in removing dental plaque and food from the teeth, assists in suppressing halitosis and delivers active ingredients (most commonly fluoride) to help prevent tooth decay (dental caries) and gum disease (gingivitis) (Darby M L and Walsh M M. 2010). Salt and sodium bicarbonate (baking soda) are among materials that can be substituted for commercial toothpaste. Swallowing of tooth paste is not recommended due to the presence of fluoride (Verkaik M *et.al.*, 2011).

Adhesions of group of microorganisms on the surfaces of the teeth which are in general called as biofilm and since are related to the teeth are called as dental plaque. They are commonly found between the teeth, on the front of teeth, behind teeth, on chewing surfaces etc.... Bacterial plaque is one of the major causes for dental decay and gum disease (Darby M L and Walsh M M. 2010). Further development of dental plaque will leads to the destruction of the teeth enamel and tissues due to the production of acids by the microorganisms and leads to the periodontal problems due to the inflammation and swelling of the gums (called as gingivitis) and destruction of the bone which are supporting the teeth (called as periodontitis) (Verkaik M *et.al*., 2011). If the oral hygiene is not maintained, these biofilms leads to the dental caries (demineralisation) and there can be chances of hardening of the biofilms due to calcification called as dental calculus or tartar (Verkaik M *et al.*, 2011). Dental biofilms will be composed of Gram –ve such as *Vibrios, Fusobacterias* and Gram +ve bacterias such as S. *Mutans* (Kolenbrander PE. 2000).

As mentioned in the above paragraph, toothpastes are meant to maintain the oral hygiene. They might be having sodium fluoride as an anti-caries agent and to avoid the mouth odour. As thickeners they might be having carboxymethyl cellulose, carrageenan (sulphated polysaccharides) or hydrated silica. Propylene glycol will be used as a stabilizers and sodium hydroxide to control the pH. Sodium saccharin and sorbitol will be used as sweeteners. Glycerine will be used as a hydrator and titanium dioxide as a colorant. Detergents like sodium lauryl sulphate used as surfactants and cleansers. Triclosan, which blocks the fatty acid synthesis, will be used as preservatives due to their low allergic reactions.

Some toothpaste will have herbal formulations based on plant enzyme technology that gently removes surface strains on the teeth. The enzymes can be cysteinyl proteases like papains and bromelain, isolated from papaya and pineapple respectively, which

safely whiten teeth. Herbal formulations can be pomegranate fruit rind, neem, triphala, bishop's weed, false black pepper etc. Pomegranate fruit rind has astringent, antibacterial and antioxidant properties. Studies have shown that they are enriched with polyphenols. Recent studies have shown that Pomegranate also helps to combat dental plaque (Singh *et al.*, 2002). Indian Gum Arabic Tree's fresh twigs are used to protect gums and teeth. The bark extract has tannins which are known for their antioxidant properties. Neem has been used for hundreds of years as an extremely effective method of total oral hygiene. Neem bark possesses phenolic and antioxidant activity and also has anti-inflammatory and antimicrobial properties. Triphala is an astringent which can stop the gum bleeding. Since they are the combination of three fruit extracts are enriched in tannins and phenolic compounds like gallic acid, chebulinic acid etc. Due to the phenolics they possess antioxidant properties, and helps in healing mouth ulcers (Ayurvedic pharmacopoeia committee). Bishop's Weed, a collection of group of plants, is used as a home-remedy for various ailments. It has mouth-freshening, antiseptic, astringent and carminative properties. False Black Pepper (*Embelia ribes*) which are rich in flavonoids has anti-inflammatory properties, which are effective in treating swollen gums.

There is some toothpaste in the market which has the capacity to ameliorate the sensitivity of the teeth. Along with the absorbent, detergents, thickeners, artificial sweeteners as mentioned in the earlier paragraphs, they have potassium nitrate for desensitization of the teeth. The potassium ion from potassium nitrate hyperpolarizes the nerve and stops it from firing. The nerve impulses are thus desensitized and there is no pain. Strontium based toothpastes (acetate and chloride) which share a similar chemical structure to calcium are therefore able to replace some of the lost calcium and block the exposed tubules in the dentinal tissue. This blockage of the exposed tubules prevents the movement of the fluid within the tubules in response to a sensitivity stimulus that could otherwise cause tooth pain (Ursula and Margaret, 2015).

Oral mucosa, where polyphenols (PPs) reach highest concentration with respect to all other tissues is constantly exposed to oxidative stress from environment and diet (Halliwell B *et al.*, 2005, Johnson IT, 2004). Catechins from tea inhibit production of important metalloproteinase, thus potentially reduces invasion and migration, inducing apoptosis and growth arrest in both oral cancer and oral leukoplakia cell lines. PPs may also contribute to increase the anti-oxidant activity of oral fluids. When there is disequilibrium between oxidative stress and anti-oxidant activity, periodontal tissue destruction may appear. This suggests that antioxidant rich diets might inhibit periodontal disease development and progression (Petti S and Scully C, 2009). Effect of PPs against dental caries has been generally investigated indirectly. PPs act against mutants *Streptococci* by inhibiting glucosyl-transferase (GTF) activity and insoluble glucan synthesis, adherence inhibition on hard surfaces, inhibition of acid production from sucrose or glucose, bacteriostatic activity against mutants *Streptococci* and down regulation of essential enzymes for *Streptococcus* metabolism. High consumption of coffee, barley coffee, tea and wine show lower *Lactobacilli* and mutants *Streptococci* levels in plaque and saliva and lower dental plaque scores than low/ non-consumers. In an *in-vivo* study, conducted to study the effect of mouth wash containing oolong tea (OTE) (oolong tea is traditional Chinese tea produced through process including withering plant under strong sun and oxidation before curling and twisting) extract was effective even after plaque deposition in human volunteers; the results showed that while OTE significantly inhibited plaque deposition, it has no significant effect on *Streptococci* mutants. It was concluded that, OTE had strong anti-plaque properties due to its ability to inhibit insoluble glucan synthesis (Ciardi JE and BowenGR, 1978).

In the present study, three different paste samples have been selected. Selection of these paste samples were based on their difference in the composition.

2. MATERIALS AND METHODS:

Preparation of paste sample: Different paste sample of 1 gram in 9 ml of dist. water and centrifuged at 5000 rpm for 5 minutes and the supernatant was collected. Obtained supernatant is used as the source of paste sample for the further study.

1. Anti-Microbial Assay:

A variety of laboratory methods can be used to evaluate or screen the *in vitro* antimicrobial activity of an extract or a pure compound. The most known and basic are the disk-diffusion and broth or agar dilution methods.

Preparation of media:

The 0.225 g beef extract and 0.375 g of peptone were dissolved in 150 mL of dist. water. Then transfer 50mL of this mixture to different test tubes and autoclaved. Then check pH of the medium and adjust to pH 7.0, if necessary, using HCl and/ or NaOH. Adding the agar in next step will not appreciably change pH. Add 2 g of agar powder to the flask. Heat to just boiling for 1-2 minutes while stirring constantly (Mounyr B *et. al.*, 2016).

Preparation of stock solution of organisms

Autoclaved 50 mL of beef extract and peptone solution taken in LAF and take one loop full of two different organisms (*E.Coli* and *S.aureus*) and mixed thoroughly in solution and kept for 24 hours in 37ºC (Mounyr B *et. al.*, 2016).

Inhibition study:

3 mL of broth was taken and 20 µL of microorganism was added. Then 5g of paste sample was added and mixed well. The absorbance was measured at 560 nm for every 60 minutes (Mounyr B *et. al.*, 2016).

Inhibition study using spread plate method:

Methodology

Pour pre-autoclaved agar medium to pre-sterilized six different petriplates named as control, blank and with sample of three different toothpastes. After streaking the plates with organisms, 10µL of toothpaste samples were loaded above the streak part of stock solution in sample plate using punching small paper without overflow to the stock of solution of two different organisms. Then was kept it for incubation at 37^0C for 24 hours and 48 hours and observed the zone of inhibition (Mounyr B *et. al.*, 2016).

Anti-oxidant study:

DPPH Assay:

1mM DPPH was prepared in ethanol. Tooth paste sample was by 1 gram of sample in 10 mL of ethanol and centrifuged at 5000 rpm for 5 minutes. The supernatant was used for further study.

Methodology:

Tooth paste samples of 10µL of three different paste samples added with 2.5mL of stock DPPH solution and incubated for 20 min at room temperature. Readings were noted at 517 nm. Based on the value and colour changed antioxidant is determined (Blois, 1958).

Superoxide anion scavenging assay:

Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of Nitroblue Tetrazolium (NBT). In these experiments, the superoxide radicals were generated in 3 ml of Tris-HCl buffer (16 mM, pH 8.0)

containing 1 ml of NBT (50 mM) solution, 1 ml NADH (78 mM) solution and sample solution of fraction (25 – 500 mg/ml) in ethanol. The reaction started by adding 1 ml of Phenozine Methosulphate (PMS) solution (10 mM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min; the absorbance was read at 560 nm by spectrophotometer (Schimadzu UV-Vis 1700) against blank samples using ascorbic acid as a control. Decreased absorbance of the reaction mixture indicated the increasing of superoxide anion scavenging activity (Pawan GN, 2013).

Estimation of Total polyphenols:

Total phenolic compounds in three different toothpaste samples were quantified by using Folin- Ciocalteu (FC) method.

Methodology

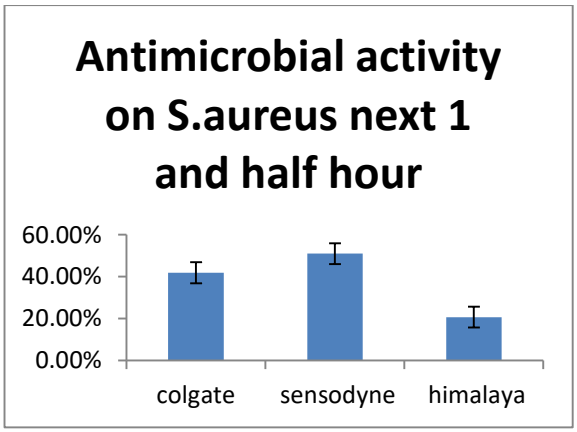
200µl of paste sample was taken and water was added to makeup to the volume 1 ml. Add 0.5 ml of FC reagent (1:1), mixed thoroughly for 3 minutes and later was added with 2 ml of 20% (w/v) sodium carbonate and incubated for 60 minutes. Gallic acids used as a reference compound (0-50ml/mL). The absorbance was measured using colorimeter at 650 nm (Shrawan S, 2011).

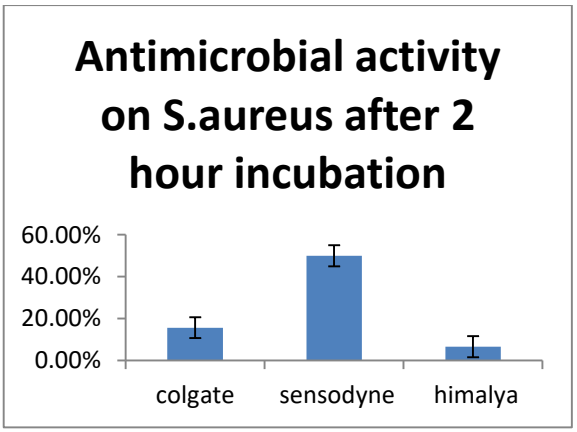
Effect of Paste samples on squamous epithelial cells:

Isolation of epithelial cells from buckle part of mouth is done by wet moulting method (Rufus MC, 1970). Dry the ethanol soaked tooth pick in air and scrape gently the inner side of cheek. A large number of cells will come on the tooth pick. Gently rub the tooth pick on slide in one direction to make a spread of cells. Dry the cells on slide so that the cells will not get washed away while staining. Put a few drops of methylene blue stain and leave for 5 min. Methylene blue is a vital stain and thus even a diluted solution of stain can be easily picked up by the living cells. In this case we are not staining the living cells. After 5 min of staining, rinse cells once with distilled water so that complete stain is not gone and a diluted stain remains. Mount the cells in a drop of distilled water with a cover glass and observe under the bight field students microscope.

2. RESULTS AND DISCUSSION:

Toothpaste is having good anti-microbial activity against both *S.aureaus* and *E.coli*. From spread plate study, Colgate shows only 20% zone of inhibition, Himalaya show good antimicrobial activity of almost 80% of zone of inhibition. But sensodyne and chloremphenicol show less inhibition and more growth of organism (microbial activity). Himalaya>colgate>>sensodyne and chloremphenicol (Antibiotic disc).





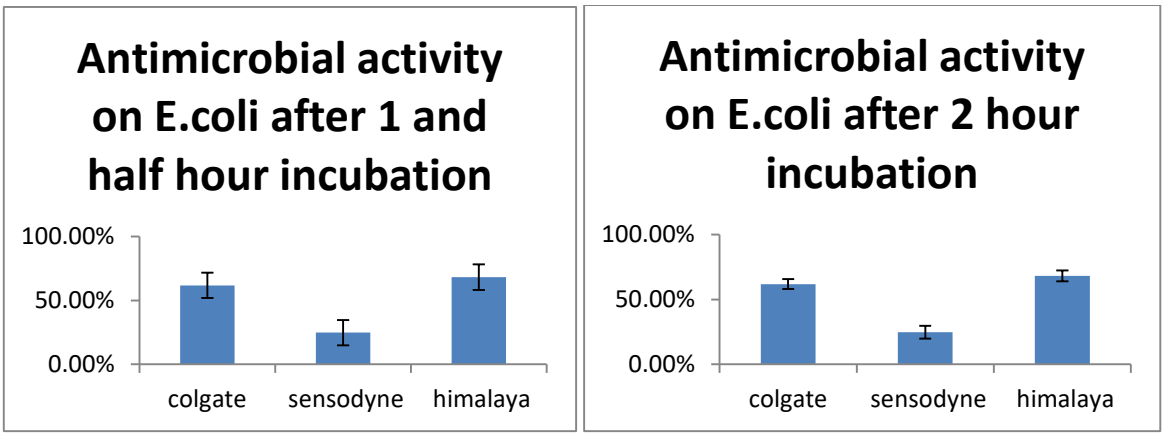


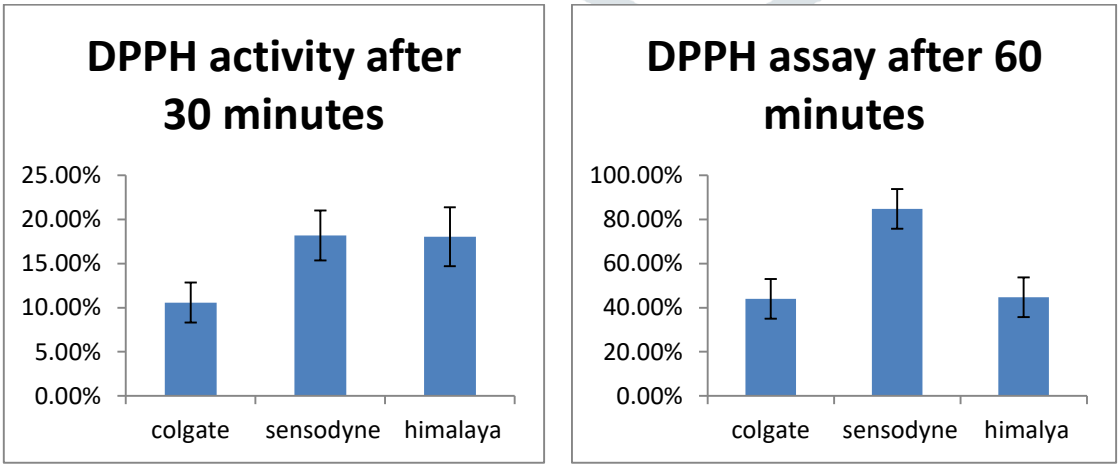
Fig. 1. Anti-microbial activity of the tooth paste samples. X- axis-% Inhibition Y-axis- Tooth paste samples.

In broth culture, *S.aureus* and *E.coli* as control and three paste samples incubated shows antimicrobial activity ranging from one and half hour and two hour of different values in spectrophotometer. Where *S.aureus* with three paste sample show respective percentage of inhibition. From fig 1-After 1 and half hour colgate-73.40%, sensodyne-49.03% and Himalaya-79.46% inhibition. Finally after 2 hour colgate-87.40%, sensodyne-57.08% and Himalaya-93.75%.

In *E.coli* organism three sample show different inhibition. From fig 1, after one and half hour, colgate-60.67%, sensodyne-23.59% and himalaya-67.11% and finally after two hour of incubation colgate-78.86%, sensodyne-23.59% and Himalaya-87.15%.

This experiment show sensodyne have good antimicrobial activity against *S.aureus* than in Colgate and Himalaya. Colgate total contains which kills a wide range of bacteria. Triclosan binds to teeth with the assistance of a copolymer, providing 12 hours of protection. Also decreasing oral bacteria enables continues fresh breath.

DPPH activity of three paste extracts reveals that they have got profound antioxidant activity. The DPPH antioxidant assay is based on the ability of DPPH a stable free radical to decolourize the presence of antioxidant. The DPPH radial contain odd electron, which is responsible for absorbance at 517nm and for visible deep purple colour. When DPPH accepts, an electron donated by antioxidant compound, the DPPH is decolourized which can be quantitatively measured from the change in absorbance.



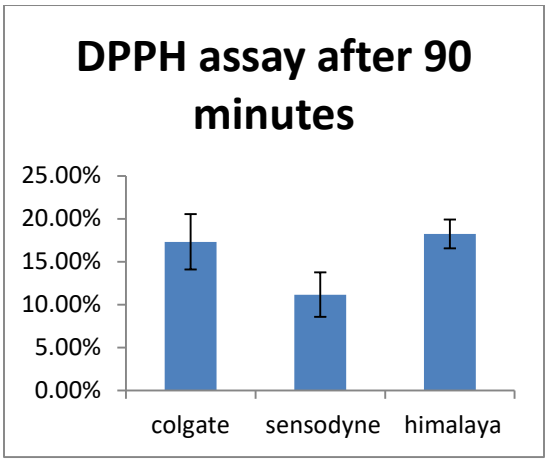


Fig. 2. Anti-microbial activity of the tooth paste samples. X- axis-% Inhibition Y-axis- Tooth paste samples.

The paste extract of three different paste sample show different activity with respect to fig (2) every half an hour incubation colgate-10.58%, sensodyne-18.18% and Himalaya-18.08%. After 1 and half hour incubation colgate-44.06%, sensodyne-84.85% and himalaya-44.67% and finally after 2 hour colgate-17.32% sensodyne-11.16% and Himalaya-18.24%. From these studies we conclude Himalaya is showing good DPPH radical scavenging activity at 90 min. This may be due to high phenolic content in Himalaya.

Estimation of Total polyphenols: Total phenolic compounds in three different toothpaste samples were quantified by using Folin- ciocalteu method.

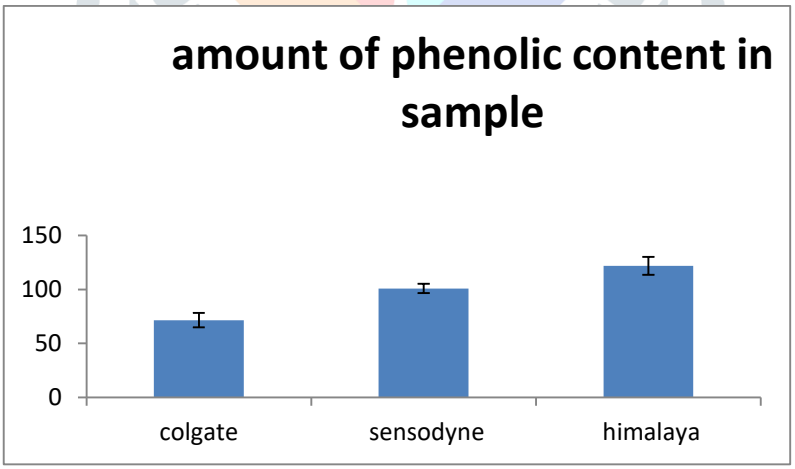


Fig. 4. Phenolic content of the tooth paste samples. X- axis-% Inhibition Y-axis- Tooth paste samples.

According to our study (Fig. 4), the phenolic content in Himalaya is high. Therefore it has high free radical scavenging activity compare to colgate and colgate has good phenol cantent than Sensodyne (Balaji R et al., 2002).

Superoxide anion scavenging assay:

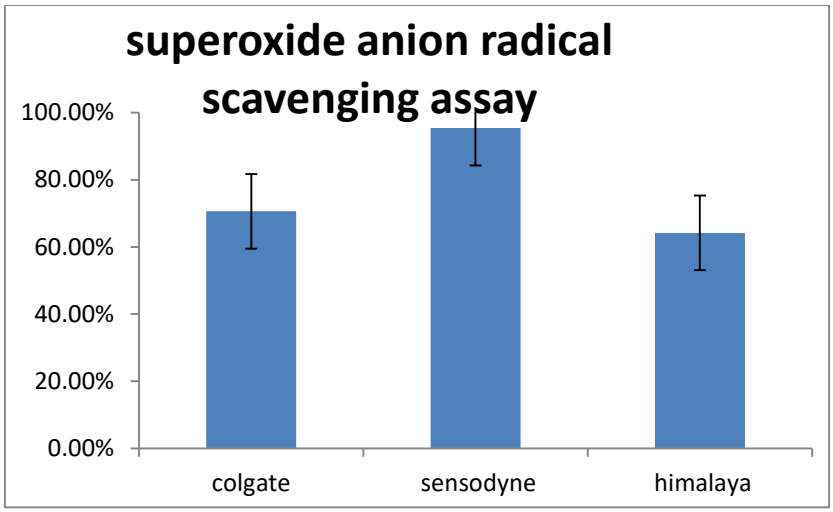


Fig 6.5. Superoxide anion radical scavenging assy. X-axis- % Phenolic content in µg. Y-axis- Tooth paste samples.

% Inhibition=A_1 x 100/A_0 =100- xvalue = y value where x resembles activity of sample and y value resembles percentage of inhibition. Figure 6.5 shows that for Sensodyne it is 95.38%, for Himalaya, 63.59% and for Colgate it is 69.78%.

Effect on squamous epithelial cells by paste samples:

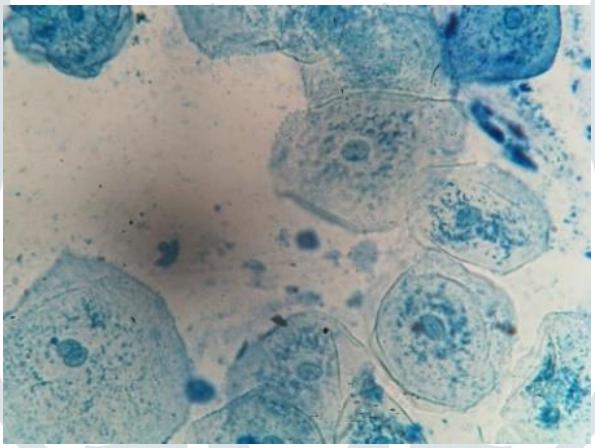


Fig. 6.6. Effect on squamous epithelial cells by paste samples.

Fig. 6.6 shows that, all the paste samples are having no effect on squamous epithelial cells. Present study showed that paste sample upon incubation with epithelial cells, there is no damage of epithelial cells on incubation and show clear existence in microscope.

From the result of present study, Sensodyne with NF shows good anti-microbial activity against *S.aureus* and *E.coli* than colgate and Himalaya. These paste samples based on their composition, shows good anti-oxidant and super oxide anion scavenging activity.

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