RESEARCH ARTICLE

Comparative antimicrobial efficacy study of different commercially available toothpaste in India: An in vitro study

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Received: 12.09.2021

Accepted(Online first): 08.11.2021

Vol./Issue/Year: 2(2), 2021

Competing interests: Author(s) stated no compete of interest.

Edited by: Lowy, A.D., Mátyás, B.

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How to cite: Dhakal et al. "Comparative antimicrobial efficacy study of different commercially available toothpaste in India: An in vitro study" DRC Sustainable Future 2021, 2(2): 122-131, DOI: 10.37281/DRCSF/2.2.5

ABSTRACT

Antimicrobial chemotherapeutic agents have been recommended for lowering oral bacteria growth. The main purpose of this study is to examine the efficacy of different toothpaste formulations in providing complete oral cavity protection against oral pathogens. By using a modified well agar diffusion assay, twenty kinds of toothpaste were examined for antimicrobial efficacy against two oral pathogens: *Streptococcus aureus* and *Escherichia coli*. The examination indicated that the majority of the non-herbal dentifrices and combinations of herbal and chemical-based dentifrices chosen for the investigation were viable against both microbial strains, however, to differing degrees. TP1 and TP17 were found the best against *E. coli* and *S. aureus*, respectively, with 21.553 mm and 23.443 mm as the zone of inhibition. From the herbal dentifrices, TP15 was found to have significant effect on *E. coli*, followed by TP19 for *S. aureus*. Nevertheless, toothpaste TP15 and TP19 were not effective against *S. aureus* and *E. coli*, respectively. In correlation, the inhibition zones of every single other dentifrice were found to be less. Antimicrobial activity against test organisms was stronger in a sodium lauryl sulphate-based dental formulations, when combined with fluoride. A formulation including TP15 exhibited substantial activity against the tested bacterium *E. coli* among herbal dentifrices. Statistical analysis demonstrated that the effectiveness against Gram-negative bacteria was greater than against Gram-positive bacteria. Furthermore, herbal toothpaste can be incorporated with chemotherapeutic agents to enhance its effectiveness against pathogens present in the oral microbiome. This comparison aids in the identification of the toothpaste's shortcomings and benefits over other formulations, widening the scope of more potent toothpaste products.

Keywords: Antimicrobial efficacy, toothpaste, dentifrices, cyclooxygenase, extracellular polymeric substances, *Escherichia coli*, and *Staphylococcus aureus*.

Abbreviations: (ANOVA) Analysis of Variance, (COX) Cyclooxygenase, (EPS) Extracellular Polymeric Substances, (FDA) U.S. Food and Drug Administration, (MRSA) Methicillin-Resistant *Staphylococcus aureus*

1.Introduction

Oral microbiome is the collective genome of microorganisms found in the oral cavity (Deo and Deshmukh, 2019). The oral cavity is a complex system that comprises teeth, tongue, and buccal mucosa; it consists of over 700 different species of bacteria and is home to 6 billion bacteria (Deo and Deshmukh, 2019). Bacteria that live in human's mouths help the gums and teeth digest food (Loesche, 1996). Bacterial plaque causes dental disease because of release of acidic waste, which weakens and decays the teeth (Marsh, 2006). Toothpaste is used to maintain oral hygiene while also delivering active chemicals that protect teeth from dental caries and gum disease. Fluoride, surfactants (detergents), antimicrobials, and bleaching agents are all active constituents in chemical-based toothpaste for teeth whitening. Aside from the oral benefits, these compounds can be dangerous if consumed in significant amounts (Davis *et al.* 2003). Hence, using toothpaste to brush teeth helps to remove oral biofilm and reduce bacterial attacks. Some common examples of bacteria responsible for dental cavities are *Staphylococcus aureus* (Torlak *et al.*, 2017), *Escherichia coli* (Patra *et al.*, 2014), *Streptococcus mutans* (Kawada-Matsuo and Komatsuzawa, 2017), *Streptococcus sobrinus* (Liu *et al.*, 2020), *Lactobacilli* sp. (Caufield *et al.*, 2015), and *Porphyromonas gingivalis* (Patra *et al.*, 2014).

Mechanism of tooth decay by dental biofilm formation

Dental caries is mainly caused by the action of acids on the enamel surface. It results from the accumulation of plaque and from different biochemical reactions of complex microorganisms (Torlak *et al.*, 2017). Dental biofilms are colonies of microorganisms that attach to the surface of the tooth and multiply rapidly (Figure 1).

When left unchecked, they cause several oral diseases, such as dental caries, gingivitis, periodontitis, cavities, sensitive teeth, reversible pulpitis, and halitosis (Nimako-Boateng *et al.*, 2016). Bacteria that form biofilms have higher resistance to antibiotics and are much more difficult to be destroyed by the immune system of the host (Gurenlian, 2007). The slimy extracellular polymeric substances (EPS) produced by these bacteria offer them protection and mechanical support and also help in the binding of vital nutrients needed for the growth of specific microbes (Saini *et al.*, 2011). Biofilms are formed when a thin film of salivary glycoproteins, known as a dental pellicle, attaches to the surface of a tooth. Bacteria then adhere to the pellicle and release EPS, which helps bacteria to stick together and further enhance their growth. Bacterial colonies grow and aggregate to produce mature biofilms which later disperse to form new colonies (Huang *et al.*, 2011; Chen *et al.*, 2020). Acids produced by biofilm-forming bacteria have been recently investigated to have oral carcinogenic effects. These acids promote the breakdown of enamel, by this initiating the process of tooth decay. Once the enamel is demineralized, the acids diffuse deeper into the tooth, causing damage to the dentin and pulp. Inflammation of dental pulp is known as pulpitis. If the dental pulp is breached, bacteria gain access to the bloodstream, a phenomenon known as bacteremia. When biofilm-forming bacteria dominate the gingival sulcus, supragingival biofilm is formed, which causes gingival inflammation, leading to the onset of gingivitis. If the supragingival biofilm is not removed and is left to spread, it forms the subgingival biofilm, leading to periodontitis (Larsen and Fiehn, 2017; Radaic and Kapila, 2021). Such complexities due to dental biofilm can be prevented by maintaining good oral hygiene.

Figure 1. A graphical overview of tooth decay (plaque) mechanism and toothpaste working

History of toothpaste

The invention of toothpaste is popularly credited to Egyptians, who started using a paste made of oxen hooves, myrrh, eggshells, pumice, and water (Li *et al.*, 2011). The reason for this invention was to provide a solution to teeth problems faced even today, such as keeping teeth clean, preventing cavities, and freshening breath. It worked by removing the layer of plaque on the teeth.

Around the same time, Romans and Greeks also invented novel forms of toothpaste by adding more abrasives and flavouring agents, such as charcoal and bark to help with bad breath (Vranic *et al.*, 2004a). China and India were employing a similar powdered form with native ingredients, including ginseng, mint, and salt. (Buzalaf *et al.*, 2011) In China, toothpaste and toothbrushes were made from *Salvadora persica* plants (Needham and Lu, 1959).

Until around 1945, toothpaste was sold in jars in powder form with soap being an essential component. After 1945, soap was replaced by sodium lauryl sulphate, which gave the toothpaste a smoother texture (Neel *et al.*, 2016). Major components present in toothpastes are sodium lauryl sulphates, fluorides, triclosan, coloring and flavoring agents, and sweeteners (Weatherly and Gosse, 2017). Various special toothpastes are also sold, which offer solutions to specific problems, such as teeth whitening, gingivitis, and sensitivity (Xiao *et al.*, 2020; Vaz *et al.*, 2019)

Modern toothpastes

Modern chemical-based toothpastes contain fluorides and triclosan because of their antibacterial properties (Wang and Ren, 2017). 1000-1500 ppm of fluoride used daily has been found to reduce the chances of dental caries (Sridhar *et al.*, 2014). Tooth enamel undergoes periodic demineralization and remineralization, with hydroxyapatite being the primary mineral (Figure 1). Fluoride ions act by inhibiting demineralization of the enamel (Shubha and Hiremath, 2010). The presence of bacteria converts sugars to lactic acid, causing the pH to fall. When it falls below pH 5.5, demineralization takes place (Neel *et al.*, 2016). Fluoride prevents this by protecting the crystals and even altering the bacterial physiology, which affects membrane permeability (Al-Ayed *et al.*, 2016).

Triclosan is a broad-spectrum antimicrobial chemical that was recently banned in soaps by the U.S. Food and Drug Administration (FDA), but continues to remain an important component of toothpastes (Sukkarwalla *et al.*, 2013a). Comparative studies have revealed that toothpaste containing triclosan is more effective at controlling microbial flora in the mouth than non-triclosan toothpaste (Blinkhorn *et al.*, 2009). It blocks lipid biosynthesis, thereby damaging bacterial cell

membranes, leading to their destruction. It also plays a role in reducing inflammation by inhibiting the cyclooxygenase (COX) and lipoxygenase pathways, although studies in this area have been limited, so far (Xiao *et al.*, 2020)

Bacteria and oral cavity

E. coli are Gram-negative bacteria primarily found in the intestine. They are also present in abundance in infants prior to their first tooth eruption. After tooth eruption takes place, microorganisms present in the oral cavity colonize various regions, such as the teeth, tongue, and mucosa (Padhiary *et al.*, 2013). *E. coli* infections are prevalent in the gastrointestinal tract, urinary tract, and biliary tract, but rare cases of osteomyelitis in the maxillary region have also been reported. Primary causes are contamination and contact with faecal matter (Wang and Ren, 2017).

S. aureus is a Gram-positive bacterium primarily found in the nasal and ear cavities, but it can also be isolated from the oral cavity. It is also known to cause tooth decay and infections, such as osteomyelitis (Patil *et al.*, 2013). This is an inflammation that affects the region in the proximity of mandible, with rare cases seen within the mandible. Chronic osteomyelitis are generally caused by mixed microbial population consisting of staphylococcus and actinomycetes (Sridhar *et al.*, 2014). Methicillin-Resistant *S. aureus* (MRSA) is known to be resistant to a multitude of antimicrobial treatments.

In this study, solution properties of twenty dentifrice formulations were screened for *in vitro* antibacterial efficacy to compare their effectiveness toward tooth decaying bacteria *E. coli* and *S. aureus*. *E. coli* and *S. aureus* were used because of their presence and colonization in the oral microbiome. One additional reason for investigating these bacteria is the increasing number of reports on their role in afflictions, such as osteomyelitis. Most plant extracts added to the herbal-based toothpaste possess medicinal value. Antibacterial efficacies of such herbal and chemical-based toothpaste were compared and evaluated.

Based on this information, we conducted the investigation of antimicrobial efficacy of different kinds of toothpastes by using the standard agar well diffusion method. Relevant comparison of several toothpaste formulations aids in the identification of the toothpaste's shortcomings and benefits over other formulations, widening the scope of more potent toothpaste products.

2. Methodology

A total of twenty kinds of toothpastes (Table 1) were tested for their antimicrobial activity against two oral pathogens, *E. coli* and *S. aureus*. Nutrient agar was used as a nutrient medium (Valgas *et al.*, 2007).

Table 1. List of different sample toothpaste and their composition

Labelling	Name	Type	Composition
TP1	Colgate® Strong Teeth	Cavity protection	Calcium carbonate, sorbitol, water, silica, sodium lauryl sulphate, flavour, arginine, cellulose gum, carrageenan, sodium silicate, titanium dioxide, sodium monofluorophosphate, sodium bicarbonate, sodium saccharin, sodium benzoate, benzyl alcohol, limonene, potassium nitrate
TP2	Pepsodent® G	Gum Care	Water, sorbitol, hydrated silica, sodium lauryl sulphate, PEG-32, zinc citrate, flavour, cellulose gum, sodium fluoride, sodium saccharin, C177891
TP3	Himalaya Herbals® Complete Care	Cavity protection	Sorbitol, aqua, glycerin, hydrated silica, silica, potassium sorbate, titanium dioxide, sodium lauryl sulphate, sodium benzoate, flavour, sodium saccharin, xanthan, sodium fluoride, methanol, punica granatum pericarp extract, calcium fluoride, zanthoxylum alatum fruit extract, acacia arabica stem bark extract, terminalia bellerica fruit extract, emblica officinalis fruit extract, emblica ribes fruit extract, azadirachta indica bark extract, vitex negundo extract, thymol, citric acid, salvadora persica stem extract, acacia farnesiana flower/stem extract, acacia catechu bark powder, mimosops elengi flower extract
TP4	Vicco® Vajradanti	Herbal	Babhula, jambhul, lavang, manjishtha, dalcchini, bor, vajradanti, acord, khair, patang, bakul, jeshthamadh, kavab-chini, anantmul, maifal, trifala-akkalkadha, excipients q.s
TP5	Lever Ayush®	Herbal	<i>Eugenia caryophyllus</i> , Dasanakanthi choornam, <i>Acacia Nilotica</i> , <i>Acacia catechu</i> , ferric oxide, <i>Piper longum</i> , <i>Glycyrrhiza glabra</i> , <i>Syzygium Aromaticum</i> , Daruharidari <i>Berberis aristata</i> , <i>Piper nigrum</i> , <i>Myristica fragrans</i> , <i>Myristica Fragrans</i> , <i>Piper cubeba</i> , <i>Elettaria cardamomum</i> , <i>Cinnamomum zeylanicum</i> , <i>Cinnamomum camphora</i> , <i>Rose centifolia</i>
TP6	K. P. Nambodirisi®	Herbal	Calcium carbonate, glycerine, water, dicalcium phosphate dihydrate, sodium lauryl sulphate, extract of ginger, black pepper, long pepper, <i>Terminalia chebula</i> , gooseberry
TP7	Himalaya Herbals® Sparkling White	Whitening	Water, sorbitol, hydrated silica, sodium lauryl sulphate, glycerin, bromelain, xanthan gum, titanium dioxide, flavour, sodium saccharin, sodium benzoate, potassium sorbate, papain, menthol, salvadora persica stem extract, sodium citrate, <i>Prunus amygdalus</i> shell extract, <i>Cinnamomum zeylanicum</i> bark oil, <i>Eugenia caryophyllus</i> bud oil, thymol, citric acid.
TP8	Close Up®	Whitening	Water, sorbitol, hydrated silica, sodium lauryl sulphate, PEG-32, zinc sulphate, cocamidopropyl betaine, lavor, cellulose gum, sodium fluoride, sodium saccharin, C177891, C1 1625, C1 16035, synthetic fluorophlogopite, C116035, <i>Melaleuca alternifolia</i> , <i>Eucalyptus globulus</i> , eugenol
TP9	Sensodyne® Whitening	Whitening	Water, sorbitol, hydrated silica, sodim saccharin, sodium fluoride, sodium hydroxide, glycerin, pentasodium triphosphate, potassium nitrate, PEG-6, aroma, titanium dioxide, cocamidopopyl betaine, xanthum gum, sodium hydroxide, sucralose, sodium methyl cocoyl taurate
TP10	Colgate® Sensitive Plus	Sensitivity	Calcium carbonate, sorbitol, arginine bicarbonate, sodium lauryl sulphate, flavour, sodium monofluorophosphate, sodium carboxymethyl cellulose, sodium bicarbonate, tetrasodium pyrophosphate, titanium dioxide, sodium saccharin, benzyl alcohol, xanthan gum, limonene, in aqueous base
TP11	SENSODYNE® Deep Clean	Sensitivity	Aqua, hydrated silica, sorbitol, glycerin, pentasodium triphosphate, potassium nitrate, PEG-6, sodium hydroxide, cocamidopropyl betaine, sodium saccharin, sodium fluoride, FD&C
TP12	Colgate® Sensitive	Sensitivity	Sorbitol, glycerin, silica, potassium nitrate, polyethylene glycol 600, sodium lauryl sulphate, sodium carboxymethyl cellulose, flavour, tetrasodium pyrophosphate, sodium saccharin, xanthan, sodium fluoride, C142090, in aqueous base
TP13	Dabur Red Toothpaste	Herbal	<i>Piper nigrum</i> , <i>Piper longum</i> , <i>Zingiber officinale</i> , Tejovati/Tomer, <i>Zanthoxylum armatum</i> , <i>Syzygium aromaticum</i> , <i>Mentha arvensis</i> , gairic powder, sodium saccharin, methyl paraben, propyl paraben, sodium benzonate.
TP14	Pepsodent® Germi Check+	Cavity protection	Calcium carbonate, water, sorbitol, hydrated silica, sodium lauryl sulphate, kaolin, flavour, sodium monofluorophosphate, cellulose gum, potassium nitrate, benzyl alcohol, sodium saccharin, cetylpyrimidine, C1 45430, limonene
TP15	Dabur® Meswak Complete Oral Care	Gum Care	Calcium carbonate, sorbitol, water, silica, sodium lauryl sulphate, flavour, miswak extract, cellulose gum, carrageenan, sodium silicate, PVM/MA copolymer, sodium saccharin, zinc gluconate, sodium benzonate, benzyl alcohol, C177891, p-thymol
TP16	Colgate® Active Salt	Salt	Calcium carbonate, sorbitol, silica, sodium lauryl sulphate, sodium silicate, sodium monofluorophosphate, sodium carboxymethyl cellulose, sodium chloride, sodium bicarbonate, sodium saccharin, xanthan gum, benzyl alcohol, pigment blue (C1 74160), flavour, in aqueous base
TP17	Colgate® Salt (Neem)	Salt	Calcium carbonate, sorbitol, water, silica, sodium lauryl sulphate, flavour, xanthum gum, sodium carboxy methylcellulose, sodium monophosphate, sodium silicate, eucalyptus oil, thymol, clove oil, sodium saccharin, sodium benzonate, eugenol, d-limonene, benzyl alcohol, C177492, C177491, C174260, lemon oil, neem oil, basil oil
TP18	Patanjali® Dant Kanti	Herbal	<i>Anacyclus pyrethrum</i> , <i>Azadirachta indica</i> , <i>Acacia arabica</i> , <i>Xanthoxylum alatum</i> , <i>Mentha spicata</i> , <i>Syzygium aromaticum</i> , <i>Piper sylvaticum</i> , <i>Barleria prionitis</i> , <i>Mimosops elengi</i> , <i>Embelia ribes</i> , <i>Curcuma longa</i> , <i>Salvadora persica</i> , <i>Quercus infectoria</i>
TP19	Himalayan® Dental Cream	Herbal	<i>Carum copticum</i> , fluorspar, <i>Azadirachta indica</i> , <i>Xanthoxylum alatum</i> , <i>Punica granatum</i> , <i>Acacia arabica</i> , <i>Vitex negundo</i> , <i>Embelia ribes</i> , Triphala comprises of <i>Embelia officinalis</i> , <i>Terminalia chebula</i> and <i>Terminalia bellerica</i>
TP20	Sensodyne® Fresh Mint	Sensitive Teeth and Cavity Protection	Potassium nitrate, Sodium fluoride w/v fluoride ion, Water, hydrated silica, sorbitol, glycerin, pentasodium triphosphate, PEG-8, flavour, titanium dioxide, sodium methyl cocoyl taurate, cocamidopropyl betaine, xanthan gum, sodium hydroxide, sodium saccharin, sucralose

Procedure

The antimicrobial activity of dentifrices of different toothpastes was measured by the modified agar well diffusion method (Balouiri, Sadiki and Ibensouda, 2016). Antimicrobial activity was interpreted as the diameter of the zone of inhibition (Shubha and Hiremath, 2010).

Cleaning and sterilization

The equipment required for the experiment was cleaned and rinsed using 75% ethanol and detergent. The equipment was dried in a hot air oven (at 80 °C, for 3 h) and placed in an autoclave for sterilization (121 °C, 15 psi, for 15 min) (Sridhar *et al.*, 2014).

Preparation of nutrition media

Nutrient media were prepared by adding 15 g of nutrient agar to 1000. mL distilled water. The flask was thoroughly mixed until the powder was completely dissolved. For sterilization, the medium was autoclaved. After that, the medium was poured into Petri dishes (25 mL). A spread plate approach was used to inoculate the bacterial strains (Sridhar *et al.*, 2014).

Inoculation of toothpaste sample

A sterile 4 mm/8 mm cork-borer was used to cut six wells at equidistance from each other, and labelled in each Petri dish containing bacteria. A micropipette was used to inoculate 150 µL of a 1:1 dilution of toothpaste and water mixture into the wells, and the plates were sealed and incubated for 18-24 h at 37 °C. Each dentifrice's zone of inhibition (mm) was determined (Prasanth, 2011). Experiments were repeated three times and all plates were prepared in triplicate.

Statistical analysis

To compare the mean differences in the zone of clearance across samples and strains, a two-tailed analysis of variance (ANOVA) with replication was used. A two-tailed Student's t-test was used to compare the differences between sample averages of two strains. All statistical computations were performed with the Microsoft Excel 2016 program, and all sample analyses were completed with a 95% confidence level at $p < 0.05$ (John and Rajan, 2021).

3.Results

In vitro antimicrobial analysis of different toothpaste formulations was carried out to determine their growth inhibition ability against two common Gram-positive and Gram-negative bacteria species, *S. aureus* and *E. coli*, respectively. Comparison of the zones of inhibition formed by the toothpaste formulations, due to the active antimicrobial agents present in the toothpaste, was used to analyze the antimicrobial efficiency.

For *E. coli*, toothpaste TP1 demonstrated the maximum zones of inhibition with a mean diameter of 21.553 mm (Figure 1). Other non-herbal toothpaste with broad zones of inhibition included TP2 and TP14 with mean diameters of 19.550 mm and 16.333 mm, respectively (Figure 1). In the non-herbal variety of toothpaste, TP17 had the lowest zone of inhibition with a diameter of 2.22 mm, followed by TP20, which had a zone of inhibition of 5.997 mm (Figure 1).

Except for TP15 with a zone of inhibition of 17.663 mm (Figure 1), all other herbal toothpaste demonstrated lesser but comparable zones of inhibition to non-herbal toothpaste, indicating weaker antimicrobial activity and lesser efficiency at inhibiting *E. coli* microbial growth. Among these, TP13 showed no zone of inhibition. TP16, which is a combination of chemical and herbal ingredients, exhibited an inhibition zone of 16.440 mm, which is comparable to TP15, an entirely herbal toothpaste. TP17 inhibited bacteria *S. aureus* the most effectively, with zones of inhibition of 23.44 mm in diameter, followed by TP1 and TP14 with 19.643 mm and 18.440 mm in mean diameter, respectively. In addition to the standard chemical ingredients found in toothpaste, TP17 also consisted of herbal ingredients, such as eucalyptus oil, clove oil, lemon oil, neem oil, and basil oil. Specifically, among the herbal toothpaste, TP19 provided best results, as it had the greatest zone of inhibition with a diameter of 12.330 mm (Figure 1), whereas other herbal toothpastes exhibited a low to nil zone of inhibition.

4.Discussion

Herbal toothpaste had conflicting outcomes. Pure herbal-based toothpaste was not found to be highly effective at performing all actions required for good oral hygiene; instead, it was less toxic. Furthermore, the combined action of many plant extracts has improved the efficiency of herbal-based toothpaste. Clove oil and mentha species commonly boost antibacterial properties of herbal toothpastes. Neem leaf extract, for example, possesses natural antimicrobial properties and provides comprehensive oral hygiene (Radafshar *et al.*, 2010; Anushree *et al.*, 2015). Several studies indicated significant result, with a larger zone of inhibition, while others showed no significant effect over non-herbal toothpaste (Lakshmi *et al.*, 2015; Chaudhary *et al.*, 2020).

Ingredients common to TP1, TP2, and TP14 were sodium benzoate, sodium saccharin, sodium lauryl sulphate, and sorbitol. Sodium saccharin is a sweetening agent, which does not provide any health benefits, and is mainly used for enhancing flavor and taste (Vranic *et al.*, 2004b).

Figure 2. Efficacy of different kinds of toothpastes against *E. coli* and *S. aureus*; positive controls (Amp) = ampicillin and (Ery) = Erythromycin, negative control (D/W) = distilled water

Figure 3. Student's t-Test: Paired two samples for means between *E. coli* (E) and *S. aureus* (S); Rejected null hypothesis for the above samples as there is a significant increase in efficacy toward Gram-negative bacteria

Previously thought to be linked to bladder cancer, sodium saccharin was listed as a potential carcinogen, but because of lack of consistent evidence, it has been delisted (Uçar and Yılmaz, 2015). Sodium lauryl sul-

phate is a synthetic detergent and antibacterial agent that is used to lower the surface tension and loosen the food debris and contaminants, thereby making it easier to rinse them out along with the foam generated. None-

theless, sodium lauryl sulphate is known to be a skin irritant and is linked to the increase in severity of recurrent aphthous ulcers (Vranic *et al.*, 2004b; Baskran, 2021). Sodium benzoate is used as a preservative in toothpaste; it also possesses antibacterial properties and has been shown to induce the growth of helpful bacteria, like Bifidobacterium and inhibit *E. coli* growth (Gerasimidis *et al.*, 2020). But it has several side effects, e.g., it may cause dyspnea, swelling of the nasal mucosa, and in severe cases, even can lead to anaphylactic shock. It exhibited low levels of cytotoxicity at standard concentrations (Tabatabaei *et al.*, 2019). Sorbitol plays a double role of humectant and sweetening agent, and helps hold the toothpaste formulation together. It does not possess strong antibacterial properties, but studies have revealed that it assists in the slowing down of dental caries, decreasing the production of lactic acid and inhibiting *S. mutans*. Nevertheless, a sorbitol-xylitol mixture had better results in inhibiting dental caries in children (Smolarek *et al.*, 2015). Overdose of sorbitol produces a laxative effect, causing abdominal pain and severe diarrhoea (Awuchi, 2017).

TP15 consists of extracts from the plant *Salvadora persica* L., which has been used as a natural toothbrush for centuries. In the meanwhile, TP15 has been scientifically proven to exhibit significant antibacterial activity, and can inhibit plaque formation (Khatak *et al.*, 2010). It consists of various phytochemical components, including salvadorine, flavonoids, saponins, tannins, sterol, resins, alkaloids, fluoride, chloride, sulfur, vitamin C, and benzyl isothiocyanate (Al-Ayed *et al.*, 2016; Balto *et al.*, 2017). Both alkaloids and sulfur have been known to exert bactericidal effects; fluoride helps in tooth remineralization and tannins promote saliva production (Sukkarwalla *et al.*, 2013b). Thus, TP15 is an exception among herbal toothpastes and was able to indicate zones of inhibition similar to non-herbal formulations.

Among herbal toothpastes, TP19 had the greatest zone of inhibition against *S. aureus*. This is believed to the result of various herbal extracts present in it. For many years, *Punica granatum* L has been employed as both food and medicine. Major phytoconstituents include ellagic acid, ascorbic acid, puniceic acid, ellagitannins, anthocyanins, and flavones. It has been scientifically proven that extracts of this plant are effective against *S. aureus* and *E. coli*, and have antibacterial, anti-inflammatory and antioxidant effects (Rath *et al.*, 2014; Pagliarulo *et al.*, 2016). *Zanthoxylum alatum* is popularly known as “toothache tree” and is found in the sub-tropical Himalayas; it has been shown to possess antioxidant and antimicrobial properties. The essential oil extract of this plant was demonstrated to inhibit bacterial growth of *S. aureus* and *E. coli*. The major active constituents of the oil include linalool, methyl cinnamate, limonene, 2-decanone, sabinene, α -pinene, and β -phellandrene (Guleria *et al.*, 2013). Neem (*Azadirachta indica*) has been widely used in traditional medicine for decades, owing to its antibacterial, antifungal, antiseptic, antimalarial, and antiviral benefits. Its major phytoconstituents include azadirachtin, nimbin, gedunin, β -Sitos-terol, and quercetin (Alzohairy, 2016). Research evidenced that the extracts of this plant are highly effective against *S. aureus* and *E. coli* (Francine *et al.*, 2015). Ajwain (*Trachyspermum ammi*), also known as Bishop’s weed, is a popular spice used in Southern Asia; it possesses antimicrobial and anti-inflammatory properties. The major active constituents of ajwain oil include thymol and carvacrol. Though not much effective against *S. aureus*, studies have shown that the essential oil of this plant is efficacious in inhibiting the growth of *E. coli* (Nouri Gharajalar, 2021). Hence, TP19 displayed a greater zone of inhibition relative to other herbal formulations (Table 2), which exhibited a low to nil zone of inhibition.

Table 2. Efficacy of different toothpaste against different *E. coli* and *S. aureus*

Toothpaste →	TP 1	TP 2	TP 3	TP 4	TP 5	TP 6	TP 7	TP 8	TP 9	TP 10	TP 11	TP 12	TP 13	TP 14	TP 15	TP 16	TP 17	TP 18	TP 19	TP 20	
<i>E. coli</i> (zone of clearance) (mm)	24.33	14.00	13.00	13.66	7.30	7.00	6.60	11.00	11.60	18.66	14.00	11.66	0.00	21.66	16.33	15.66	3.30	14.00	14.00	9.30	Total mean (<i>E. coli</i>)
	20.33	16.00	13.66	11.00	9.00	7.00	10.33	9.60	14.00	18.33	13.00	10.66	0.00	19.66	18.00	18.66	3.30	16.00	10.00	4.30	
	20.00	19.00	12.00	11.00	8.30	10.33	11.00	0.00	7.30	14.33	9.30	7.30	0.00	17.33	18.66	15.00	0.00	15.00	10.00	4.30	
Mean	21.55	16.33	12.89	11.89	8.20	8.10	9.30	6.80	10.98	17.11	12.11	9.80	0.00	19.55	17.66	16.44	2.20	15.00	11.33	6.00	11.67
<i>S. aureus</i> (zone of clearance) (mm)	16.60	13.00	0.00	0.00	0.00	0.00	12.66	17.66	11.66	11.33	14.00	15.00	9.30	20.00	14.00	12.33	21.33	2.00	11.00	8.00	Total mean (<i>S. aureus</i>)
	18.33	13.30	0.00	0.00	0.00	0.00	11.33	14.66	6.00	3.30	13.66	7.60	9.00	17.66	0.00	12.33	24.00	0.00	14.33	3.30	
	24.00	13.00	0.00	0.00	0.00	0.00	12.00	4.60	0.00	0.00	12.00	0.00	5.00	17.66	0.00	11.00	25.00	0.00	11.66	0.00	
Mean	19.64	13.10	0.00	0.00	0.00	0.00	8.00	14.77	7.40	4.80	13.22	7.50	7.70	18.44	4.60	11.89	23.44	0.60	12.33	3.70	8.58

A higher probability of increased efficiency against Gram-negative bacteria (*E. coli*) than those against Gram-positive bacteria (*S. aureus*) (Figure 2) was confirmed for TP3, TP4, TP5, TP6, TP10, TP13, TP16, TP17, and TP18. This might be due to the active ingredients present in the primarily herbal toothpaste. TP3, TP4, TP5, TP6, and TP18 were found moderately effective against Gram-negative bacteria (*E. coli*), but they were ineffective for Gram-positive bacteria (*S. aureus*). Nevertheless, the toothpaste TP13 and TP17 were shown to be more effective in inhibiting the growth of Gram-positive bacteria (*S. aureus*). TP10 and TP16, chemical-based toothpaste had a moderate effect on both bacterial strains, indicating that it might work efficiently against a wide range of bacteria present in the oral microbiome.

5. Conclusions

Antimicrobial properties of twenty dentifrices were concentrated in vitro, and we presumed that non-herbal grown toothpaste was increasingly viable against both *E. coli* and *S. aureus* strains.

Our study has shown that toothpaste containing chemical antimicrobial agents, mainly sodium lauryl sulphate, was more effective in controlling microbial growth in the mouth, as compared to toothpaste containing natural antimicrobial agents at the same dilution levels. The only exception to this was the herbal and chemical combination toothpaste TP15, which showed similar zones of inhibition as the non-herbal toothpaste.

Dental biofilm production is a normal process that must be maintained with frequent brushing with the standard toothpaste formulation to increase the efficacy of biofilm control and avoid the development of plaques, which leads to dental cavities and periodontal disease.

Our study can further be advanced and additional experimentation can be done by comparing herbal and non-herbal toothpastes, at different concentrations to check their antimicrobial activity against other common oral microbes. Furthermore, different kinds of toothpastes could be mixed to possibly obtain an improved composition that is effective against a wide range of oral microbes. Studies on the less effective toothpaste can be performed to determine what components do they lack, or how much change in the concentration of their components is required to enhance their antimicrobial activity. Moreover, toothpaste can be tested for the extent they can slow down the progression of a growing cavity or how quickly and effectively it can reverse gingivitis.

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