JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Antimicrobial effect of natural kinds of toothpaste on oral pathogenic bacteria

Serdar Demir[1], Gül Keskin[1], Neşe Akal[2], Yasemin Zer[3]

[1] *Department of Pedodontics, Faculty of Dentistry, Gaziantep University, Gaziantep, Turkey*
[2] *Department of Pedodontics, Faculty of Dentistry, Gazi University, Ankara, Turkey*
[3] *Department of Medical Microbiology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey*

Abstract

Introduction: Because of the adverse effects on human health of some antimicrobial ingredients in traditional toothpaste, consumers are increasingly turning to toothpastes with natural ingredients. This study evaluates the antimicrobial effect of toothpastes containing different natural active agents against three oral pathogens: *Streptococcus mutans, Streptococcus sanguinis,* and *Enterococcus faecalis*.

Methodology: This study tested one traditional toothpaste and seven different natural toothpastes containing theobromine, aloe vera, miswak, propolis, chitosan, enzymes and probiotics. The agar-well diffusion method was used to test the antimicrobial effect. Inhibition zones formed around toothpastes after 24 hours of incubation were measured and the data collected were statistically analyzed.

Results: Toothpastes containing theobromine and chitosan and the traditional toothpaste showed antimicrobial efficacy for all tested bacteria. Toothpastes containing aloe vera, miswak, and propolis were only effective on *S. mutans*, while toothpastes containing probiotics and enzymes did not show any antimicrobial effect on the bacteria. Among toothpastes with natural ingredients, the theobromine-containing toothpaste showed the highest efficacy on *S. mutans*, while the aloe vera- and propolis-containing toothpastes had the lowest efficacy ($p < 0.05$).

Conclusions: Theobromine- and chitosan-containing toothpastes, which showed antimicrobial activity against all bacteria, can be recommended as alternatives to traditional toothpastes.

Key words: Toothpaste; *Streptococcus mutans*; *Streptococcus sanguinis*; *Enterococcus faecalis*; antimicrobial effect.

J Infect Dev Ctries 2021; 15(10):1436-1442. doi:10.3855/jidc.14966

(Received 25 February 2021 – Accepted 23 April 2021)

Introduction

Dental caries is a local, progressive and infectious bacterial dynamic disease caused by acidic byproducts of bacterial metabolism that lead to the dissolution of dental hard tissues [1]. The formation of caries typically depends on multiple factors, including cariogenic microflora, fermentable carbohydrates, plaque formation and time [2]. Dental plaque, an oral microbial biofilm formed on dental surfaces, consists of a wide variety of oral microbial strains and species [3]. *Streptococcus mutans* and *Streptococcus sanguinis* are two of the main bacteria involved in dental biofilm formation and responsible for dental hard tissue destruction [4]. *S. mutans*, a member of the group of mature dental plaques, has the ability to synthesize soluble and insoluble glucans from dietary sucrose mediated by glucosyltransferase enzymes, allowing extracellular aggregation for stable biofilm formation on the tooth surface [5]. *S. sanguinis* is one of the first colonizing streptococci in the course of biofilm formation. These two species may be more dominant in certain environments due to bacteriocins produced by *S. mutans* and hydrogen peroxide produced by *S. sanguinis* [6]. *Enterococcus faecalis* has not been considered as part of the normal oral microbiota and is regarded an infectious microorganism associated with unsuccessful endodontic treatments. However, *E. faecalis* has been found in different layers of the oral biofilm [7]. In addition, *E. faecalis* can contribute to collagen and periodontal destruction, leading to the progression of the disease in patients with chronic periodontitis [8]. Due to its resistance to effective antibiotic groups such as vancomycin, ciprofloxacin and erythromycin, the World Health Organization has reported *E. faecalis* as a "high priority resistant pathogen" for the development of antibiotics [9].

Reducing the number and activity of cariogenic microorganisms assists in the prevention of plaque-related dental and gingival diseases. Therefore, the use of mechanical plaque control and oral hygiene products containing antimicrobial agents is recommended [10]. Toothpastes are a widely used agent for dental biofilm control [11]. However, toothpastes can contain potentially harmful components that may lead to local

side effects such as irritation and desquamation of the oral mucosa (stomatitis, glossitis, gingivitis, buccal mucositis) and systemic side effects such as allergic and toxic reactions (acute or chronic). These effects have mainly been associated with fluoride, sodium lauryl sulfate (SLS) and triclosan [12].

Secondary metabolites from plants, microorganisms and seafood have long been recognized as valuable sources of new molecules with drug development potential in many biomedical fields [13]. They show anti-inflammatory, analgesic [14], antimicrobial [15], antineoplastic [16] and anticariogenic [17] effects. The development and introduction of oral care products claiming to be prepared from natural agents has been continually increasing [18]. Despite the recent increase in consumption of oral care products that claim to be healthy and contain natural agents, there is scant evidence of their benefits [19]. It has also been reported that the antimicrobial activities of these natural ingredients may be altered due to their interactions with other ingredients in commercial preparations [20].

Although many studies have investigated the remineralization and antiplaque effects of natural toothpastes, few studies examined their antimicrobial efficacy. Therefore, this study aims to determine and compare the antimicrobial efficacy of natural toothpastes that have been in use in recent years.

Methodology

Eight toothpastes were tested in this study: toothpastes containing theobromine (Theodent, LLC, Louisiana, USA), aloe vera (Urtekram, International A/S, Mariager, Denmark), miswak (Eyüp Sabri Tuncer, Istanbul, Turkey), propolis (Aagaard Propolis, Sanhelios, Bremen, Germany), chitosan (Chitodent, Helmuth Focken Biotechnik e.K., Sindelfingen, Germany), probiotics (PerioBiotic, Designs for Health, Inc., Suffield, Canada), enzymes (Curaprox Enzycal Zero, Curaden AG, Kriens, Switzerland), and a traditional toothpaste (Colgate Total 12, Colgate-Palmolive Co., Ltd., China). Table 1 lists the contents of the tested toothpastes. Furthermore, a mouthwash containing 0.2% chlorhexidine digluconate (Klorhex

Table 1. Contents of the toothpastes tested in the study.

Toothpaste	Product name (Manufacturer)	Contents
Theobromine-containing toothpaste	Theodent (LLC, Lousiana, USA)	Active Ingredients: theobromine, calcium acetate and sodium hydrogen phosphate. Inactive Ingredients: purified water, hydrated silica, sorbitol, xylitol, glycerin, sodium lauroylsarcosinate, xanthan gum, titanium dioxide, citric acid, and spearmint oil, sodium benzoate, stevia extract, sodium bicarbonate, and sugar-free vanilla extract.
Aloe Vera-containing toothpaste	Urtekram (International A/S, Mariager, Denmark)	Calcium carbonate, aqua, glycerin, *Aloe barbadensis* leaf extract, xanthan gum, citrus reticulata peel oil* (aroma), commiphoramyrrha oil, *Magnolia officinalis* bark extract, limonene, linalool. (ingredient from organic farming, made using organic ingredients). Natural chalk, water, glycerine**, aloe vera*, polysaccharides, mandarine oil*, myrrh, magnolia bark extract. * = Organic farming. ** = Made using organic ingredients.
Miswak-containing toothpaste	Eyüp Sabri Tuncer (İstanbul,Turkey)	Aqua, Sorbitol, Hydrated Silica, Glycerin, Xylitol, Polysorbate 20, Cocamidopropyl Betaine, Disodium Phosphate, Aroma, Xanthan Gum, Menthol, Thymol, *Stevia rebaudiana* Extract, *Mentha piperita* Oil (Naneyağı), *Eucalyptus globulus* Leaf Oil, *Salvadora persica* bark / root extract, Phenylpropanol, Caprylyl Glycol, Propanediol, Tocopherol).
Propolis-containing toothpaste	Aagaard Propolis (Sanhelios, Bremen, Germany)	Aqua (Water), sodium metaphosphate, sorbitol, silica, alcohol, sodium alginate, capryl/ capramidopropyl betaine, aroma (fragrance), *Propolis cera*, sodium chloride, *Chamomilla recutita* flower extract (matricaria), ascorbic acid, sodium hydroxide, sodium saccharin, *Salvia officinalis* Leaf Extract, CI 77891 (titanium dioxide).
Chitosan-containing toothpaste	Chitodent (Helmuth FockenBiotechnike.K. Sindelfingen, Germany)	Aqua, sorbitol, hydrated silica, gycerin, cocoamidopropyl betaine, chitosan, lactic acid, aroma, hydroxypropyl guar, Sodium, saccharin, CI 42051(colorant bleu), natrium hydroxid.
Probiotic-containing toothpaste	PerioBiotic (Designs for health, Inc. Suffield, Canada)	Water, hydrated silica, calcium glycerophosphate, glycerin, xylitol, spearmint oil (*Menthaviridis*), carrageenan,Dental-Lac (*Lactobacillus paracasei*), potassium sorbate, yucca (*Yucca filamentosa*) powder, stevia leaf extract (*Eupatorium rebaudianumbertoni*), zinc chloride.
Enzymes-containing toothpaste	CuraproxEnzycal Zero (Curaden Ag, Kriens, Switzerland)	Aqua, sorbitol, hydrated silica, glycerin, steareth-20, titanium dioxide, aroma, sodium hydrogen phosphate, carrageenan, sodium chloride, citric acid, sodium benzoate, potassium thiocyanate, glucose oxidase, amyloglucosidase, lactoperoxidase.
Traditional toothpaste	Colgate Total 12 White (Colgate-Palmolive Co., Ltd. China)	Hydrated silica, water, glycerin, sorbitol, PVM/MA copolymer, sodium lauryl sulfate, flavor, sodium hydroxide, carrageenan, propylene glycol, sodium fluoride, sodium saccharin, triclosan, cellulose gum, CI 77891(titanium dioxide), CI 77019(sericite mika), CI 42090(Colorant Bleu brillant FCF), Active Ingredients: Sodium Fluoride 0.32% w/w (1450 ppm F), Triclosan 0.3% w/w.

mouth rinse fluid, Drogsan İlaçları Sanayi ve Ticaret A.Ş., Ankara, Turkey) was used as the positive control group, while 0.85% NaCl dilution fluid sterile saline (ID Broth, Becten, Dickinson and Company, USA) was used as the negative control group.

Three lyophilized microorganism strains from the American Type Culture Collection (ATCC) were used to test the antimicrobial effectiveness of the toothpastes. These were: *S. mutans*-ATCC 25175, *S. sanguinis*-ATCC 10556, and *E. faecalis*-ATCC 29212. Microorganisms stored inactively at 4 ± 1 °C were inoculated into separate media and activated and multiplied by keeping in an incubator at 37 ± 1 °C for 24 hours. Mueller Hinton Fastidious Agar plates (MH-F, Becton Dickinson GmbH, Heidelberg, Germany) with a diameter of 9 cm and a thickness of 5 mm as recommended by the European Antibiotic Susceptibility Committee (EUCAST) were used to determine antimicrobial activity, and the agar-well diffusion method was preferred. For each bacteria and toothpaste one separate agar plate was used. In each agar plate a sterile cork borer (Cork Borer-Isolab, İnterlab. Ürünleri San. Tic. A.Ş., Istanbul, Turkey) was used to bore 3 wells with 6mm diameter and 5mm depth, equidistant from each other and from the edges. One well each was used for the toothpaste sample, the positive control and the negative control (Figure 1.A). Microorganism suspension adjusted to 0.5 McFarland (1.5×10^8 CFU/mL) in activated fresh bacterial cultures 0.85% NaCl solution was inoculated by spreading over the entire surface of the agar plates in all directions with the help of sterile swabs. Each well was filled with an equal amount (0.2ml) of toothpaste and control group materials using sterile syringes and incubated for 24 hours at 37 ± 1 °C in 5% CO_2 (Figure 1.B). This procedure was repeated three times. At the end of 24 hours, the inhibition zone diameters around the tested materials were measured using a digital caliper (Insize 1108-200 digital caliper, Insize Co. Ltd., Jiangsu Province, PRC) randomly and the readings were recorded (Figure 1.C).

Statistical Analyses

SPSS v22.0 was used for statistical analysis of the data obtained in the study. The normality of the distribution was tested using the Shapiro-Wilk test. Intergroup comparisons of non-normally distributed data was performed with Kruskal-Wallis and post-hoc tests. The descriptive statistics are given as mean value ± SD. A p-value of < 0.05 was considered significant.

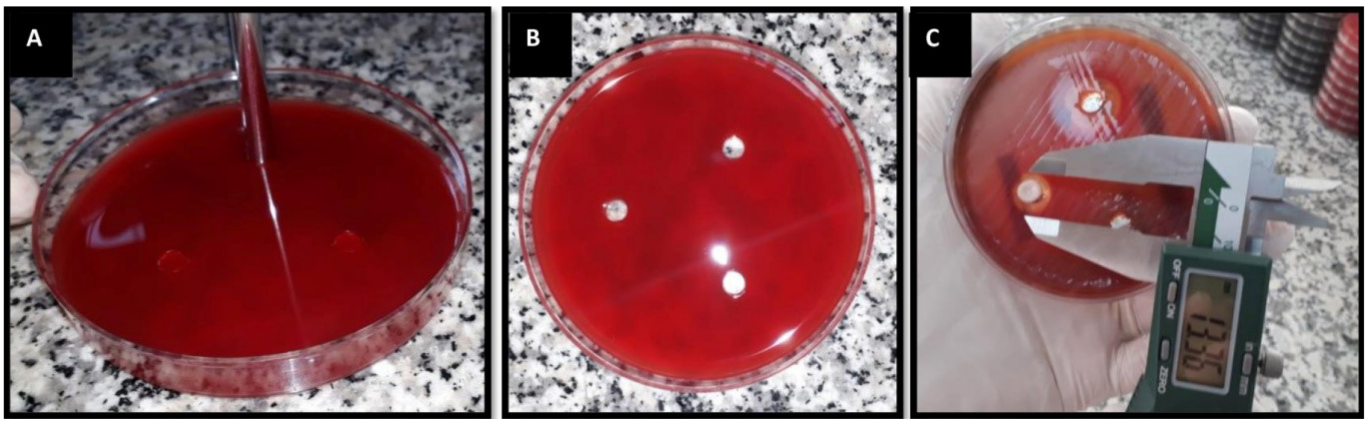
Results

Inhibition zone diameters formed by the tested toothpastes against *S. mutans, S. sanguinis* and *E. faecalis* after 24 hours of incubation are shown in Table 2. The traditional toothpaste and theobromine- and chitosan-containing toothpastes showed antimicrobial efficacy for all tested bacteria, while probiotic- and enzyme-containing toothpastes did not have antimicrobial efficacy. The 0.2% chlorhexidine digluconate positive control group showed the highest antimicrobial efficacy on all bacteria ($p < 0.05$). The negative control group showed no antimicrobial efficacy on any of the bacteria.

The toothpaste with traditional ingredients showed the highest antimicrobial efficacy against *S. mutans*, followed by toothpastes containing theobromine, miswak, chitosan, aloe vera and propolis, respectively. No statistically significant differences were found between the traditional toothpaste and the theobromine-containing toothpaste ($p > 0.05$).

The traditional toothpaste and theobromine- and chitosan-containing toothpastes showed antimicrobial efficacy on *S. sanguinis*. The traditional toothpaste

Figure 1. Representative images about performing the agar-well diffusion method. (A) Wells with 6mm diameter and 5mm depth. (B) Placing the tested materials to the wells. (C) Measurement of the inhibition zone by a digital caliper.



showed the highest effect, followed by the chitosan- and theobromine-containing toothpastes. Statistically significant differences were found between all groups ($p < 0.05$).

The traditional toothpaste and theobromine- and chitosan-containing toothpastes showed antimicrobial efficacy on *E. faecalis*. The highest antimicrobial efficacy on *E. faecalis* was seen in the toothpaste with traditional ingredients ($p < 0.05$), followed by toothpastes containing theobromine and chitosan, respectively. Statistically significant differences were found between the theobromine- and chitosan-containing toothpastes ($p > 0.05$).

Discussion

This study was designed to evaluate the antimicrobial efficacy of toothpastes containing natural ingredients on *S. mutans, S. sanguinis* and *E. faecalis*. The results showed that theobromine- and chitosan-containing toothpastes had antimicrobial efficacy on all three pathogenic microorganisms; however, toothpastes containing aloe vera, miswak and propolis were effective on *S. mutans* only. Toothpastes containing probiotics and enzymes did not have any antimicrobial efficacy on the tested bacteria.

A 0.2% chlorhexidine digluconate solution was used as a positive control group. This solution is accepted as the gold standard due to its ability to bind microorganisms to negatively charged cell walls and disrupt their osmotic balance, its prolonged broad-spectrum antimicrobial activities, and its plaque prevention potential [21]. The highest antimicrobial efficacy on *S. mutans, S. sanguinis* and *E. faecalis* was seen in the positive control group, followed by traditional toothpaste. In the positive control group and all other groups, *S. sanguinis* and *E. faecalis* were more resistant against the antimicrobial agents tested in the study compared with *S. mutans*. Previously published studies support this conclusion, and this resistance could be associated with the ability of these strains to block the entry of antimicrobial molecules due to outer cell layers that form an impermeable barrier with bacterial spores [22,23].

In this study, theobromine-containing toothpastes showed antimicrobial efficacy for all tested bacteria. Theobromine is a natural alkaloid of white crystalline structure and bitter taste, found mainly in cocoa beans and hence in chocolate, of which it is an active ingredient, but also in tea, guarana, cola and mate leaves [24]. The anti-caries effect of theobromine has been associated with active ingredients in its structure such as anti-glycosyltransferase, oleic and linoleic acid, flavonoids and polyphenols. The anti-glycosyltransferase could inhibit the glycotransferase substance produced by *S. mutans* by adhering to the plaque [25]. Flavonoids and polyphenols could perform a bacteriostatic effect on *S. mutans* and diminish its activity, thus reducing caries formation by preventing the development of biofilm [26]. Similar to the present study, Lakshmi *et al*. [27] reported that theobromine-containing toothpaste showed antimicrobial efficacy. They also reported that theobromine was effective on inhibition of *S. mutans, Lactobacillus acidophilus*, *E. faecalis*, and *Candida albicans.*

Chitosan is a natural polycationic linear polysaccharide derived from the partial deacetylation of chitin. The polysaccharide structure of chitosan consists of β- (1-4) linked D-glucosamine and N-acetyl-D-glucosamine randomly distributed in the polymer [28]. Chitosan is used in medicine due to its diverse characteristics such as biocompatibility, non-toxicity, low allergenicity and biodegradability. In addition, chitosan has been reported to have other biological properties such as antitumoral, antimicrobial, and antioxidant efficacy [29]. Chitosan is a beneficial ingredient in toothpaste that prevents streptococci from adhering to tooth surfaces and is less abrasive [30]. Furthermore, in patients with poor oral hygiene,

Table 2. Mean values of inhibition zones (mm).

Groups	*S. mutans*	*S. sanguinis*	*E. faecalis*
	Mean (Std. Deviation)	Mean (Std. Deviation)	Mean (Std. Deviation)
Theobromine	16.30 (0.29)^{b}	11.65 (0.07)^{d}	11.36 (0.03)^{c}
Aloe vera	10.64 (0.08)^{d}	0	0
Miswak	13.16 (0.13)^{c}	0	0
Propolis	10.49 (0.06)^{d}	0	0
Chitosan	12.53 (0.09)^{c}	13.04 (0.04)^{c}	10.99 (0.01)^{d}
Probiotic	0	0	0
Enzymes	0	0	0
Traditional	17.04 (0.03)^{b}	16.96 (0.03)^{b}	12.61 (0.02)^{b}
Positive control	24.24 (0.53)^{a}	19.99 (0.17)^{a}	19.82 (0.67)^{a}
Negative control	0	0	0

Different superscript letters (a,b,..) mean statistically differences in same column (Group comparision) ($p < 0.05$).

chitosan can reduce the demineralization of enamel and prevent excessive dentin sensitivity and tooth decay [31]. In this study, chitosan-containing toothpaste showed a significant antimicrobial effect on the microorganisms tested. This effect can be explained by the ability of chitosan to disrupt the cell structure by binding to the bacterial cell wall and changing membrane permeability or by binding to bacterial DNA and inhibiting DNA replication, causing cell death [32]. In addition, one of the suggested mechanisms for its antimicrobial efficacy is that chitosan acts as a chelating agent that selectively binds metal elements that cause toxin production and inhibit microbial growth [33].

In this study, toothpastes containing aloe vera, miswak, and propolis showed an antimicrobial effect only on *S. mutans*. Although different results have been reported in the literature regarding the antimicrobial activity of these three natural ingredients, most of the researchers agree that they are effective on *S. mutans*. One study reported that a toothpaste containing aloe vera had a high antimicrobial effect on *S. mutans*, *C. albicans*, *S. sanguinis* and *Actinomyces viscosus* [34]. This result, which runs contrary to our study, may be related to the presence of different active agents (SLS, sodium lauroyl sarcosinate) in the aloe vera-containing toothpaste. Surfactants such as SLS, cocamidopropyl betaine and sodium lauroyl sarcosinate added to toothpastes have been reported to increase the antibacterial effect [20]. Carvalho *et al*. reported no antimicrobial efficacy of aloe vera-containing toothpaste in their study [35]. However, the concentration of the antimicrobial agent has been shown to significantly affect its antimicrobial efficacy [36].

In the present study, toothpaste containing miswak showed an antimicrobial effect on *S. mutans*, a result which is in line with the literature [37,38]. This efficacy may be due to substances such as fluoride, silica, resins, sulfur compounds, sodium bicarbonate, tannic acid, alkaloid, chloride and vitamin C [39]. Fatin-Majdina *et al*. [38] reported that a miswak-containing mouthwash was effective also on *S. sanguinis,* although to a lesser extent than its efficacy on *S. mutans.* Researchers proposed that this might be because *S. sanguinis*, as a hydrophobic bacteria, adheres more tightly to the biofilm.

Smoralek *et al*. [40] evaluated the antimicrobial efficacy of a combination of propolis and tea tree oil in toothpastes and a mixture of propolis, SLS, and NaF on *S. mutans*, *E. faecalis* and *Pseudomonas aeruginosa*. They reported that a combination of propolis and tea tree oil in toothpaste had an antimicrobial effect on only *S. mutans*, while toothpaste containing propolis, SLS, and NaF had an antimicrobial effect on *S. mutans* and *E. faecalis*. Taken together, the previous and current data suggest that different concentrations and origins of propolis in toothpastes might affect its antimicrobial efficacy.

In the current study, toothpastes containing probiotics and enzymes did not show antimicrobial efficacy on any of the three microorganisms. Probiotics prevent pathogenic microorganisms from adhering to surfaces in the mouth and from forming biofilm by competing with existing pathogenic microorganisms and by producing chemicals (such as bacteriocin and hydrogen peroxide) that inhibit them [41]. Furthermore, in in-vivo plaque control studies, toothpaste containing probiotics has been reported to reduce the incidence of gingivitis and the number of pathogenic microorganisms [42]. These studies suggest that probiotics may be more effective together with mechanical plaque control.

The enzyme-containing toothpaste used in this study contained lactoperoxidase, amyloglucosidase, and glucose oxidase enzymes. Lactoperoxidase, an enzyme secreted primarily in saliva, milk and other body fluids, has an antimicrobial effect against bacteria, fungi and viruses [43]. Amyloglucosidase and glucose oxidase work together to produce hydrogen peroxide from polyglycans in the environment, and when sufficient hydrogen peroxide is formed, lactoperoxidase shows an antimicrobial effect by catalyzing the conversion of thiocyanate to hypothiocyanite [44]. Accordingly, the antimicrobial effect of the toothpaste may differ in the oral environment. Studies have reported that lactoperoxidase is not a bactericide but rather is bacteriostatic on streptococci and lactobacilli [45].

This study has some limitations. Toothpastes are compound products consisting of more than one ingredient. When their effectiveness is tested, it is possible to achieve different results depending on the concentration of active ingredients, surfactants or the agent with antimicrobial efficacy. Although products with only one active agent listed by the manufacturer were selected when choosing toothpastes with natural ingredients, when the content of the tested pastes is examined, different active agents are found, for instance chamomilla in addition to propolis, magnolia in addition to aloe, and mentha in addition to miswak. These agents, which may not be explicitly stated by the manufacturer, may increase the antimicrobial activity of the toothpaste. Additionally, sodium bicarbonate [46], titanium dioxide [47], glycerin [48] and xylitol

[49] have been identified in toothpastes and may also show antimicrobial activity. For this reason, different results regarding antimicrobial efficacy can be found in literature. Therefore, to protect and maintain oral and dental health, it is important to test the antimicrobial efficacy of these products that are used as alternatives to traditional toothpastes.

Conclusions

Toothpaste is a personal hygiene product that is widely used to ensure and maintain oral and dental health. Natural ingredients toothpastes are increasingly being used to prevent caries formation and to maintain gingival and periodontal health. This study evaluated the effectiveness of toothpastes with natural ingredients on pathogenic microorganisms that cause dental and oral health deterioration. According to the obtained data, toothpastes containing theobromine and chitosan showed antimicrobial activity against all bacteria and can be recommended as an alternative to traditional toothpastes. However, additional studies are needed to evaluate their effectiveness in clinical practice.

Acknowledgements

The study was supported by the Scientific Research Project Coordination Unit of Gaziantep University (DHF.DT.19.12).

References

1. Featherstone JDB (2008) Dental caries: a dynamic disease process. Aust Dent J 53: 286-291.
2. Selwitz RH, Ismail AI, Pitts NB (2007) Dental caries. Lancet 369: 51–59.
3. Struzycka I (2014) The oral microbiome in dental caries. Polish J Microbiol 63: 127–135.
4. Santiago KB, Piana GM, Conti BJ, Cardoso EO, Andrade BFMT, Zanutto MR, Rall VLM, Fernandes A, Sforcin JM (2018) Microbiological control and antibacterial action of a propoliscontaining mouthwash and control of dental plaque in humans. Nat Prod Res 32: 1441–1445.
5. Veloz JJ, Alvear M, Salazar LA (2019) Antimicrobial and antibiofilm activity against *Streptococcus mutans* of individual and mixtures of the main polyphenolic compounds found in Chilean propolis. Biomed Res Int 2019: 7602343.
6. Kreth J, Merritt J, Shi W, Qi F (2005) Competition and coexistence between *Streptococcus mutans* and *Streptococcus sanguinis* in the dental biofilm. J Bacteriol 187: 7193–7203.
7. Kouidhi B, Zmantar T, Mahdouani K, Hentati H, Bakhrouf A (2011) Antibiotic resistance and adhesion properties of oral Enterococci associated to dental caries. BMC Microbiol 11: 155.
8. Kunthur Chidambar C, Maji Shankar S, Raghu P, Badravalli Gururaj S, Shashi Bushan K (2019) Detection of *Enterococcus faecalis* in subgingival biofilms of healthy, gingivitis, and chronic periodontitis subjects. J Indian Soc Periodontol 23: 416–418.
9. Espíndola LCP, do Nascimento MVMR, do Souto RM, Colombo APV (2021) Antimicrobial susceptibility and virulence of *Enterococcus* spp. isolated from periodontitis-associated subgingival biofilm. J Periodontol. [Epub ahead of print]
10. Khalil MA, El-Sabbagh MS, El Naggar EB E-ER (2019) Antibacterial activity of *Salvadora persica* against oral pathogenic bacterial isolates. Niger J Clin Pr 22: 1378–1387.
11. Verkaik MJ, Busscher HJ, Jager D, Slomp AM, Abbas F, Van Der Mei HC (2011) Efficacy of natural antimicrobials in toothpaste formulations against oral biofilms in vitro. J Dent 39 :218–224.
12. Tadin A, Gavic L, Govic T, Galic N, Zorica Vladislavic N, Zeljezic D (2019) In vivo evaluation of fluoride and sodium lauryl sulphate in toothpaste on buccal epithelial cells toxicity. Acta Odontol Scand 77: 386–393.
13. Cragg GM, Newman DJ (2013) Natural products: a continuing source of novel drug leads. Biochim Biophys Acta - Gen Subj 1830: 3670–3695.
14. Aitken-Saavedra J, Chaves Tarquinio SB, De Oliveira Da Rosa WL, Fernandes Da Silva A, Almeida MacHado BME, Santos Castro I, Oliveira Wennesheimer A, Morales-Bozo I, Uchoa Vasconcelos AC, Neutzling Gomes AP (2020) Effect of a homemade salivary substitute prepared using chamomile (*Matricaria chamomilla* L.) flower and flax (*Linum usitatissimum* L.) seed to relieve primary burning mouth syndrome: a preliminary report. J Altern Complement Med 26: 799–806.
15. Shui Y, Li J, Lyu X, Wang Y (2021) Phytotherapy in the management of denture stomatitis: a systematic review and meta-analysis of randomized controlled trials. Phyther Res 35: 4111–4126.
16. Lazarević M, Milošević M, Petrović N, Petrović S, Damante G, Milašin J, Milovanović B (2019) Cytotoxic effects of different aromatic plants essential oils on oral squamous cell carcinoma- an in vitro study. Balk J Dent Med 23: 73–79.
17. de Oliveira Carvalho I, Purgato GA, Píccolo MS, Pizziolo VR, Coelho RR, Diaz-Muñoz G, Alves Nogueira Diaz M (2020) In vitro anticariogenic and antibiofilm activities of toothpastes formulated with essential oils. Arch Oral Biol 117: 104834.
18. Braga AS, Girotti LD, de Melo Simas LL, Pires JG, Pelá VT, Buzalaf MAR, Magalhães AC (2019) Effect of commercial herbal toothpastes and mouth rinses on the prevention of enamel demineralization using a microcosm biofilm model. Biofouling 35: 796–804.
19. Freires IA, Rosalen PL (2016) How natural product research has contributed to oral care product development? A critical view. Pharm Res 33: 1311–1317.
20. Guven Y, Ustun N, Tuna EB, Aktoren O (2019) Antimicrobial effect of newly formulated toothpastes and a mouthrinse on specific microorganisms: An in vitro study. Eur J Dent 13: 172–177.
21. Prasanth M (2011) Antimicrobial efficacy of different toothpastes and mouthrinses: an in vitro study. Dent Res J (Isfahan) 8: 85–94.
22. Lee SY, Lee SY (2019) Susceptibility of oral streptococci to chlorhexidine and cetylpyridinium chloride. Biocontrol Sci 24: 13–21.
23. Cieplik F, Jakubovics NS, Buchalla W, Maisch T, Hellwig E, Al-Ahmad A (2019) Resistance toward chlorhexidine in oral bacteria-is there cause for concern? Front Microbiol 10: 507.

24. Thorn AK, Lin WS, Levon JA, Morton D, Eckert GJ, Lippert F (2020) The effect of theobromine on the in vitro de- and remineralization of enamel carious lesions. J Dent X 3: 100013.
25. Osawa K, Miyazaki K, Shimura S, Okuda J, Matsumoto M, Ooshima T (2001) Identification of cariostatic substances in the cacao bean husk: their anti-glucosyltransferase and antibacterial activities. J Dent Res 80: 2000–2004.
26. Cevallos González FM, dos Santos Araújo EM, Lorenzetti Simionato MR, Kfouri Siriani L, Armas Vega ADC, Studart Medeiros I, Bona Matos A (2019) Effects of theobromine addition on chemical and mechanical properties of a conventional glass ionomer cement. Prog Biomater 8: 23–29.
27. Lakshmi A, Vishnurekha C, Baghkomeh PN (2019) Effect of theobromine in antimicrobial activity: an in vitro study. Dent Res J (Isfahan) 16: 76–80.
28. Venkatesan J, Kim SK (2010) Chitosan composites for bone tissue engineering. An overview. Mar Drugs 8: 2252–2266.
29. Cheung RCF, Ng TB, Wong JH, Chan WY (2015) Chitosan: an update on potential biomedical and pharmaceutical applications. Mar Drugs 13: 5156–5186.
30. Ozalp S, Tulunoglu O (2014) SEM-EDX analysis of brushing abrasion of chitosan and propolis based toothpastes on sound and artificial carious primary enamel surfaces. Int J Paediatr Dent 24: 349–357.
31. Arnaud TMS, De Barros Neto B, Diniz FB (2010) Chitosan effect on dental enamel de-remineralization: an in vitro evaluation. J Dent 38: 848–852.
32. Divya K, Vijayan S, George TK, Jisha MS (2017) Antimicrobial properties of chitosan nanoparticles: Mode of action and factors affecting activity. Fibers Polym 18: 221–230.
33. Yilmaz Atay H (2020) Antibacterial activity of chitosan-based systems. In Jana S, Jana S, editors. Functional chitosan: drug delivery and biomedical applications. Singapore: Springer. 457–489.
34. Lee SS, Zhang W, Li Y (2004) The antimicrobial potential of 14 natural herbal dentifrices: results of an in vitro diffusion method study. J Am Dent Assoc 135: 1133–1141.
35. Carvalho FG, De Cássia Negrini T, Sacramento LVS, Hebling J, Spolidorio DMP, Duque C (2011) The in vitro antimicrobial activity of natural infant fluoride-free toothpastes on oral micro-organisms. J Dent Child 78: 3–8.
36. Jain S, Rathod N, Nagi R, Sur J, Laheji A, Gupta N, Agrawal P, Prasad S (2016) Antibacterial effect of aloe vera gel against oral pathogens: an in-vitro study. J Clin Diagnostic Res 10: ZC41–ZC44.
37. Jenner F, Abdul Jaleel V, Kulshrestha R, Maheswar G, Krishna Rao P, Kranthi J (2013) Evaluating the antimicrobial activity of commercially available herbal toothpastes on microorganisms associated with diabetes mellitus. J Contemp Dent Pract 14: 924–929.
38. Fatin-Majdina N, Zubaidah HAR, Munirah ARM, Marina MB (2014) Effects of Salvadora persica extract on the bacterial population in single-species biofilm. Sains Malaysiana 43: 1889–1893.
39. Tubaishat RS, Darby ML, Bauman DB, Box CE (2005) Use of miswak versus toothbrushes: oral health beliefs and behaviours among a sample of Jordanian adults. Int J Dent Hyg 3: 126–136.
40. De Camargo Smolarek P, Esmerino LA, Chibinski AC, Bortoluzzi MC, Santos EB, Kozlowski VA (2015) In vitro antimicrobial evaluation of toothpastes with natural compounds. Eur J Dent 9: 580–586.
41. Seminario-Amez M, López-López J, Estrugo-Devesa A, Ayuso-Montero R, Jané-Salas E (2017) Probiotics and oral health: a systematic review. Med Oral Patol Oral Cir Bucal 22: e282–e288.
42. Maden EA, Altun C, Polat GG, Basak F (2018) The In vitro Evaluation of the effect of xyliwhite, probiotic, and the conventional toothpastes on the enamel roughness and microhardness. Niger J Clin Pract 21: 306–311.
43. Magacz M, Kędziora K, Sapa J, Krzyściak W (2019) The significance of lactoperoxidase system in oral health: application and efficacy in oral hygiene products. Int J Mol Sci 20: 1443.
44. Daly S, Seong J, Newcombe R, Davies M, Nicholson J, Edwards M, West N (2019) A randomised clinical trial to determine the effect of a toothpaste containing enzymes and proteins on gum health over 3 months. J Dent 80: S26–S32.
45. Seifu E, Buys EM, Donkin EF (2005) Significance of the lactoperoxidase system in the dairy industry and its potential applications: a review. Trends Food Sci Technol 16: 137–154.
46. Dobay O, Laub K, Stercz B, Kéri A, Balázs B, Tóthpál A, Kardos S, Jaikumpun P, Ruksakiet K, Quinton PM, Zsembery A (2018) Bicarbonate inhibits bacterial growth and biofilm formation of prevalent cystic fibrosis pathogens. Front Microbiol 9: 2245.
47. López de Dicastillo C, Guerrero Correa M, B. Martínez F, Streitt C, José Galotto M (2021) Antimicrobial effect of titanium dioxide nanoparticles. In Mares M, Lim SHE, Lai K, editors. Antimicrobial resistance. A one health perspective. London: IntechOpen. 1–-18.
48. Nalawade T, Sogi SP, Bhat K (2015) Bactericidal activity of propylene glycol, glycerine, polyethylene glycol 400, and polyethylene glycol 1000 against selected microorganisms. J Int Soc Prev Community Dent 5: 114–119.
49. Cocco F, Cagetti MG, Majdub O, Campus G (2020) Concentration in saliva and antibacterial effect of Xylitol chewing gum: in vivo and in vitro study. Appl Sci 10: 2900.

Corresponding author

Serdar Demir, PhD
Department of Pedodontics, Faculty of Dentistry, Gaziantep University. Üniversite Blv., Diş Hekimliği Fakültesi, 27310 Şehitkamil Gaziantep/TURKEY
Phone: +90 342 360 96 00/4801
Mobile: +905447252775
Fax: +90 342 361 03 46
E-mail: phdserdardemir@gmail.com

Conflict of interests: No conflict of interests is declared.