



Effectiveness of Antimicrobial Mouthwashes in Caries Biofilm

Elif YILMAZ¹, Ayşe Dina ERDILEK^{2*}

¹ Restorative Dentistry, Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, elify1979@gmail.com

( <https://orcid.org/0000-0001-5713-4286>)

² Restorative Dentistry, Dentistry Faculty, Istanbul University, Istanbul, derdilek@istanbul.edu.tr

( <https://orcid.org/0000-0002-7988-5678>)

Received: 28.09.2023

Accepted: 30.10.2023

Published: 31.12.2023

*Corresponding author

Review Article

Abstract

Background/aim: The objective of this paper is to examine the efficiency of antimicrobial mouthwashes in fighting caries biofilm and sustaining oral health. The antibacterial qualities of mouthwashes and their significance in reducing tooth plaque and gingivitis are emphasized. The study focuses on the role of various ingredients in mouthwash composition, including chlorhexidine, zinc, essential oils, and fluoride. Furthermore, the introduction of propolis and xylitol in traditional mouthwashes is investigated due to their antibacterial qualities and extra health advantages, with the potential for these ingredients to be promising alternatives assessed.

Materials and methods: This study synthesizes results from other research and publications, concentrating on the components of mouthwashes and their value in oral health. Chlorhexidine, zinc, essential oil, fluoride, propolis, and xylitol are the chemicals being studied for their antibacterial qualities and advantages in avoiding dental disorders.

Results: The findings indicate that the mentioned components play a crucial part in the formulation of mouthwashes, each of which contributes to the prevention of dental plaque and gingivitis. Propolis and xylitol, in particular, are being studied as potential attractive replacements to typical mouthwash components due to their extra health advantages.

Conclusion: The findings imply that the mentioned components play an important role in the formulation of mouthwashes, with each contributing to the prevention of dental plaque and dental disorders. Propolis and xylitol are being studied as potential attractive replacements for typical mouthwash components due to their extra health advantages.

Keywords: antimicrobial mouthwashes, oral health, dental plaque, caries biofilm, streptococcus mutans

1. Introduction

Dental health is an integral part of overall health and encompasses much more than simply having healthy teeth (Petersen, 2003). The bacteria found in the oral biofilm often lead to common dental caries and periodontal diseases. Thus, the control of oral biofilms therefore plays an essential role in the prevention of dental diseases (Baehni & Takeuchi, 2003). Various oral hygiene procedures have been developed and introduced in recent years to control biofilms in the oral environment (Çintan & Koçak, 2019). Using interdental brushes, dental floss, or regular tooth brushing helps remove plaque from the mouth, thus reducing the risk of dental disease (Daly, 2009; Kiger, Nylund, & Feller, 1991). However, studies have shown that these hygiene tools are insufficient for preventing tooth decay, plaque formation, and gum diseases (Asadoorian, 2016).

In cases where adequate oral hygiene cannot be achieved by mechanical cleaning alone, antimicrobial mouthwashes may also be necessary for plaque control. With the use of these antimicrobial agents, both the amount of plaque is reduced, and the structure of the plaque is disrupted, making it easier to remove (Rosling et al., 1997; Serrano et al., 2015). In recent years, the use of mouthwash is often recommended following basic mechanical cleaning methods to ensure oral biofilm control (BAĞIŞ & BAĞIŞ, 2019). The use of antiseptic mouthwashes has become a part of patients' daily oral hygiene practices today.

2. Chlorhexidine-Containing Mouthwashes

Chlorhexidine was first introduced as an antiseptic agent in England in 1953. It has been used for the treatment of infections and general disinfection in humans and animals since 1957 (Fardal & Turnbull, 1986; Løe, 1973). As of the 1970s, it has been widely used not only in medicine but also in dentistry (Mandel, 1994).

The Chlorhexidine (CHX) molecule (C₂₂H₃₀Cl₂N₁₀) consists of 2 symmetric 4-chlorophenol rings and 2-biguanides attached to the central hexamethylene chain (Basrani & Lemonie, 2005). Chlorhexidine gluconate is the salt of chlorhexidine and gluconic acid. It has broad antimicrobial activity against gram (+) and gram (-) bacteria, facultative anaerobes and aerobes, dermatophytes, fungi, and some lipophilic virus types (Lee, 2009; Davies, 1973). However, their activity against bacterial spores, mycobacteria, and some fungal species is limited (Broadley et al., 1991; Russell, 1990; Weller, 2000).

Although the mechanism of the antimicrobial action of chlorhexidine is still unclear, the most widely accepted view is that it is a cationic agent and interacts with the negatively charged bacterial cell membrane (Denton, 1991). Its efficacy is achieved by adsorbing onto the cell walls of microorganisms (Hernandez et al., 2005). At low concentrations, it exhibits a bacteriostatic effect, increasing cell permeability and causing leakage of cell contents. At higher concentrations, it has a bactericidal effect, causing precipitation in bacterial cytoplasm, leading to bacterial death. Due to its cationic property, it can adhere to tooth surfaces and oral mucosa (Lindhe, Lang, & Karring, 2008). It has a greater antibacterial effect on gram-positive cocci than on gram-negative bacteria (Mensi et al., 2018).

Chlorhexidine-containing solutions have been reported to be most stable at a pH range of 5-8. In acidic environments where the pH is below 5, it gradually degrades, while at pH=8 and above, chlorhexidine precipitates, losing its effectiveness (Russell & Day, 1993). The antibacterial activity is at its highest when the pH is between 5.5-7 (Lang et al., 1982).

Chlorhexidine has high sensitivity in oral tissues. Due to this property, it can be detected in increased levels in saliva even hours after its use (Tomás et al., 2008). Its antimicrobial activity continues due to its retention on oral surfaces and slow release. Additionally, the prolonged action time of chlorhexidine allows for longer interaction with microorganisms, enhancing its bactericidal effectiveness (Killooy, 1988).

Chlorhexidine can be used in the oral environment in the form of toothpaste, gel, spray, mouthwash, and polish (Mandel, 1994). The effectiveness of chlorhexidine-containing mouthwashes on plaque and gingivitis has been demonstrated in many studies (Abdallah et al., 2016). The mouthwash form was first prepared in 0.2% and 0.1% concentrations and later 1.2% concentration was introduced (Van der Weijden et al., 2005). Today, the amount of chlorhexidine in mouthwashes varies between 0.12%-0.2% (Van Rijkom et al., 1996).

Streptococcus mutans and *S. sobrinus*, known to cause initial caries, are more sensitive to chlorhexidine among oral streptococci (Dalli et al., 2010). Chlorhexidine is considered to have caries-preventive potential as it is an antiseptic agent that can suppress the growth of *Streptococcus mutans* (Emilson, 1994; Van Rijkom et al., 1996). A 0.2% CHX solution is considered a clinically effective mouthwash that prevents supragingival plaque formation and consequently the development of gum disease and caries (Wilken et al., 2001). Benzylamine hydrochloride has been

added to chlorhexidine-containing mouthwashes to provide analgesic and anti-edema effects (Erdemir, Şengün, & Ülker, 2007). This nonsteroidal anti-inflammatory agent helps to treat inflammation and its side effects by providing analgesic, antipyretic, and anti-edema effects in mouthwash (Serrano et al., 2015). However, there are also studies reporting that the use of chlorhexidine reduces the number of *Streptococcus mutans*, but its caries preventive effect is insufficient (Spets-Happonen et al., 1991; Spets-Happonen et al., 1985). Studies have reported that the cytotoxic effects of chlorhexidine mouthwashes used orally at concentrations between 0.12% and 2% are at low levels (Löe et al., 1976; Tanomaru Filho et al., 2002).

It is crucial to use chlorhexidine mouthwashes according to the instructions for use. Improper use or excessively high doses may cause undesirable adverse effects (Hatipoğlu, Güncü, & Şengün, 2007). In long-term use, yellow-brown discoloration of dental tissues, desquamative lesions on the oral mucosa, ulcerations, impaired taste sensation, and sensitization of the mucosa may be observed (Serrano et al., 2015; Balci & Buduneli, 2015; Arabacı et al., 2013). In summary, it can be said that Chlorhexidine-containing mouthwashes are still considered the gold standard among antimicrobial mouthwashes (Lee, 2009).

3. Zinc-Containing Mouthwashes

Zinc, a fundamental trace element found in muscle tissue, bone, and skin in the body, plays a crucial role in maintaining oral health (Christianson, 1991; Thomas & Bishop, 2013). It is naturally present in saliva, teeth, and dental plaque (Kim, Kim, & Kho, 2010; Lynch, 2011). Zinc (Zn^{2+}) is a sulfhydryl reactive agent, and its reaction is considered to have an antimicrobial effect (Cummins, 1991).

It possesses broad-spectrum antibacterial activity (Finney, Walker, Marsh, & Brading, 2003). It primarily acts by targeting glycolytic enzymes and bacterial cell cytoplasm and inhibiting the glycolysis process. Zinc has antimicrobial properties against various bacteria, such as *Streptococcus mutans*. Phan et al. (Phan et al., 2004) conducted various experiments on the effectiveness of zinc against *Streptococcus mutans* and proved zinc's ability to block the glycolysis process. In addition to its good retention on oral surfaces, it also persists in saliva and plaque for hours following its application (Gilbert & Ingram, 1988). Repeated zinc applications create an accumulation effect in plaque (Hall et al., 2003; Afseth, Helgeland, & Bonesvoll, 1983). Numerous in vivo and in vitro studies have proven zinc's ability to inhibit and limit acid production in dental plaque (Harrap, Best, & Saxton, 1984). It has also been shown that zinc application prevents dentin demineralization and supports remineralization (Windisch, 2001). It has been reported that their preferred use in mouthwashes can be attributed to their properties such as their lack of compound-forming toxicity and lack of coloration (Basavaraj & Khuller, 2011).

Zinc is used in mouthwashes in various salt forms such as zinc oxide, zinc chloride, zinc citrate, zinc sulfate, and zinc lactate (Taşdemir & Alkan, 2016). A zinc salt's inhibitory effect on microbial glycolysis is pH-dependent and bacterial-specific. Inhibition is highest at pH 7, as seen in *Streptococcus salivarius* and *Streptococcus sobrinus*. Additionally, zinc salts help reduce bad breath by inhibiting the production of volatile sulfur compounds (He, Pearce, & Sissons, 2002; Addy, Richards, & Williams, 1980).

4. Essential Oil-Containing Mouthwashes

The introduction of mouthwashes containing essential oils derived naturally and prepared from plants has taken their place as a different method of providing oral hygiene. Mouthwashes containing essential oils include eucalyptol, thymol, menthol, and methyl salicylate (Stoeken, Paraskevas, & van der Weijden, 2007). In these mouthwashes, alcohol can be used as a solvent and carrier for essential oils and other components (Lee, 2009).

Several laboratory studies have demonstrated the effectiveness of essential oil mouthwashes in killing a variety of oral organisms, including pathogens and opportunistic bacteria (Ross, Charles, & Dills, 1989). Due to its broad spectrum, it is an effective antiseptic against yeasts, and gram-negative and positive bacteria (Petersen, 2003). With their antimicrobial properties, they slow plaque maturation and reduce bacterial density (Balci & Buduneli, 2015). The mechanisms by which essential oils work against bacteria are complex. The disruption of the cell wall results in the precipitation of cell proteins at high concentrations, whereas the inactivation of essential enzymes occurs at low concentrations. They are also suggested to exhibit anti-inflammatory effects due to their antioxidant activity. Studies on mouthwashes containing essential oils have shown significant reductions in odor-causing bacteria found in the gum line, as well as significant reductions in plaque bacteria on interproximal and buccal tooth surfaces (Daly, 2009; Kiger, Nylund, & Feller, 1991). Another clinical study on *Streptococcus mutans* showed that mouthwash containing essential oils significantly reduced the density of *Streptococcus mutans* in dental plaque (Fine et al., 2000).

Mouthwashes containing essential oils have very few negative effects. First of all, essential oils can cause tooth staining (Baehni & Takeuchi, 2003; Çintan & Koçak, 2019). Second, they have poor retention on oral surfaces and may cause a bad taste and burning sensation (Taşdemir & Alkan, 2016). Apart from these two negative effects, essential oil mouthwashes can penetrate the biofilm and with their anti-inflammatory, antiplaque, and anti-gingivitis properties, essential oils help to reduce gingival diseases (Balci & Buduneli, 2015). In particular, the efficacy and safety of a mouthwash containing a fixed combination of essential oils have been demonstrated in several long-term clinical studies, both in terms of reduction of existing supragingival plaque and gum inflammation and inhibiting the formation of new plaque and gum inflammation (Lamster, Alfano, Seiger, & Gordon, 1983). Mouthwashes containing essential oils are available on the market under the commercial name 'Listerine'.

5. Fluoride Mouthwashes

Fluorides (sodium fluoride, amine fluoride, sodium monofluorophosphate, stannous fluoride) prevent mineral loss, remineralize tooth enamel, and help prevent caries by reducing acid-forming plaque formation (Hitz Lindenmüller & Lambrecht, 2011). The fluoride ion replaces the hydroxide anion in tooth structure, forming a more resilient structure called fluorapatite (Ca_3F) (Çağlar & Cengiz, 1996).

Fluoride has been added to mouthwash formulations due to its effect on caries prevention and tooth remineralization (Giertsen, 2004; Maggio et al., 2010). Fluoride mouthwashes aim to increase the fluoride level in saliva and maintain it at a certain level (Ercan & Taş Özyurtseven, 2011). Fluoride-containing mouthwashes enhance protection against caries by increasing the fluoride reserve created by toothpaste. They also function in interdental spaces that toothbrushes can't reach (Hitz Lindenmüller & Lambrecht, 2011).

The recommended procedure is to use a low-concentration fluoride solution, once or twice a day, or a more concentrated solution, once a week or once every two weeks. The most commonly used fluoride compound in mouthwash is sodium fluoride (Marinho et al., 2016). The World Health Organization recommends daily use of a 0.05% sodium fluoride mouthwash (230 ppmF) or weekly use of a 0.2% sodium fluoride mouthwash (900 ppmF) for caries prevention (O'Mullane et al., 2016).

For individuals at high risk of caries and patients undergoing orthodontic treatment, fluoride mouthwashes are considered part of the protective treatment against caries (Petersson et al., 2007). It has also been shown that fluoride mouthwashes significantly reduce the formation of white spot lesions during orthodontic treatment (Ogaard, 2008).

Zero et al. (Zero et al., 1992) demonstrated in their study that the amount of fluoride in saliva reached higher levels with the use of fluoride mouthwashes and recommended the use of fluoride mouthwashes in addition to the use of fluoride paste to reduce caries formation. It has been reported that the combined use of fluoride toothpaste and mouthwash is effective in remineralizing root caries (Petersson et al., 2007).

In a study investigating the effect of fluoride mouthwashes by Altenburger et al. (Altenburger et al., 2007), it was reported that the use of mouthwashes containing 250 ppm fluoride twice a day significantly increased remineralization on approximal surfaces. Daily use of mouthwashes containing 0.05% NaF has been found to be very successful in preventing the progression of initial caries when used in conjunction with other fluoride-containing preparations (Marinho et al., 2016).

Boyd (Boyd et al., 1993) reported in his study that a 0.4% tin fluoride mouthwash showed similar values in terms of caries prevention effect with 0.05% sodium fluoride mouthwash. Another study examined mouthwashes containing chlorhexidine and amine fluoride/tin fluoride and found them to be effective against *Streptococcus mutans* and *Porphyromonas gingivalis* (Karlı & Taş Özyurtseven, 2021).

In recent years, fluoride mouthwashes have been widely used to prevent dental caries in children. Studies have shown that fluoride mouthwashes have a high caries preventive effect (Gaffar et al., 1998). It is recommended to use a fluoride mouthwash for children at risk of caries, for individuals with inadequate tooth brushing habits, for patients receiving radiotherapy, for patients undergoing orthodontic treatment and for those unable to brush properly. As fluoride is dangerous to swallow, these mouthwashes should not be used by children under six. (Bacaksız & Tulunoğlu, 2012).

6. Xylitol Mouthwashes

Xylitol is a five-carbon sugar alcohol primarily derived from birch trees. Unlike six-carbon sugar, xylitol is not easily metabolized by oral bacteria (Santiago et al., 2018).

In recent years, its use as a natural sweetener has become widespread. In addition to its use in gum and toothpaste, xylitol is also used in mouthwashes. Xylitol-containing mouthwashes are a natural and effective tool for maintaining oral health. It contributes significantly to the oral care routine by preventing dental caries, reducing dental plaque, and promoting remineralization (Ozan et al., 2007).

It has been shown in previous studies that xylitol inhibits the growth of differing strains of *Streptococcus mutans*. According to ongoing research, this is also the case for other oral streptococci, and xylitol inhibits both bacterial reproduction and acid production when glucose is used as an energy and carbon source. Oral streptococci do not produce acid from xylitol, as is well known. Researchers have shown that xylitol reduces the incidence of caries both in rats and in humans (Hayacibara et al., 2005).

Xylitol is best known for its dental benefits, such as reducing the risk of tooth decay. It is believed to function through three mechanisms: xylitol can replace cariogenic sucrose, stimulate salivation, and may have specific inhibitory effects on *Streptococcus mutans*, the main cause of dental caries. Although a recent meta-analysis concluded that high-quality studies on the dental health benefits of xylitol are needed, the same study concluded that xylitol is an effective strategy as a self-administered caries prevention agent (Wåler & Rølla, 1983).

Xylitol reduces the incidence of dental caries by raising pH and salivary flow and decreasing the number of periodontopathogenic and cariogenic bacteria, xerostomia, plaque levels, dental erosion and gingival inflammation (Salli et al., 2019; Mäkinen, 2000).

Research has been conducted to determine the viability of *Streptococcus sanguis* and *Streptococcus mutans* in the early stages of biofilm formation when xylitol and chlorhexidine are combined in a mouthwash compared to xylitol and chlorhexidine alone. Chlorhexidine and xylitol combined inhibit streptococci more effectively than either agent alone (Nordblad et al., 1995).

When mouthwashes prepared with some herbal ingredients considered healthy were evaluated alone or in combination with xylitol, mouthwashes prepared with xylitol showed a marked superiority in their antibacterial activity on saliva (Decker et al., 2008).

There are also studies showing that the combined use of xylitol and fluoride is more successful in providing remineralization. In the study by Gaffar et al., the combined use of xylitol+NaF was compared with the use of xylitol or F alone, and the combined use of xylitol+NaF was found to be more successful in terms of remineralization (Murray et al., 1997).

Considering the recent literature, limited research has been conducted on the synergistic effects of xylitol and fluorides, chlorhexidine, and other products that promote oral health, such as probiotics. (Ozan et al., 2007).

7. Propolis Mouthwashes

Propolis is a naturally occurring bee product. It is a dark-colored, sticky and dense combination of beeswax and resin, and contains a variety of active natural ingredients such as essential oils, phenolic acids, plant balsams, aromatic alcohols, flavonoids, fatty acids, vitamins and mineral salts. Several laboratory studies have demonstrated that propolis has some properties such as antibacterial, anti-inflammatory, antiviral, antioxidant, antitumor, antifungal, and hepatoprotective properties. The chemical composition of propolis consists of 50-60% resin, 30-40% beeswax, 5-10% essential oils, 5% pollen, and some trace elements (Saeed et al., 2021; Abbasi et al., 2018).

Staphylococcus aureus, which is a Gram-positive bacterium, and *Salmonella*, which is a Gram-negative bacterium, show particular effectiveness in terms of antibacterial activity. In evaluating propolis' antibacterial properties against certain anaerobic oral pathogens, researchers found that it is effective against *Prevotella oralis*, *Lactobacillus acidophilus*, *Porphyromonas gingivalis*, *Prevotella melaninogenica*, *Fusobacterium nucleatum*, as well as *Veillonella parvula*. It consists primarily of aromatic compounds such as caffeic acid and flavonoids (Dodwad & Kukreja, 2011).

Due to its antimicrobial, antioxidant, anti-inflammatory, and antiproliferative properties, propolis has great potential in dentistry, oral health management, and medicine. Propolis has been used for treating caries, throat infections, and oral infections since the 12th century, and was mentioned in one of the earliest descriptions of the preparation. (Ercan et al., 2015).

Propolis has many clinical applications in dentistry. It can be used to treat dental diseases such as denture ulcers and stomatitis, bad breath, periodontal pocket/abscess, cervical, dentinal, and root caries sensitivity, lichen planus, candidal infections, angular cheilitis, dry mouth, pericoronitis (Hayacibara et al., 2005). It is known that propolis extract has antimicrobial activity against *Streptococcus mutans*, a Gram-positive coccus facultative anaerobic bacterium commonly found in the human oral cavity and causing tooth decay. This anti-caries effect has been shown to involve two different mechanisms: a direct activity against *Streptococcus mutans* and inhibition of glucosyltransferase activity leading to the inhibition of bacterial adhesion. Propolis extract can be used as an alternative measure to prevent dental caries (Wåler, 1983; Decker et al., 2008).

Propolis-containing mouthwashes are among the herbal mouthwashes used for oral hygiene. It has been shown that propolis mouthwash can be an alternative to chemical rinsing and when used in subgingival irrigation, better improvement in microbiological and clinical parameters was obtained compared to conventional treatment (Santiago et al., 2018).

In a study by Duailibe et al., twenty volunteers were asked to rinse their mouths with propolis extract for one minute to examine the effect of mouth rinsing with propolis extract. When saliva samples before and after rinsing were compared, a 62% reduction in *Streptococcus mutans* colony count was detected. The researchers also recorded a significant reduction after a 7-day follow-up when volunteers were asked to rinse their mouths with propolis extract every day (Santiago et al., 2018).

An experiment by Santiago and colleagues compared the effectiveness of a mouthwash that contains 2.6% propolis with a mouthwash that contains 0.12% chlorhexidine on dental plaque accumulation. It was found that the mouthwash containing propolis was as effective as the chlorhexidine mouthwash in reducing plaque accumulation during the 14-day trial period (Ozan et al., 2007).

Ozan et al. evaluated the effects of different concentrations of propolis solutions and 0.2% chlorhexidine on oral microorganisms and human gingival fibroblasts by using propolis mouthwash at concentrations of 1%, 2.5%, 5%, and 10%. This study showed that chlorhexidine was more effective against oral microorganisms, and on the contrary, chlorhexidine showed more cytotoxicity against gingival fibroblasts (Ozan et al., 2007).

Propolis is used to protect oral health and prevent cavities due to its antibiotic and plaque-preventing properties. A simple propolis mouthwash can be made by adding 10-50 drops of propolis to a glass of warm water. Unlike commonly used mouthwashes, propolis mouthwash can be swallowed for more benefits (Dodwad & Kukreja, 2011).

8. Discussion

Mouthwashes have long been an essential of oral hygiene routines, providing an additional line of protection against dental disorders. The antibacterial properties of mouthwashes, as stated in this article, play a critical role in preventing the formation and advancement of dental plaque and gingivitis. Chlorhexidine has been employed for decades in mouthwashes due to its broad-spectrum antibacterial action. However, long-term usage might have negative effects, underlining the need for alternate therapies.

Fluoride and essential oils have demonstrated potential; however, their efficacy varies depending on concentration and formulation. The use of propolis as a mouthwash component is very noteworthy. Because of its natural origins and powerful antibacterial characteristics, it has the potential to be a game-changer in oral health care. Furthermore, the fact that propolis is safe to consume provides two benefits: dental hygiene and overall health.

While the agents mentioned in the article have demonstrated efficacy against caries biofilm, other aspects like as patient compliance, possible allergic responses, and long-term consequences must be considered. Future research should concentrate on improving formulations in order to enhance benefits while avoiding negative effects. Natural compounds such as propolis may pave the way for more holistic and safer dental care solutions in the future.

References

- Abbasi, A. J., Mohammadi, F., Bayat, M., Gema, S. M., Ghadirian, H., Seifi, H., Bayat, H., & Bahrami, N. (2018). Applications of Propolis in Dentistry: A Review. *Ethiopian Journal of Health Sciences*, 28(4), 505–512.
- Abdallahman, B. M., Holmes, H., Peck, M. T., & Basson, N. J. (2016). In vitro antimicrobial comparison of three commercially available chlorhexidine-based oral rinses. *South African Dental Journal*, 71(7), 304-307.
- Addy, M., Richards, J., & Williams, G. (1980). Effects of a zinc citrate mouthwash on dental plaque and salivary bacteria. *Journal of Clinical Periodontology*, 7, 309-15.
- Afseth, J., Helgeland, K., & Bonesvoll, P. (1983). Retention of Cu and Zn in the oral cavity following rinsing with aqueous solutions of copper and zinc salts. *Scandinavian Journal of Dental Research*, 91, 42–45.
- Altenburger, M. J., Schirrmeister, J. F., Wrbas, K. T., & Hellwig, E. (2007). Remineralization of artificial interproximal carious lesions using a fluoride mouthrinse. *American Journal of Dentistry*, 20(6), 385-9.
- Arabacı, T., Türkez, H., Çiçek, Y., Geyikoğlu, F., Tatar, A., Keleş, M. S., et al. (2013). Analyzing of the effects of chlorhexidine digluconate on the antioxidant enzyme levels in human peripheral blood cultures. *Cumhuriyet Dental Journal*, 16(3), 197-202.

- Asadoorian, J. (2016). Therapeutic oral rinsing with commercially available products: Position paper and statement from the Canadian Dental Hygienists Association. *Canadian Journal of Dental Hygiene*, 3, 126–139.
- Bacaksiz, A., & Tulunoğlu, Ö. (2012). Fluorid Kullanımı, Dental Fluorozis ve Tedavisinde Güncel Yaklaşımlar. *ADO Klinik Bilimler Dergisi*, 6(1), 1137-1148.
- Baehni, P. C., & Takeuchi, Y. (2003). Anti-plaque agents in the prevention of biofilm-associated oral diseases. *Oral Diseases*, 9(Suppl. 1), 23-29.
- Bağış, Y. H., & Bağış, N. (2019). Farklı Ağız Gargalarının Antibakteriyel Etkilerinin İn-Vitro Olarak İncelenmesi. *Ankara Üniversitesi Diş Hekimliği Fakültesi Dergisi*, 46(1), 1-6.
- Balci, N., & Buduneli, N. (2015). Anti-Inflammatory Agents in Mouth-Rinses For Periodontal Treatment. *Clinical Anti-Inflammatory & Anti-Allergy Drugs*, 2(1), 14-20.
- Basavaraj, P., & Khuller, N. (2011). Halitosis: a review. *Indian Journal of Stomatology*, 2(3), 183-6.
- Basrani, B., & Lemonie, C. (2005). Chlorhexidine gluconate. *Australian Endodontic Journal*, 31(2), 48-52.
- Boyd, R. L. (1993). Comparison of three self-applied topical fluoride preparations for control of decalcification. *Angle Orthodontist*, 63(1), 25-30.
- Broadley, S. J., Jenkins, P. A., Furr, J. R., & Russell, A. D. (1991). Anti-mycobacterial activity of biocides. *Letters in Applied Microbiology*, 13(3), 118-122.
- Christianson, D. W. (1991). Structural biology of zinc. *Advances in Protein Chemistry*, 42, 281–355.
- Cummins, D. (1991). Zinc citrate/Triclosan: a new anti-plaque system for the control of plaque and the prevention of gingivitis: short-term clinical and mode of action studies. *Journal of Clinical Periodontology*, 18, 455-61.
- Çağlar, M. K., & Cengiz, A. B. (1996). Pediatride Flor ve Önemi. *Türkiye Klinikleri Journal of Pediatrics*, 5(1), 1-9.
- Çintan, S., & Koçak, N. A. (2019). Kimyasal plak kontrolü. In M. M. Akkaya (Ed.), *Cerrahi Olmayan Periodontal Tedavi* (pp. 8-14). Ankara: Türkiye Klinikleri.
- Dalli, M., Ercan, E., Zorba, Y. O., İnce, B., Şahbaz, C., Bahşi, E., et al. (2010). Effect of 1% chlorhexidine gel on the bonding strength to dentin. *Journal of Dental Science*, 5(1), 8-13.
- Daly, C. G. (2009). Prescribing good oral hygiene for adults. *Australian Prescriber*, 32, 72-75.
- Davies, A. (1973). The mode of action of chlorhexidine. *Journal of Periodontal Research*, 8(12), 68-75.
- Decker, E. M., Maier, G., Axmann, D., Brex, M., & von Ohle, C. (2008). Effect of xylitol/chlorhexidine versus xylitol or chlorhexidine as single rinses on initial biofilm formation of cariogenic streptococci. *Quintessence International*, 39(1), 17–22.

- Denton, G. W. (1991). Chlorhexidine. In S. S. Block (Ed.), *Disinfection, Sterilization and preservation* (4th ed., pp. 274-289). Philadelphia: Lea & Febiger.
- Dodwad, V., & Kukreja, B. J. (2011). Propolis mouthwash: A new beginning. *Journal of Indian Society of Periodontology*, 15(2), 121–125.
- Emilson, C. G. (1994). Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. *Journal of Dental Research*, 73(3), 682-691.
- Ercan, E., & Taş Özyurtseven, B. (2011). Diş hekimliğinde topikal florür uygulama metotları. *Cumhuriyet Dental Journal*, 13(1), 27-33.
- Ercan, N., Erdemir, E. O., Ozkan, S. Y., & Hendek, M. K. (2015). The comparative effect of propolis in two different vehicles; mouthwash and chewing-gum on plaque accumulation and gingival inflammation. *European Journal of Dentistry*, 9(2), 272–276.
- Erdemir, E. O., Şengün, A., & Ülker, M. (2007). Cytotoxicity of mouthrinses on epithelial cells by micronucleus test. *European Journal of Dentistry*, 1(2), 80-85.
- Fardal, O., & Turnbull, R. S. (1986). A review of the literature on use of chlorhexidine in dentistry. *Journal of the American Dental Association*, 112(6), 863-869.
- Fine, D. H., Furgang, D., Barnett, M. L., Drew, C., Steinberg, L., Charles, C. H., & Vincent, J. W. (2000). Effect of an essential oil-containing antiseptic mouthrinse on plaque and salivary *Streptococcus mutans* levels. *Journal of Clinical Periodontology*, 27, 157–161.
- Finney, M., Walker, J. T., Marsh, P. D., & Brading, M. G. (2003). Antimicrobial effects of a novel Triclosan/zinc citrate dentifrice against mixed culture oral biofilms. *International Dental Journal*, 53(Suppl. S1), 371–378.
- Gaffar, A., Blake-Haskins, J. C., Sullivan, R., et al. (1998). Cariostatic effects of a xylitol/NaF dentifrice in vivo. *International Dental Journal*, 48(1), 32-9.
- Giertsen, E. (2004). Effects of mouthrinses with triclosan, zinc ions, copolymer, and sodium lauryl sulphate combined with fluoride on acid formation by dental plaque in vivo. *Caries Research*, 38(5), 430-5.
- Gilbert, R. J., & Ingram, G. S. (1988). The oral disposition of zinc following the use of an anticalculus toothpaste containing 0.5% zinc citrate. *Journal of Pharmacy and Pharmacology*, 40, 399–402.
- Hall, P. J., Green, A. K., Horay, C. P., de Brabander, S., Beasley, T. J., Cromwell, V. J., Holt, J. S., & Savage, D. J. (2003). Plaque antibacterial levels following controlled food intake and use of a toothpaste containing 2% zinc citrate and 0.3% Triclosan. *International Dental Journal*, 53(Suppl. S1), 379–384.
- Harrap, G., Best, J., & Saxton, C. (1984). Human oral retention of zinc from mouthwashes containing zinc salts and its relevance to dental plaque control. *Archives of Oral Biology*, 29, 87-91.

- Hatipoğlu, H., Güncü, G. N., & Şengün, D. (2007). Klorheksidin içeren ağız gargarasının hatalı kullanımı sonucu gözlenen deskuamatif lezyonlar: Olgu Raporu [Desquamative lesions observed as a result of incorrect use of mouthwash containing chlorhexidine: Case Report].
- Hayacibara, M. F., Koo, H., Rosalen, P. L., Duarte, S., Franco, E. M., Bowen, W. H., Ikegaki, M., & Cury, J. A. (2005). In vitro and in vivo effects of isolated fractions of Brazilian propolis on caries development. *Journal of Ethnopharmacology*, 101(1-3), 110–115.
- He, G., Pearce, E., & Sissons, C. (2002). Inhibitory effect of ZnCl₂ on glycolysis in human oral microbes. *Archives of Oral Biology*, 47, 117-29.
- Hernandez, E. P., Botero, T. M., Mantellini, M. G., McDonald, N. J., & Nör, J. E. (2005). Effect of ProRoot MTA mixed with chlorhexidine on apoptosis and cell cycle of fibroblasts and macrophages in vitro. *International Endodontic Journal*, 38(2), 137-143.
- Hitz Lindenmüller, I., & Lambrecht, J. T. (2011). Oral care. *Current Problems in Dermatology*, 40, 107–115.
- Karlı, E. D., & Taş Özyurtseven, B. (2021). Diş hekimliğinde ağız gargaraları kullanımı. In C. Alpaslan (Ed.), *Diş Hekimliğinde Kanıt Dayalı İlaç Kullanımı* (1st ed., pp. 34-41). Ankara: Türkiye Klinikleri.
- Kiger, R. D., Nylund, K., & Feller, R. P. (1991). A comparison of proximal plaque removal using floss and interdental brushes. *Journal of Clinical Periodontology*, 18(9), 681-684.
- Killooy, W. J. (1988). The use of local-delivered chlorhexidine in the treatment of periodontitis. Clinical results. *Journal of Clinical Periodontology*, 25, 953-958.
- Kim, Y. J., Kim, Y. K., & Kho, H. S. (2010). Effects of smoking on trace metal levels in saliva. *Oral Diseases*, 16, 823–830.
- Lamster, I. B., Alfano, M. C., Seiger, M. C., & Gordon, J. M. (1983). The effect of Listerine® Antiseptic on reduction of existing plaque and gingivitis. *Clinical Preventive Dentistry*, 5, 12–16.
- Lang, N. P., Hotz, P., Graf, H., Geering, A. H., Saxer, U. P., Sturzenberger, O. P., & Meckel, A. H. (1982). Effects of supervised chlorhexidine mouthrinses in children. A longitudinal clinical trial. *Journal of Periodontal Research*, 17, 101–111.
- Lee, S. S. (2009). *Breath: Causes, Diagnosis, and Treatment of Oral Malodor* (2nd ed., Vol. II). San Bernardino: Culminare Inc.
- Lindhe, J., Lang, N. P., & Karring, T. (2008). Chemical supragingival plaque control. In M. Addy & J. Moran (Eds.), *Clinical Periodontology and Implant Dentistry* (5th ed., p. 748-760). Mungsgaard: Oxford.
- Löe, H. (1973). Does chlorhexidine have a place in the prophylaxis of dental diseases? *Journal of Periodontal Research Supplement*, 12, 93-99.

Löe, H., Schiött, C. R., Karring, G., & Karring, T. (1976). Two years oral use of chlorhexidine in man. I. General design and clinical effects. *Journal of Periodontal Research*, 11(3), 135-144.

Lynch, R. J. (2011). Zinc in the mouth, its interactions with dental enamel and possible effects on caries; a review of the literature. *International Dental Journal*, 61(Suppl. S3), 46–54.

Maggio, B., Guibert, R. G., Mason, S. C., Karwal, R., Rees, G. D., Kelly, S., et al. (2010). Evaluation of mouthrinse and dentifrice regimens in an in situ erosion remineralisation model. *Journal of Dentistry*, 18(Suppl. 3), S37-44.

Mäkinen, K. K. (2000). The rocky road of xylitol to its clinical application. *Journal of Dental Research*, 79, 1352–1355.

Mandel, I. D. (1994). Antimicrobial mouthrinses: overview and update. *Journal of the American Dental Association*, 125, 2-10.

Marinho, V. C., Chong, L. Y., Worthington, H. V., & Walsh, T. (2016). Fluoride mouthrinses for preventing dental caries in children and adolescents. *The Cochrane Database of Systematic Reviews*, 7(7), CD002284.

Mensi, M., Cochis, A., Sordillo, A., Uberti, F., & Rimondini, L. (2018). Biofilm Removal and Bacterial Re-Colonization Inhibition of a Novel Erythritol/Chlorhexidine Air-Polishing Powder on Titanium Disks. *Materials*, 11.

Murray, M. C., Worthington, H. V., & Blinkhorn, A. S. (1997). A study to investigate the effect of a propolis-containing mouthrinse on the inhibition of de novo plaque formation. *Journal of Clinical Periodontology*, 24(11), 796–798.

Nordblad, A., Suominen-Taipale, L., Murtomaa, H., Vartiainen, E., & Koskela, K. (1995). Smart Habit xylitol campaign, a new approach in oral health promotion. *Community Dental Health*, 12, 230–234.

Ogaard, B. (2008). White spot lesions during orthodontic treatment: mechanisms and fluoride preventive aspects. *Seminars in Orthodontics*, 14, 183-93.

O'Mullane, D. M., et al. (2016). Fluoride and oral health. *Community Dental Health*, 33(2), 69-99.

Ozan, F., Sümer, Z., Polat, Z. A., Er, K., Ozan, U., & Deger, O. (2007). Effect of mouthrinse containing propolis on oral microorganisms and human gingival fibroblasts. *European Journal of Dentistry*, 1(4), 195-201.

Petersen, P. E. (2003). The World Oral Health Report 2003: continuous improvement of oral health in the 21st century--the approach of the WHO Global Oral Health Programme. *Community Dentistry and Oral Epidemiology*, 31(Suppl 1), 3-23.

Petersson, L. G., Hakestam, U., Baigi, A., & Lynch, E. (2007). Remineralization of primary root caries lesions using an amine fluoride rinse and dentifrice twice a day. *American Journal of Dentistry*, 20(2), 93-96.

- Phan, T. N., Buckner, T., Sheng, J., Baldeck, J. D., & Marquis, R. E. (2004). Physiologic actions of zinc related to inhibition of acid and alkali production by oral streptococci in suspensions and biofilms. *Oral Microbiology and Immunology*, 19, 31–38.
- Rosling, B., Wannfors, B., Volpe, A. R., Furuichi, Y., Ramberg, P., & Lindhe, J. (1997). The use of a triclosan/copolymer dentifrice may retard the progression of periodontitis. *Journal of Clinical Periodontology*, 24, 873-880.
- Ross, N. M., Charles, C. H., & Dills, S. S. (1989). Long-term effects of Listerine antiseptic on dental plaque and gingivitis. *Journal of Clinical Dentistry*, 1, 92–95.
- Russell, A. D. (1990). Bacterial spores and chemical sporicidal agents. *Clinical Microbiology Reviews*, 3(2), 99-119.
- Russell, A. D., & Day, M. J. (1993). Antibacterial activity of chlorhexidine. *Journal of Hospital Infection*, 25(4), 229-238.
- Saeed, M. A., Khabeer, A., Faridi, M. A., & Makhdoom, G. (2021). Effectiveness of propolis in maintaining oral health: a scoping review. *Canadian Journal of Dental Hygiene*, 55(3), 167–176.
- Salli, K., Lehtinen, M. J., Tiihonen, K., & Ouwehand, A. C. (2019). Xylitol's Health Benefits beyond Dental Health: A Comprehensive Review. *Journal of Oral Biosciences*, 63(1), 23–34.
- Santiago, F. S., Piana, G. M., Conti, B. J., Cardoso, E. M., Rall, V. L. M., Fernandes, A., Jr, & Sforcin, J. M. (2018). Microbiological control and antibacterial action of a propolis-containing mouthwash and control of dental plaque in humans. *Natural Product Research*, 32(12), 1441–1445.
- Serrano, J., Escribano, M., Roldán, S., Martín, C., & Herrera, D. (2015). Efficacy of adjunctive antiplaque chemical agents in managing gingivitis: a systematic review and meta-analysis. *Journal of Clinical Periodontology*, 42(16), 106-138.
- Spets-Happonen, S., Luoma, H., Forss, H., Kentala, J., Alaluusua, S., & Luoma, A.-R. (1991). Effects of a chlorhexidine-fluoride-strontium rinsing program on caries, gingivitis and some salivary bacteria among Finnish schoolchildren. *Scandinavian Journal of Dental Research*, 99(2), 130-138.
- Spets-Happonen, S., Markkanen, H., Pollanen, L., Kauppinen, T., & Luoma, H. (1985). Salivary *Streptococcus mutans* count and gingivitis in children after rinsing with a chlorhexidine-fluoride solution with and without strontium. *Scandinavian Journal of Dental Research*, 93(4), 329-335.
- Stoeken, J. E., Paraskevas, S., & van der Weijden, G. A. (2007). The long-term effect of a mouthrinse containing essential oils on dental plaque and gingivitis: a systematic review. *Journal of Periodontology*, 78, 1218–1228.
- Tanomaru Filho, M., Leonardo, M. R., Silva, L. A., Aníbal, F. F., & Faccioli, L. H. (2002). Inflammatory response to different endodontic irrigating solutions. *International Endodontic Journal*, 35, 735-739.

Taşdemir, Z., & Alkan, B. A. (2016). Plak Kontrolü Sağlayan Antimikrobiyal Ajanlar.

Thomas, B., & Bishop, J. (2013). *Manual of Dietetic Practice*. John Wiley & Sons: Hoboken, NJ, USA.

Tomás, I., Cousido, M. C., Tomás, M., Limeres, J., García-Caballero, L., & Diz, P. (2008). In vivo bactericidal effect of 0.2% chlorhexidine but not 0.12% on salivary obligate anaerobes. *Archives of Oral Biology*, 53(12), 1186-1191.

Van der Weijden, G. A., Timmerman, M. F., Novotny, A. G. A., Rosema, N. A. M., & Verkerk, A. A. J. (2005). Three different rinsing times and inhibition of plaque accumulation with chlorhexidine. *Journal of Clinical Periodontology*, 32(1), 89-92.

Van Rijkom, H. M., Truin, G. J., & van't Hof, M. A. (1996). A meta-analysis of clinical studies on the caries-inhibiting effect of chlorhexidine treatment. *Journal of Dental Research*, 75(2), 790-795.

Wåler, S. M. (1983). Effect of xylitol on dental plaque in vivo during carbohydrate challenge. *Scandinavian Journal of Dental Research*, 91(4), 256–259.

Weller, P. J. (2000). Chlorhexidine. In A. H. Kibbe (Ed.), *Handbook of Pharmaceutical Excipients* (3rd ed., p. 121-5). Washington DC: Pharmaceutical Press.

Wilken, R., Botha, S. J., Grobler, A., & Germishuys, P. J. (2001). In vitro cytotoxicity of chlorhexidine gluconate, benzydamine-HCl and povidone iodine mouthrinses on human gingival fibroblasts. *South African Dental Journal*, 56(10), 455-460.

Windisch, W. (2001). Homeostatic reactions of quantitative Zn metabolism on deficiency and subsequent repletion with Zn in ⁶⁵Zn-labeled adult rats. *Trace Elements and Electrolytes*, 18, 122–128.

Zero, D. T., Raubertas, R. F., Fu, J., Pedersen, A. M., Hayes, A. L., & Featherstone, J. D. (1992). Fluoride concentrations in plaque, whole saliva, and ductal saliva after application of home-use topical fluorides. *Journal of Dental Research*, 71(11), 1768-75.