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THE ANALYSES OF ANTIMICROBIAL EFFICACY OF VARIOUS HERBAL TOOTHPASTES

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ABSTRACT

Objective: *The aim of this in-vitro study is to evaluate the antibacterial efficacy of different toothpastes on Streptococcus mutans, Lactobacillus acidophilus, Actinomyces viscosus, Candida albicans, Staphylococcus aureus and Escherichia coli.*

Material and Method: *Three conventional (Signal Expert Protection, Klorhex Daily Care, Klorhex Intensive Care), two conventional with herbal ingredients (Gumgumix, Colgate Hemp Seed Oil), one experimental based, and six experimental herbal (hemp seed oil, hemp extract, and various metal nanoparticles) toothpastes were tested to determine antimicrobial activity through the agar-well diffusion method.*

Streptococcus mutans, Lactobacillus acidophilus, Actinomyces viscosus, Staphylococcus aureus, Escherichia coli and Candida albicans strains were prepared at 10^8 CFU/mL in physiological saline, then spread on agar media. Wells with 6 mm diameter were punched and the toothpaste samples were transferred. Paper discs containing 0.2% chlorhexidine digluconate were used as the positive control. After incubation at 37°C for 24-48 hours, the inhibition zones that formed around the wells were measured.

Results: *It was observed that Klorhex Intensive Care and one experimental toothpaste (containing Ag nanoparticle, hemp oil, and hemp extract) were effective against all the microorganisms used in the study. The herbal toothpaste that contained ginger didn't perform any antimicrobial activity against the microorganisms. Signal Expert Protection, Colgate Hemp Seed Oil, and Klorhex Daily Care toothpastes were effective against all of the study's microorganisms except E. coli.*

Conclusion: *The addition of hemp seed oil and extract increased antibacterial activity in some experimental toothpastes.*

Keywords: *Toothpaste, herbal, hemp, streptococcus mutans, nanoparticles.*

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FARKLI BİTKİSEL DİŞ MACUNLARININ ANTİMİKROBİYAL ETKİNLİĞİNİN ANALİZİ

ÖZET

Amaç: Bu in vitro çalışmanın amacı, farklı diş macunlarının Streptococcus mutans, Lactobacillus acidophilus, Actinomyces viscosus, Candida albicans, Staphylococcus aureus ve Escherichia coli üzerindeki antibakteriyel etkinliğini değerlendirmektir.

Materyal ve Metot: Üç geleneksel (Signal Expert Protection, Klorhex Günlük Bakım, Klorhex Yoğun Bakım), iki bitkisel içerikli geleneksel (Gumgumix, Colgate Hemp), bir deneysel baz ve altı deneysel bitkisel (kenevir tohumu yağı, kenevir ekstraktı ve farklı metal nanopartiküller) diş macunu, agar-well difüzyon yöntemi yoluyla antimikrobiyal aktiviteyi belirlemek için test edildi.

Streptococcus mutans, Lactobacillus acidophilus, Actinomyces viscosus, Staphylococcus aureus, Escherichia coli ve Candida albicans suşları fizyolojik salin içerisinde 10^8 CFU/mL'de hazırlandı, ardından agar ortamına yayıldı. 6 mm çapında kuyucuklar açılarak diş macunu örnekleri aktarıldı. Pozitif kontrol olarak %0,2 klorheksidin diglukonat içeren kağıt diskler kullanıldı. 37°C'de 24-48 saat inkübasyonun ardından kuyucukların etrafında oluşan inhibisyon zonları ölçüldü.

Bulgular: Klorhex Yoğun Bakım ve bir adet deneysel diş macununun (Ag nanopartikülü, kenevir yağı ve kenevir ekstraktı içeren) çalışmada kullanılan tüm mikroorganizmalara karşı etkili olduğu gözlendi. Zencefil içeren bitkisel diş macunu mikroorganizmalara karşı herhangi bir antimikrobiyal aktivite göstermedi. Signal Expert Protection, Colgate Hemp Seed Oil ve Klorhex Günlük Bakım diş macunları, *E. coli* dışında çalışmadaki tüm mikroorganizmalara karşı etkiliydi.

Sonuç: Kenevir tohumu yağı ve ekstraktının eklenmesi bazı deneysel diş macunlarında antibakteriyel aktiviteyi artırdı.

Anahtar kelimeler: Diş macunu, bitkisel, kenevir, streptococcus mutans, nanopartiküller.

INTRODUCTION

The brushing of the teeth is the basis of daily oral care, and toothpaste is the first and main aid in cleaning teeth. With the addition of chemical ingredients in their contents, toothpastes gain effects that prevent caries and cause remineralization, as well as whitening and antimicrobial properties. These chemical ingredients include fluoride compounds, sodium lauryl sulfate (SLS), parabens, triclosan, silica, sweeteners, and preservatives.[1]

The potentiality of intoxication and the various side effects of chemical-based products have led to a demand and increase in products that are marketed as natural in recent years. The manufacturers add herbal ingredients to toothpastes to meet the demands of consumers. Although most of these toothpastes contain plant extracts, they also contain synthetic additives and preservatives. However, some manufacturers add active herbal ingredients by removing fluoride, SLS, and paraben from the toothpaste content.

Ginger and hemp seed oil are some of the herbal ingredients that are added to toothpastes to increase antibacterial activity. Ginger (Zingiber officinale Roscoe), used in herbal toothpastes, has been used as a herbal remedy for centuries. It is reported that more than 100 compounds have been isolated from ginger. It is accepted as "safe" (generally recognized as safe, GRAS) by the US Food and Drug Administration (FDA).[2] There are many studies on its antimicrobial effects in oral cavities.

Hemp seeds contain vitamin E, thiamin (B1), riboflavin (B2) and many minerals such as Ca, Na, Mg, Fe, K. Hemp seed oil is a rich source of essential fatty acids, linoleic acid, and α linoleic acid.[3,4] Manosroi *et al.* found that hemp leaf extract has antibacterial effects on S. mutans and hemp seed oil has antibacterial effects on S. aureus.[5]

Metallic nanoparticles are used in many fields of medicine. In particular silver nanoparticles, are known for their antibacterial properties and low toxicity, and they are frequently used in dentistry.[6]

There are many different types of microorganisms in the oral cavity. When the balance between them is disturbed, many diseases, especially dental caries and periodontal diseases can occur. Despite this rich diversity, the microorganisms related to caries are Streptococcus mutans, Lactobacillus, and Actinomyces species. Streptococcus and, Lactobacillus can produce large amounts of acid (acidogenic), can tolerate acidic environment (aciduric), are strongly stimulated by sucrose, and are considered to be the main organisms associated with caries.[7] Candida albicans, a type of fungus, has a synergistic effect with Streptococcus mutans and is associated with caries. Actinomyces species also have cariogenic potential and cause both root caries and periodontal diseases.[8] Staphylococcus aureus, another oral cavity bacterium, is not a pathogenic species and causes infection when the body's immunal defense weakens.

This in-vitro study aims to evaluate the antibacterial efficacy of different toothpastes and herbal extracts on Streptococcus mutans, Lactobacillus acidophilus, Staphylococcus aureus, Candida albicans, and *E .coli*

MATERIAL AND METHOD

Three conventional, two herbal, six herbal-experimental and one experimental-base toothpaste and herbal ingredients of toothpastes in the study were tested for the evaluation of antimicrobial efficacy.

Three conventional toothpastes:

1. Signal Expert Protection (1450 ppm NaF) (Unilever, Bulgaria)
2. Dentasave Klorhex Intensive Care (0.2% chlorhexidine digluconate) (Drogsan, Türkiye)
3. Dentasave Klorhex Daily Care (0.05 % chlorhexidine digluconate) (Drogsan, Türkiye)

Two herbal toothpastes:

1. Gumgumix (0.4% ginger and 12.5% honey, Beka Drug, Türkiye)
2. Colgate Hemp Seed Oil (Colgate, Palmolive, USA)

Six herbal-experimental toothpastes (SPC Kozmetik, Türkiye):

1. 5% hemp seed oil + 5% hemp extract
2. 5% hemp seed oil + 5% hemp extract + 0.32% Ag nanoparticle
3. 5% hemp seed oil + 5% hemp extract + 0.32% Zn nanoparticle
4. 5% hemp seed oil + 5% hemp extract + 0.32% Cu nanoparticle
5. 5% hemp seed oil + 5% hemp extract + 0.32% Fe nanoparticle
6. 5% hemp seed oil + 5% hemp extract + 0.32% Ag+Zn+Cu+Fe nanoparticles

and one experimental-base toothpaste (containing mint oil, menthol, polisorbate 20, xanthan gum, aqua, glycerine, sorbitol, phenylpropanol, caprylyl glycol, stevia, hydrated silica) were tested for antimicrobial activity through the agar-well diffusion method.

The Agar-well Diffusion Medhod

Suspensions of Streptococcus mutans, Lactobacillus acidophilus, Actinomyces viscosus, Staphylococcus aureus, Escherichia coli and Candida albicans at 10^8 CFU/mL concentration were prepared in physiological saline (0.9% NaCl). All suspensions were standardized according to the 0.5 Mc Farland turbidity standard. Microorganisms were spread on the MRS agar or Sabouraud Dextrose agar media and wells with 6 mm diameter were punched on the agar medium surface. Toothpastes diluted 1:1 with distilled water were transferred to these wells in equal amounts. Paper discs containing 0.2% chlorhexidine digluconate were used as positive controls. After incubation at 37°C for 24 hours, the inhibition zones formed around the wells were measured.[9]

Characterization of Nanoparticles for Experimental Toothpastes

The leaves of the hemp plant to be used in our study were first dried under the tap and then washed with distilled water. The hemp plant leaves, which completed their physiological maturity, were thoroughly ground with a sterile grinder blender. 10 grams of thoroughly ground cannabis biomass were taken and mixed in 100 mL of distilled water at 50°C for 2 hours using a magnetic stirrer. The extraction was centrifuged at 3500 rpm for 15 minutes, filtered with the help of Whatmann number 1 filter paper and stored at +4°C for the synthesis step.

Green Synthesis and Characterization of Nanoparticles

Ag Nanoparticle (AgNP) Synthesis and Characterization

0.1 mM $AgNO_3$ aqueous solution was prepared from $AgNO_3$ salt and 200 ml of silver nitrate solution and 200 mL of hemp extract were placed in a 1000 L beaker and allowed to react at room temperature. The reaction started to change color in two hours under room conditions. As a result of the reaction, the dark colored solution was subjected to centrifugation (10000 rpm, 10 min). The solid part obtained at the end of centrifugation was washed several times with pure water and left to dry in an oven at 40°C. After

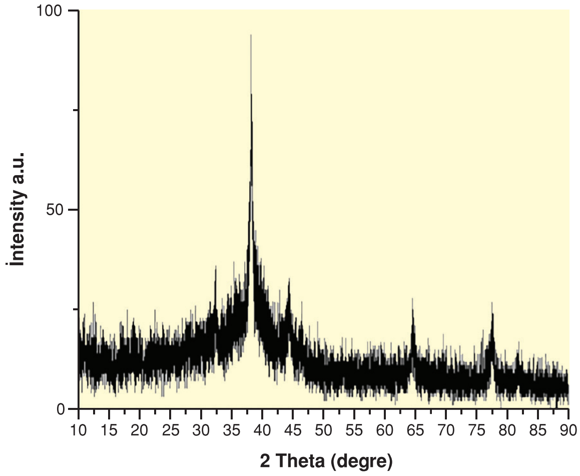

Figure 1. X-Ray diffraction graph of Ag nanoparticles.

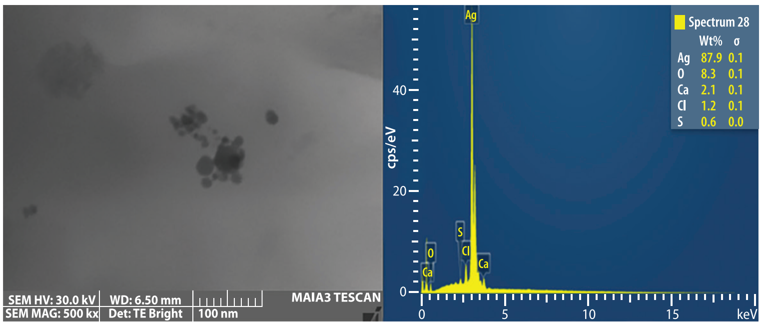

Figure 2. Scanning electron microscope image and energy dispersive X-ray analysis of Ag nanoparticles.

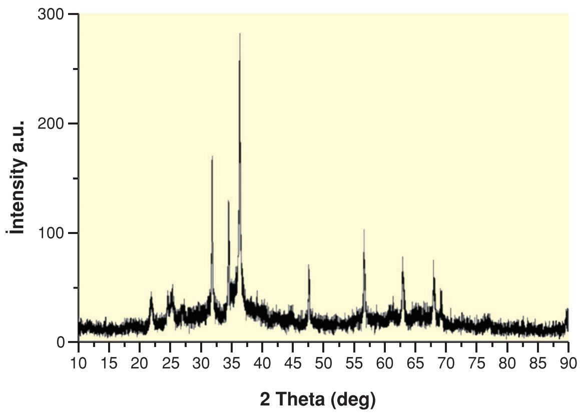

Figure 3. X-ray diffraction graph of ZnO nanoparticles.

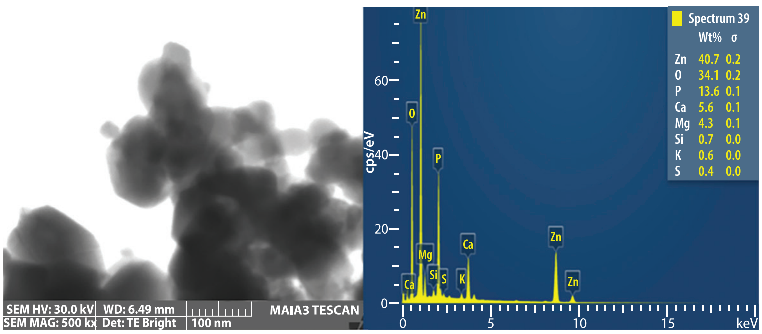

Figure 4. Scanning electron microscope image and energy dispersive X-ray analysis of ZnO nanoparticles.

the dried material was ground, Ag nanoparticles (NPs) were characterized by X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM) and Energy Dispersive X-Ray (EDX) Analysis.[10]

The crystal structure of AgNPs was made by XRD analysis. In the XRD analysis results, the peaks at 38.17, 44.36, 64.54, and 77.52, corresponding to 111°, 200°, 220° and 311° at 2θ, respectively, are sharp peaks representing the spherical crystal structure of silver (Figure 1). Using the data obtained as a result of the analysis, the crystal size of AgNPs was calculated using the Debye-Scherrer equation ($D = K\lambda/\beta \cos\theta$).[11] The average particle size was calculated as 61.3 nm as a result of the equation.

According to the SEM analysis results, it can be presumed that the AgNPs made through biosynthesis mostly have a spherical morphology (Figure 2). Again, as a result of EDX analysis, it is seen that the sample consists of 87.9% Ag, 8.3% O, and other impurities of herbal origin.

Synthesis and Characterization of ZnO NPs

20 mL of solution sulfate ($ZnSO_4$) solution (10 mM) was added to an amount of 100 mL. 20 mL of plant extract was added dropwise to this mixture. It was mixed in an ultrasonic bath for 30 minutes. 400 W microwaves for 10 minutes. The mixtures were filled into falcon tubes and rotated at 10000 rpm for 10 minutes. The resulting pellet was washed 5 times with ethanol and spun again to remove organic residues. The pellet piece was dried in an oven at 100°C for one day.[12]

The crystal structures of ZnO NPs were analyzed by XRD. The results showed that the sharp peaks at 100º (31.78), 002º (34.44), 101º (36.27), 102º (47.57), 110º (56.63), 103º (62.90), 112º (67.99), and 201º (69.13) corresponding to 2θ are the peaks that show the crystal structure of ZnO NPs (Figure 3). Using the Debye-Scherrer's equation, the nano-particle size was calculated as 96 nm.

The EDX spectrum of ZnO NPs showed peaks belonging to zinc, oxygen, and other elements thought to be of plant origin. The 40.7% zinc and 34.1% oxygen peak confirmed the synthesis of ZnO NPs. It was determined that ZnO NPs subjected to electron bombardment by scanning electron microscope were more durable and spherical (Figure 4). It was also observed that ZnO NPs had different sizes at 500 kx magnification.

Synthesis and Characterization of CuO NPs

For the synthesis of CuO nanoparticles; 100 mL of the prepared 0.1 M CuSO4 solution was taken into a clean beaker and 20 mL of hemp leaf extract was added dropwise, and stirred vigarously with the magnetic stirrer. This mixture was kept at 100°C for 4 hours, then cooled to room temperature and centrifuged at 10000 rpm for 10 minutes, and the resulting pellets were calcined at 400°C for 2 hours.[13]

The X-ray diffraction pattern of the CuO nanoparticle synthesized using leaf extract is seen in Figure 5. When the XRD graph of the CuO nanoparticle is examined, (110), (002), (111), (112), (202), (020), (202), (113), (311), (220), (311) Fourteen peaks were observed, corresponding to reflection angles corresponding to the (004), (114), (313) planes. The 2θ Angles of These Peaks Are As Follows: 2θ = 32.47°, 35.40°, 38.59°, 46.72°, 48.78°, 53.34°, 58.27°, 61.44°, 66.52°, 67.76°, 72.67°, 74.° and 82.61°. Using the Debye-Scherrer's equation, the nanoparticle size was calculated as 58 nm.

The image at 200 kx magnification obtained from the SEM analysis performed to obtain information about the surface structure of the CuO nanoparticle is given in Figure 6. From the SEM images, it can be seen that the change is in the structure and that NPs of different sizes come together in morphology in the form of high-level layers.

Synthesis and Characterization of Fe NPs

For Fe NP synthesis, 2.26 g $FeCl_3$ was dissolved in 30 mL of prepared plant extract with vigorous stirring at 80°C for 3 hours. It was seen that the extract turned black after becoming homogeneous for 3 hours. The color change is attributed in the literature to the formation of iron nanoparticles. The resulting black solution was decanted by centrifugation after cooling to room temperature. It was dried in an oven at 90°C for 12 hours. This synthesis method is similar to the synthesis methods in the literature provided.[14]

As a result of XRD analysis, it was seen that the material was synthesized as a mixture of both iron and iron oxide NPs. The XRD model of the resulting nanoparticles is shown in Figure 7. It was found that a total of ten peaks belonged to iron peaks and the other peaks belonged to iron oxides. As a result of the analysis, it was found that this substance had a cubic structure. Using the Debye-Scherrer's equation, the nanoparticle size was calculated as 116 nm.

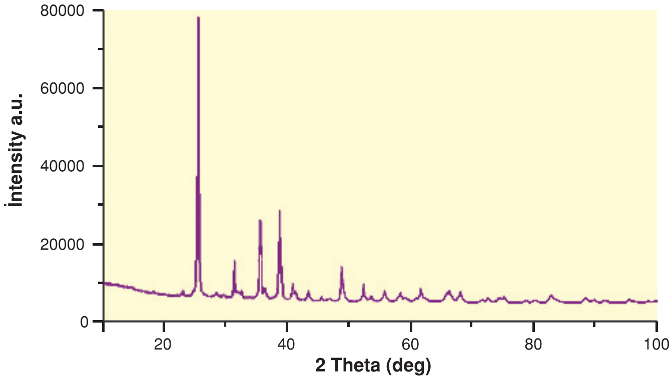

Figure 5. X-ray diffraction graph of CuO nanoparticles.

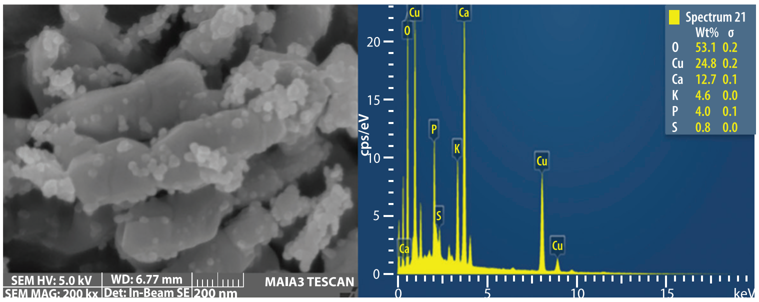

Figure 6. Scanning electron microscope image and energy dispersive X-ray analysis of Cu nanoparticles.

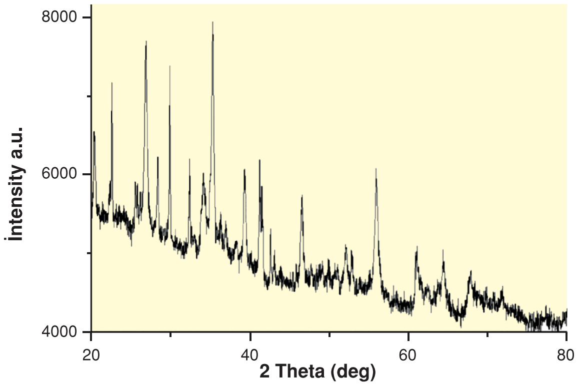

Figure 7. X-ray diffraction graph of Fe/FeO nanoparticles.

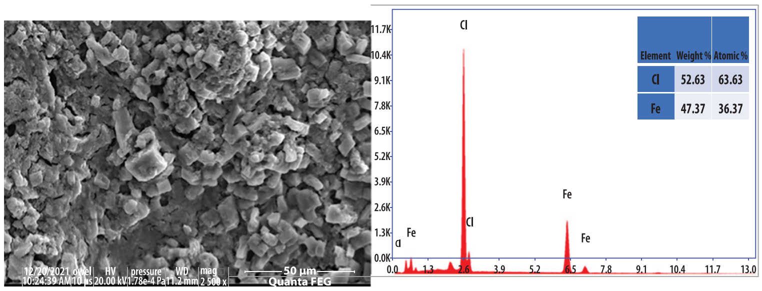

Figure 8. Scanning electron microscope image and energy dispersive X-ray analysis of Fe/FeO nanoparticles.

In the SEM image taken at 50 μm, it is seen that the nanoparticles consist of cube-like irregular sizes and structures (Figure 8). By EDX analysis, the presence of Fe element in the structure of the sample was confirmed with a Fe element ratio of 47.37%. In addition, the presence of 52.63% of the Cl element in the effluent salt is also seen as an impurity.

Table 1. Inhibition zones created by herbal ingredients of toothpastes used in the study

	Antimicrobial Efficacy (Inhibition Zones, Diameter, mm)					
	Staphylococcus aureus	Escherichia coli	Lactobacillus acidophilus	Streptococcus mutans	Actynomyces viscosus	Candida albicans
Hemp extract	0	0	0	0	0	0
Hemp seed oil	12	0	0	11	0	0
Cu Nanoparticle	0	0	0	0	0	0
Zn Nanoparticle	0	12	0	0	0	0
Ag Nanoparticle	0	0	0	0	14	7
Fe Nanoparticle	0	0	0	0	0	0
0.2 % Chlorheksidine Digluconate	21	21	18	17	18	25

Table 2. Inhibition zones created by tootpaste

	Antimicrobial Efficacy (Inhibition Zones, Diameter, mm)					
	Staphylococcus aureus	Escherichia coli	Lactobacillus acidophilus	Streptococcus mutans	Actynomyces viscosus	Candida albicans
Signal Expert Protection	21	0	30	11	12	23
Gumgumix	0	0	0	0	0	0
Colgate Hemp Seed Oil	22	0	22	14	15	29
Klorhex Daily Care	16	0	18	17	18	19
Klorhex Intensive Care	30	25	17	17	17	21
Base Toothpaste	0	0	0	0	0	0
5% Hemp Seed Oil + 5% Hemp Extract	0	0	10	12	10	0
5% Hemp Seed Oil + 5% Hemp Extract + Ag NP TP	12	12	12	14	12	17
5% Hemp Seed Oil + 5% Hemp Extract + Zn NP TP	0	0	10	12	10	7
5% Hemp Seed Oil + 5% Hemp Extract + Fe NP TP	0	0	10	11	8	0
5% Hemp Seed Oil + 5% Hemp Extract + Cu NP TP	0	0	9	11	9	0
5% Hemp Seed Oil + 5% Hemp Extract + Ag+Zn+Fe+Cu NP TP	0	0	10	11	10	0
0.2% Chlorheksidine Digluconate	21	21	18	17	18	25

RESULTS

Hemp seed oil showed an antibacterial effect against S. aureus and S. mutans. Ag nanoparticle had antimicrobial effects against A. viscosus and C. albicans. Zn nanoparticle was effective against *E. coli.* Herbal toothpaste (Gumgumix) and experimental base toothpaste containing ginger were not effective against any microorganisms used in the study (Table 1, Figure 9).

Klorhex Intensive Care toothpaste was effective against all microorganisms used in the study. The experimental toothpaste containing Ag nanoparticles, hemp oil, and hemp extract was effective against all microorganisms used in the study. Another experimental toothpaste containing hemp seed oil and hemp extract was effective against L. acidophilus, S. mutans and A. viscosus (Table 2, Figure 9).

The experimental toothpaste containing Fe nanoparticle, hemp oil and hemp extract was effective against L. acidophilus, S. mutans and A. viscosus. An Experimental toothpaste containing Zn nanoparticles, hemp oil and hemp extract was effective against L. acidophilus, S. mutans and A. viscosus. One

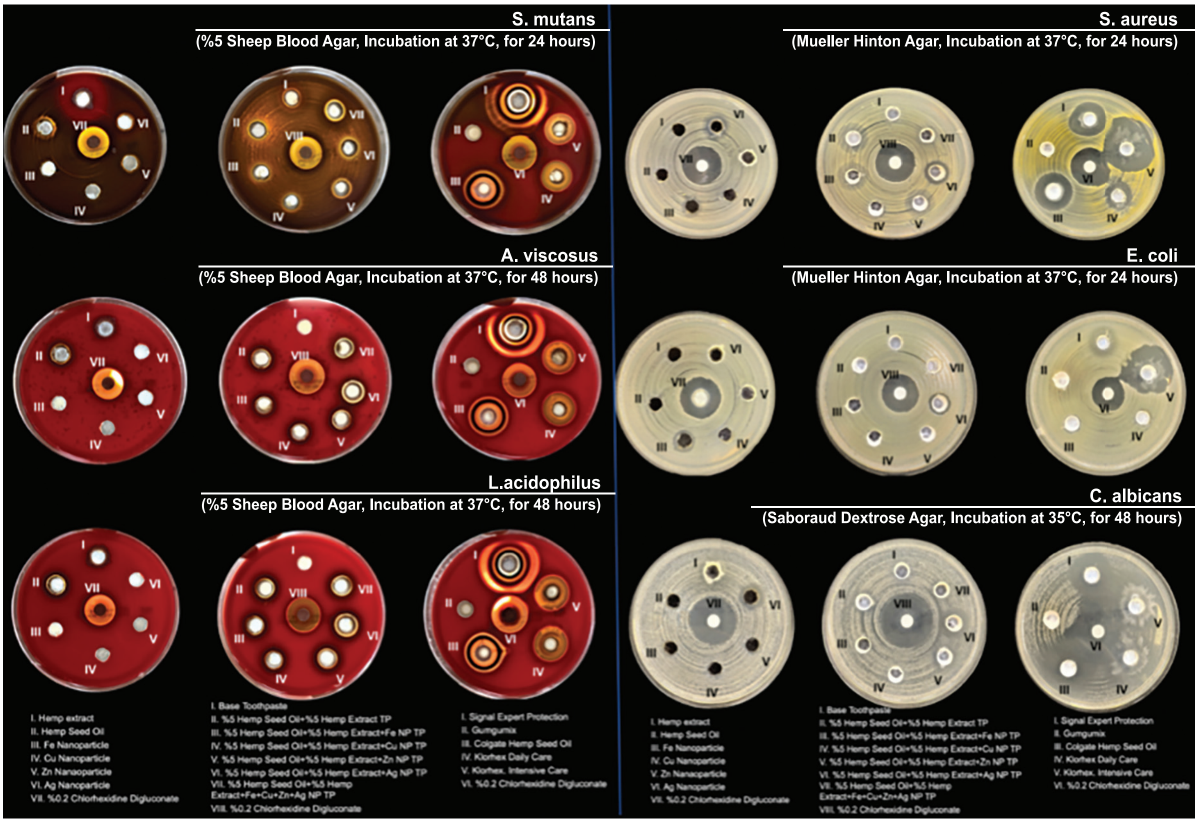

Figure 9. Inhibition zones.

of the Experimental toothpastes containing Cu nanoparticles, hemp oil, hemp extract was effective against L. acidophilus, S. mutans and A. viscosus. The Experimental toothpaste containing Fe+Cu+Zn+Ag nanoparticles, hemp oil and hemp extract was efficacious against L. acidophilus, S. mutans and A. viscosus. Signal Expert Protection, Colgate Hemp Seed Oil and Klorhex Daily Care toothpastes were effective against all microorganisms in the study except *E. Coli* (Table 2, Figure 9).

DISCUSSION

The most common dental diseases that form in the oral cavity are dental caries and periodontal diseases such as gingivitis and periodontitis. Dental caries is a multifactorial disease with a high number of cariogenic bacteria.[15] Various oral care products, especially toothpastes, are used to remove the microbial dental plaque that causes caries. Substances such as fluoride, SLS, triclosan, and hydroxylapatite are added to the contents of toothpastes to create antibacterial activity.[16] It is emphasized in various studies that SLS and different forms of fluoride increase antimicrobial activity.[17] In addition, herbal products, which are widely used in the cosmetics industry, have been added to the contents of toothpastes with an increasing momentum in our day and age. Among these herbal contents, ginger and hemp perform antibacterial activity in the oral cavity.[18,19] Many nanoparticles like the silver nanoparticle have an intrinsic antibacterial affinity that is enhanced by the affinities of various herbal extracts.[20] The nanoparticle content that is able to be added to the content of toothpastes is used in dentistry due to its antibacterial activity as seen in many substances.[6]

In the present study, the antibacterial effects of conventional toothpastes (Signal Expert Protection, Klorhex Daily Care, Klorhex Intensive Care), two herbal toothpastes containing ginger (GumGumix) and hemp seed oil (Colgate Hemp Seed Oil), and seven herbal experimental toothpastes containing hemp seed oil, hemp extract, and metal nanoparticles on S. mutans, L. acidophilus, A. viscosus, S. aureus, and C. albicans were investigated.

Since our study aims to compare the antimicrobial activities of different toothpastes, each toothpaste was tested with the same microorganisms under the same conditions, and the agar-well diffusion method was preferred to accomplish the aim of the study. Although there are many studies examining the antibacterial activity of the chemical content of toothpastes, the number of studies examining the antibacterial effect of herbal toothpastes is limited.[17,21,22]

Kengadaran *et al.* examined the antibacterial efficacy of herbal and conventional toothpastes, and found that herbal toothpaste was more effective on oral microflora.[23] According to their study, Fareen *et al.* found that the antibacterial activity of the herbal toothpaste against S. mutans was higher than the conventional toothpaste, while the results of another study showed that the conventional toothpaste was more effective against S. mutans than the herbal toothpaste.[17,24] Ahmed *et al.* examined the antibacterial activity of a toothpaste containing silver nanoparticles through the agar diffusion method, and reported that it showed higher antibacterial activity against S. mutans than the toothpaste containing fluoride.[25] In our study, the antibacterial and antifungal activity of the herbal toothpaste containing ginger and honey (Gumgumix) was not detected to be working on the microorganisms investigated in the study. Relatedly, Gumgumix is a toothpaste that has a cosmetic license but is not yet in the market. Since it is not produced regularly and frequently, it is thought that its antimicrobial effectiveness may have decreased. However, the conventional toothpaste containing hemp seed oil and herbal experimental toothpastes containing hemp seed oil and metal nanoparticles had antibacterial effects on S. mutans, A. viscosus and S. aereus. Antifungal activity on C. albicans was determined in conventional toothpastes containing SLS and fluoride, and in the experimental toothpaste containing Ag nanoparticles.

Karadaglioglu *et al.* examined the antibacterial effect of various toothpastes, adding herbal oils such as O. dubrea and C. cassia in, and employing the agar well diffusion method. It was seen that the antibacterial effect of the toothpastes increased significantly with the addition of herbal oils.[26] Similar to this study, in our study, it was observed that although the experimental based toothpaste was not effective against any bacteria, with the addition of hemp seed oil, it gained antibacterial affinity. It was determined that adding the Ag nanoparticle along with the hemp seed oil to the toothpaste enabled the experimental toothpaste to have an antimicrobial effect on all bacterial strains and C. albicans.

CONCLUSION

In conclusion, while the developments in the field of herbal toothpastes show promise, many properties of herbal toothpastes such as its antimicrobial properties, impact on remineralization, and potential toxicity need to be substantiated. For this reason, further scientific investigations are required. Recommending the right toothpaste, tailored to the needs of the patient, contributes to the betterment of oral health in society.

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*The authors declare that there are no conflicts of interest.

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