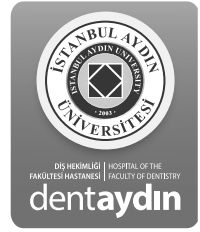




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EVALUATION OF ANTIBACTERIAL EFFECT OF TOOTHPASTES WITH DIFFERENT CONTENTS: AN IN VITRO STUDY

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ABSTRACT

Objectives: Antibacterial substances added to different toothpastes that are presented to the market with the claim of showing antibacterial effect are aimed at preventing periodontal diseases and tooth decay by controlling pathogen formation in the bacterial plaque. The aim of this in vitro study is to evaluate toothpastes with different contents for their antimicrobial activity against *Streptococcus Mutans*.

Materials and methods: Disc diffusion method was used to investigate the antibacterial effect of 11 toothpastes with different contents and different antibacterial agents (ozone, ganoderma lucidum, tea tree oil, xylitol, zinc, propolis, theobromine, triclosan, sodium lauryl sarkosinate) against *S. mutans*. 0.1 mL sample of toothpaste was placed in the centers of paper discs with syringe. Vaseline was used as control group. 3 discs were prepared for each test product. The inhibition zone diameter against the microorganism was measured after 48 hours with digital calipers. Statistical analysis was performed by One-way ANOVA and posthoc TUKEY test.

Results: In this study, it was found that Triclosan-containing toothpaste showed the highest antibacterial effect followed by toothpastes containing Ganoderma Lucidium and Xylitol respectively. Toothpastes containing Tea Tree Oil, Theobromine and Sodium Lauryl Sarkosinate were found with the similar antibacterial effects and had lowest efficacy among other antibacterial agents. Among 3 different toothpastes containing theobromine, no antimicrobial effect was observed in the toothpaste produced for children.

Conclusion: In recent years, manufacturers have been trying to use new products while using natural ingredients as well as increasing their success targets with multiple effects. However, the efficiency of antibacterial agents used for this purpose is controversial.

Keywords: Antibacterial effect, fluoride, *Streptococcus mutans*, toothpaste, zone of inhibition, natural antimicrobial agents

ÖZET

Amaç: Antibakteriyel etki gösterdiği iddiası ile piyasaya sunulan farklı diş macunlarına eklenen antibakteriyel maddelerin, bakteriyel plaktaki patojen oluşumunu kontrol altına alarak periodontal hastalık ve diş çürüğü oluşumunun önüne geçilmesi hedeflenmektedir. Bu in vitro çalışmanın amacı, *Streptococcus Mutans*'a karşı antimikrobiyal aktiviteleri için farklı içerikli diş macunlarını karşılaştırmaktır.

Gereç ve yöntemler: Disk difüzyon yöntemi, farklı içeriklere ve farklı antibakteriyel ajanlara (ozon, ganoderma lucidum, çay ağacı yağı, ksilitol, çinko, propolis, teobromin, triklosan, sodyum lauryl sarkosinat) sahip diş 11 diş macununun *S. Mutans*'a karşı antibakteriyel etkisini araştırmak için kullanılmıştır. 0.1 mL'lik diş macunu örneği, kâğıt disklerin ortasına şırınga ile yerleştirilmiştir. Vazelin ise çalışmamızda kontrol grubu olarak kullanılmıştır. Her bir test ürünü için 3 disk hazırlanmıştır. Mikroorganizma karşı inhibisyon zon çapı, 48 saat sonra dijital kaliperler ile ölçülmüştür. İstatistiksel analiz One-way ANOVA ve posthoc TUKEY testi ile yapılmıştır.

Sonuçlar: Bu çalışmada, Triklosan içeren diş macununun en yüksek antibakteriyel etkiyi, ardından Ganoderma Lucidium ve Xylitol içeren diş macunları izlediği bulunmuştur. Tea Tree Oil, Theobromine ve Sodium Lauryl Sarkosinate içeren diş macunlarına benzer antibakteriyel etkileri bulundu ve diğer antibakteriyel ajanlar arasında en düşük etkinliğe sahipti. Teobromin içeren 3 farklı diş macunu arasında, çocuklar için üretilen diş macununda antimikrobiyal etki görülmemiştir.

Sonuç: Son yıllarda üreticiler, doğal ürünlerden yararlanarak yeni ürünleri kullanmaya çalışmakta ve başarı hedeflerini çoklu etkilerle artırmaktadır. Bununla birlikte, bu amaçla kullanılan antibakteriyel ajanların etkinliği tartışmalıdır.

Anahtar Kelimeler: Antibakteriyel etki, florür, *Streptococcus mutans*, diş macunu, inhibisyon bölgesi, doğal antimikrobiyal ajanlar

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INTRODUCTION

Dental caries and related oral diseases like gingivitis and periodontitis are most common oral diseases throughout the world including both developed and developing countries, affecting people from all ages. The frequency of these oral diseases is continuously increasing with change in eating habits of people from different age groups and increased consumption of sugar.¹ Many prevention programs have been studied in order to protect the oral and dental health of the communities. These programs are based on individual or community levels. However, the implementation of prevention programs for wide range groups is much more difficult due to factors such as infrastructure deficiency, cost and ethical rules. This situation puts individual practices at the forefront in decay prevention. Therefore, daily tooth brushing with toothpaste is the key pillars to prevent caries. In this way, it is possible to improve the oral and dental health of the community at a much lower cost.

Using natural products in medicines to cure various diseases has become an increasing trend. Herbal medicine has made significant contribution to modern medical practice.² There is an increasing societal desire to rely on naturally occurring compounds for health care, which has also found its way into dentistry.³ In general, the most added therapeutic agent in toothpastes is fluoride. The fluoridated tooth pastes have the advantages of both re-mineralization of initial lesions and antibacterial effect on oral microflora.⁴ In recent years, toothpaste manufacturers have been trying to use new products while using natural ingredients as well as increasing their success targets with multiple effects. The antibacterial substances such as triclosan, chlorhexidine, propolis, tea tree extract added

to the different toothpastes presented to the market with the claim of showing antibacterial effect are aimed at preventing periodontal diseases and tooth decay by controlling the pathogen formation in the bacterial plaque.

Therefore, the aim of this in vitro study is to evaluate several toothpastes containing different components for their antimicrobial activity against *Streptococcus Mutans*.

MATERIALS AND METHODS

Toothpastes

11 commercially available toothpastes along with their ingredients as listed on the package and the manufacturer's name that were evaluated in this study are shown in Table 1. Vaseline was used as control group.

Preparation and reactivation of the microorganisms

The lyophilized microorganisms used in this research were *Streptococcus Mutans* DSM 20523. Before reactivation, each inactive microorganism was stored at 4 ± 1 °C. To start the multiplication and activation of the microorganisms, one milliliter of their specific liquid culture medium was added in each tube and inoculated at 37 ± 1 °C overnight. The specific culture mediums for *S.mutans* are TSB (Tyriptic Soy Broth). Then, to control the multiplication of the microorganisms and to check if there is any contamination; the microorganisms were inoculated in agar containing culture mediums and incubated 48 hours at 37 ± 1 °C. After all the controls, the activated microorganisms are again inoculated in their liquid culture mediums and left incubated again for 48 hours. *S.mutans* were incubated anaerobically (5 % CO₂ addition) for 48 hours at 37 ± 1 °C.

Agar disc diffusion technique

The TSA (Tryptic Soy Agar), MRS Agar and SDA (Sabouraud Dextrose Agar) culture mediums were sterilized in autoclaves (15 min, 121 °C). Twenty mL of these culture mediums was evenly distributed over the surface of 9 cm in diameter-sterile, disposable, plastic petri dishes. After the setting of the culture mediums, the petri dishes were half covered and left one hour at 37±1 °C to remove all the moisture left. Approximately 0.5 mL of the microorganism's suspension (3.6 x 10⁷ CFU/mL) was inoculated by swabbing over the agar surfaces. For each petri dish, three standardized paper discs (12 mm) were

immediately placed on the agar surface with sterile tweezers. Approximately 1 ml of the toothpaste from each sample was applied over the disc surfaces with sterile syringe. After 48 hours of incubation at 37±1 °C, diameters of circular inhibition zones produced around the paper discs were measured with a digital caliper in millimeters. (Figure 1)

Statistical Analysis

Data on antimicrobial activity were compared only between dentifrices evaluated by One-way ANOVA and post-hoc TUKEY test. A significance level of 5% was used for analyses.

TOOTHPASTES	TRADE NAME	CONTENTS	MANUFACTURER
Toothpaste 1	Colgate Total Advanced Whitening	Sodium Fluoride (1450 ppmF), Aqua, Hydrated Silica, Glycerin, Sorbitol, PVM/MA Copolymer, Sodium Lauryl Sulfate, Aroma, Sodium Hydroxide, Carrageenan, Propylene Glycol, Sodium Fluoride, Triclosan, Sodium Saccharin, Mica, Cellulose Gum, Limonene	Colgate Palmolive Co. LTD
Toothpaste 2	Dentiste Toothpaste	CDX complex (cyclodextrin complex), peppermint, licorice root, clove, eucalyptus, xylitol, cinnamon, stabilized vitamin C	Cosmeceutical Co. LTD, Thailand
Toothpaste 3	Natural Ozone Toothpaste	Sorbitol, Hydrated Silica, Deionized water, coco-glucoside, ozonated virgin olive oil, xanthan gum, aroma, vitamin E, propolis, salvadora persica extract, salvia officinalis extract, achillea millefolium extract, menthol, sodium benzoate, saccharine, potassium sorbate	Mert Cosmetic Co. LTD, Turkey

Toothpaste 4	Dentasave Toothpaste	Aqua, glycerin, hydrated silica, sodium cocoamphoacetate, potassium nitrate, sorbitol, peg 40 hydrogenated castor oil, PV M/MA copolymer cellulose gum, zinc citrat, sodium saccharin, aroma, titanium dioxide, phenaxyethanol, ethylhexylglycerin	Drogsan Pharmaceuticals Co. LTD, Turkey
Toothpaste 5	TTO Toothpaste	Aqua, sorbitol, glycerin, silica, magnesium lauryl sulfate, hyroxyethylcellulose, aroma, melaleuca alternifolia terpinen 4-ol, sodium benzoate, potassium nitrate, sodium saccharin, A. Floride, titanium dioxide	Dr. Muller Pharmaceuticals Co. LTD, Turkey
Toothpaste 6	Buccotherm Toothpaste	Castera- verduzan thermal aqua, glycerin, dicalcium phosphate dihydrate, silica, hydrogenated starch hydrolysate, water, decyl glucoside, titanium dioxide, aroma, xanthan gum, alcohol, propolis, sodium floride(1100 ppm), sodium hydroxide, stevia rebaudiana extract, propolis extract, limonane, linalool	Laboratoire ODOT23 rue du Lac, France
Toothpaste 7	Gano Fresh Toothpaste	Ganoderma Lucidum Extract	Gano excel Industries SDN BHN, Malesia
Toothpaste 8	Naturalive Toothpaste	Calcium Carbonate, aqua, sorbitol, glycerin, salvadore persica extract, propolis, decyl glucoside, aroma, menthol, tea tree extract	Naturalive cosmetics Co. LTD., Turkey

Toothpaste 9	Theodent Kids Toothpaste	Theobromine, calcium acetate, sodium hydrogen phosphate, purified water, hydrated silica, sorbitol, xylitol, glycerin, xanthan gum, citric acid, sodium benzoate, sugar-free chocolate and vanilla extract	Theodent and Rennou are registered trademark of the U.S patent and trademark office
Toothpaste 10	Theodent 300 toothpaste	Theobromine, calcium acetate, sodium hydrogen phosphate, purified water, hydrated silica, sorbitol, xylitol, glycerin, sodium lauroyl sarcosinate, xanthan gum, titanium dioxide, citric acid, spearmint oil, sodium benzoate, stevia extract, sodium bicarbonate, sugar-free vanilla extract	Theodent and Rennou are registered trademark of the U.S patent and trademark office
Toothpaste 11	Theodent Toothpaste	Theobromine, calcium acetate, sodium hydrogen phosphate, purified water, hydrated silica, sorbitol, xylitol, glycerin, sodium lauroyl sarcosinate, xanthan gum, titanium dioxide, citric acid, spearmint oil, sodium benzoate, stevia extract, sodium bicarbonate, sugar-free vanilla extract	Theodent and Rennou are registered trademark of the U.S patent and trademark office
Control	Vaseline		

Table 1: Toothpastes evaluated in the study, compositions and manufacturers



Figure 1. Measuring the inhibition zone with a digital caliper

RESULTS

Table-2 represents the inhibition zones at the end of 48 hours of the toothpastes against *St. Mutans*.

Among the eleven dentifrices tested, 6 toothpaste samples showed an antibacterial activity. The rest of the toothpaste samples did not show any inhibition zones. Toothpaste number 1 (Colgate total) was the most effective against the microorganism producing larger zone of inhibition and antibacterial effect is statistically different from the rest. This is followed by numbers 7 and 2 respectively. The antibacterial effect of these dentifrices is statistically significant when compared to other toothpastes ($p < 0.05$). There is no statistically significant difference between the antibacterial effects of 5, 10 and 11 and they have similar antibacterial effects. ($p < 0.05$).

	Averages of the groups(mm)	Standard Deviations (mm)
Toothpaste 1	35,8667	0,73793
Toothpaste 2	15,4833	1,14718
Toothpaste 3	0	0
Toothpaste 4	0	0
Toothpaste 5	5,4900	1,22111
Toothpaste 6	0	0
Toothpaste 7	30,5333	1,86436
Toothpaste 8	0	0
Toothpaste 9	0	0
Toothpaste 10	8,1200	1,35643
Toothpaste 11	7,4700	1,32367
Control (vaselin)	0	0

Table.2 The millimetric averages and standard deviations of the measurements of the inhibition zone diameter

DISCUSSION

Oral hygiene is a part of our overall health. Mechanical plaque cleaning is the most effective way to ensure oral hygiene. Tooth brushing using toothpaste introduces therapeutic agents to the oral environment in addition to mechanical cleaning. Dentifrices may have several properties and there are different contents presented to the market for antibacterial effect for plaque control.

Antibacterial agents are expected to be effective against microorganisms that threaten oral health.⁵ The use of antibacterial agents against oral pathogens contributes to the prevention of caries and periodontal diseases. The antibacterial agents added to the toothpaste should be effective in a short time since the interaction of the chemicals is minimal. However, the antibacterial agents should not be in high concentrations. The use of high concentrations may cause side effects therefore manufacturers are in need of new satisfying antibacterial agents.⁶

Dental caries is a bacteria related behavioral disease that result in the destruction of mineralized tissue of the teeth. Streptococcus mutans is one of the main opportunistic pathogens of dental caries.⁷ Streptococcus mutans is considered to be the most cariogenic of all of the oral Streptococci and leading cause of dental caries. Therefore, in this in vitro study, antibacterial activities of dentifrices with various antibacterial agents were evaluated on S. Mutans.

There were numerous dentifrices in the dental market and only 10 different commercial toothpastes were the test materials of our study. Toothpaste No.1 with triclosan (Colgate Total) produced the largest inhibition zone, and the antibacterial effect differs statistically significantly compared to others. Rossi et al.

reported that triclosan-effective toothpaste exhibited higher antimicrobial activity on S. Mutans than other antimicrobial agents including chlorhexidine, and natural extracts (chamomile extract, Echinacea extract, salvia extract, myrrh extract, Mentha piperita extract)⁸ Dülgergil et al. reported that triclosan toothpaste had a greater inhibitory effect on S. mutans and lactobacilli.⁹ Evans et al. used Colgate Total toothpaste to compare the inhibitor effect with pediatric dentifrices (500 ppm F) on the S. mutans and the inhibition zone diameter was statistically significantly higher as it was in our study.¹⁰

The second highest antibacterial effect in our study was toothpaste No.7 containing Ganoderma Lucidum (Ganoderma Fresh toothpaste). Kalyoncu et al. identified Ganoderma Lucidum as the species with the highest antimicrobial activity in the study of some wild macro-fungi species aiming to demonstrate antimicrobial activity.¹¹ However, there is no information about the ingredients of the toothpaste and the label only states Ganoderma Lucidum as the content. Therefore, the result of this sample was questionable. We think that there might be other agents with antibacterial effect.

The toothpaste No.2 (Dentiste toothpaste) contains natural antiseptic and antibacterial agents, natural ingredients such as mint, lemongrass, clove, eucalyptus, cinnamon and xylitol. According to the data of our study, it is the third toothpaste with highest antibacterial activity and statistically different from the others. It has been shown that xylitol reduces the adhesion of microorganisms to the teeth surface.¹² In an in vitro study, xylitol reduced the levels of S. Mutans in plaque and saliva by disrupting their energy production processes, leading to futile energy cycle and cell death.¹³ Within the limitations of our study, with the known properties, we may conclude that

xylitol and the other natural agents have a moderate antibacterial effect.

Toothpastes No.5 (TTO toothpaste), No.10 (Theodent 300) and No.11 (Theodent) showed the lowest antibacterial effect in our study and there was no statistically significant difference between them. The toothpaste No. 5 (TTO toothpaste) contains tea tree oil which is volatile oil obtained by vapor distillation of the leaves of *Melaleuca Alternifolia* naturally grown in Australia and is used as topical antiseptic for over 80 years in Australia. Takarada et al. have shown that this plant inhibits the adhesion of *S.mutans* and *P. gingivalis* to the teeth.¹⁴ Tea tree oil has been shown to break down bacterial permeability of the bacteria leading to lysis, which leads to ion entry into bacteria and subsequent degradation of metabolic activity. All of these effects confirm that tea tree oil has endangered the structural and functional integrity of bacterial membranes.¹⁵ The toothpaste No.10 and No.11 contains theobromine which is an alkaloid from the family of methylxanthines containing theophylline and caffeine-like compounds, and its main source is cocoa plant. Amaechi et al. have concluded that theobromine has an enhancing remineralization effect due to the ability of providing apatite formation in comparison with the standard NaF containing toothpaste.¹⁶ Kargül et al. observed that theobromine is effective in preserving the enamel surface in their study.¹⁷ However, we need more researches to make a definite statement about the antibacterial effect of theobromine.

Although Toothpaste No.9 (Theodent Kids) also contains theobromine, there was no antibacterial effect detected in our study. Therefore, we may attribute the antibacterial effect of the other 2 theobromine containing toothpastes (No. 10-Theodent 300 and No. 11-Theodent) to their sodium lauryl sarcosinate content.

In our study, no inhibition zone was observed after 48 hours against toothpastes No.3 (Natural Ozonized Toothpaste), No.4 (Dentasave Zinc Toothpaste), No 6 (Buccotherm Toothpaste), No.8 (Naturalive Toothpaste) and No.9 (Theodent Kids toothpaste). For these toothpastes, within 48 hours, the initial antibacterial effect decreased and the bacteria were able to recolonize on the surface. We think that this result might indicate the inadequate amount and concentration of the antimicrobial agents in those commercial products.

Toothpaste No.3 (Natural ozone toothpaste) contains natural ingredients such as ozone, miswak, propolis, sage, chamomile, yarrow, yellow cantorrhea extract which are mostly used for their antibacterial effect. Many seeds, plants species and animal products are reported to have antimicrobial effect. However, establishing standard concentration and long lasting effect in a manufactured product is difficult. Similarly, ozone is used in a wide range of areas in dentistry mostly as a gas, such as treating gingivitis, periodontitis, hypersensitivity, abscess, herpes infection, stomatitis-candidiasis root canal treatment, tooth whitening, wound healing and so on.¹⁸ Polydouro et al. investigated the antibacterial effect of the HealOzone (KaVo) device, which produces ozone gas.¹⁹ As a result, they found that *S. mutans* decreased significantly in ozone-applied cavity models, but they did not observe a total bacterial elimination, and only ozone use was found to be insufficient for antibacterial effect. The antibacterial effect of ozone varies depending on whether it is in the form of gas or water, the concentration of bacteria, the duration of application, the environment in which the bacteria are present.²⁰ The concentration of ozone form in the toothpaste used in this in vitro study has not been specified by the manufacturer.

Toothpaste No.6 (Buccotherm toothpaste) contains Castéra-Verduzan natural thermal spring water as its main ingredient. The manufacturer has presented this thermal water to the market to be used in oral hygiene, intraoral injuries and oral mucous membrane problems, claiming that it will heal various conditions. Alpoz et al. examined the therapeutic effect of Buccotherm oral spray on the symptoms of patients with xerostomia.²¹ As a result of this study it was reported that mouth spray was not more effective than placebo in terms of difficulty in speaking, swallowing and chewing. Also, in our study there was no antibacterial activity against *S. mutans*.

Zinc compounds, especially zinc citrate, are added to toothpastes as an antiplaque agent as it prevents bacterial growth and plaque formation. For this purpose, zinc citrate and triclosan are used together in toothpaste formulas. Because zinc compounds are absorbed in dental plaque and then released into the saliva, they can be used safely.²² Moran et al. reported that the zinc citrate formulation of toothpaste had the potential to improve oral health by both inhibiting plaque formation and reducing gingival inflammation and further investigations.²³ However, according to our in vitro study, we found that the antibacterial effect of toothpaste No.4 (Dentasave Zinc toothpaste) which contains zinc citrate compound, is not different from negative control group.

CONCLUSION

The efficiency of natural or chemical antibacterial agents used in toothpaste formulations is controversial. Further research efforts are needed to investigate new efficient natural or chemical contents for toothpastes.

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