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Comparative Evaluation of the Antimicrobial Potential of Different Toothpastes

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Abstract

Aim: The aim of this study was to compare the in vitro antimicrobial activity of toothpastes with different brands and contents, on oral pathogens. **Method:** The antimicrobial activity of three toothpastes with different contents: TTO toothpaste, Parodontax® and Theramed, on *S.mutans*, *L.acidopphilus* and *C.albicans* were investigated by the agar well diffusion test. Colgate Total® was served as the positive control. The toothpastes were diluted with distilled water and placed into the prepared wells on the agar plates which were inoculated with the test microorganisms. After incubation periods of 24 hours and 48 hours, the zones of the growth inhibition were observed and measured. Data were analyzed statistically by one-way ANOVA, Tukey and Paired sample t-test. **Results:** The results after 24 hours and 48 hours showed that, Theramed, Parodontax and Control group produced inhibition zones against *S. mutans* and *C.albicans*. But, TTO toothpaste showed no observable inhibition zones against *S. mutans* and *C.albicans*. After 48 hours, regarding *L. acidophilus*, the most effective toothpaste was Theramed. TTO toothpaste and Parodontax were significantly less effective than Theramed against *L. acidophilus (P<0.05).***Conclusion:** Compared with Colgate Total, Parodontax and Theramed, TTO toothpaste offered low antibacterial efficiency on tested microorganisms.

Key words: Toothpaste, antimicrobial, microorganism

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Amaç: Bu çalışmanın amacı farklı içeriklerdeki üç diş macununun *S.mutans, L.acidopphilus* ve *C.albicans'*e karşı antimikrobiyal aktivitelerini in vitro olarak karşılaştırmaktır. **Gereç ve Yöntem:** Çalışmada üç farklı diş macununun (TTO diş macunu, Parodontax ve Theramed) antimikrobiyal aktiviteleri karşılaştırılmış, Colgate Total pozitif kontrol grubu olarak kullanılmıştır. Agar difüzyon yöntemi kullanılan çalışmada, diş macunları distile su ile dilue edilmiş ve test mikroorganizmaları ile inoküle edilen agar üzerindeki çukurlara yerleştirilmiştir. 24 ve 48 saat sonra çukurlar etrafında oluşan inhibisyon zonları ölçülmüştür. Veriler tek yönlü varyans analizi (ANOVA), Tukey ve Paired sample t-test kullanılarak karşılaştırılmıştır. **Bulgular:** 24 ve 48 saat sonuçlarına göre Theramed, Parodontax ve kontrol grubunda *S. mutans* ve *C.albicans'a* karşı inhibisyon zonu oluşmuştur**.** Fakat TTO diş macunu *S. mutans* ve *C.albicans*'a karşı gözlenebilir inhibisyon zonu oluşturmamıştır. 48 saat sonra, Theramed'in *L. Acidophilus'a* karşı en etkili diş macunu olduğu gözlemlenmiştir. TTO diş macunu ve Parodontax *L. Acidophilus'a karşı* Theramed'den anlamlı olarak daha az etkili olduğu belirlenmiştir *(P<0.05).* **Sonuç:** TTO toothpaste; Parodontax, Theramed ve Colgate Total ile karşılaştırıldığında test mikroorganizmalarına karşı daha düşük antibakteriyel etki gösterdiği belirlenmiştir.

Anahtar kelimeler: Dişmacunu, antimikrobiyal, mikroorganizma

Introduction

Dental caries is a disease of the hard tissues of the teeth caused by an imbalance in the interactions between fermentable carbohydrates and cariogenic bacteria in dental plaque[1]. *S. mutans*, one of the main opportunist microorganism of dental caries; metabolizes sucrose in a peculiar way, producing an extra cellular adhesive polysaccharide (dextran), a sticky insoluble glucan which promotes the firm adherence of the organisms to the tooth surface contribute the formation of dental plaque, subsequently leads to localized decalcification of the enamel surface. In addition, Lactobacillus species are thought to be important pathogens in active caries lesions, *Lactobacillus acidopphilus* is the most common among of these[1] and *Candida albicans* is the most commonly isolated candida specie which is responsible for oral fungal infections[2].

In the absence of good oral hygiene, pathogen microorganisms in plaque initiate and progress dental caries. Elimination of microbial dental plaque biofilm decreases number of bacteria and prevents dental disease[3]. Toothbrushing with toothpaste is commonly practiced form of oral hygiene that can prevent the degradation of tooth enamel[1]. In addition to the mechanical plaque removal capacity, toothpastes contain fluoride and a wide range of chemicals (abrasives, detergents, flavourings, preservatives and humectants) for producing an inhibitory effect on plaque formation[4]. The success of any toothpaste, in part, lies on its ability to eliminate pathogenic oral microflora[5]. The benefits of introducing antibacterial agents in oral hygiene products have been researched in recent years[6]. It was reported that Aloe vera tooth gel and the toothpastes were effective against Candida albicans, Streptococcus mutans, Lactobacillus acidophilus. It was suggusted that Triphala, a herbal nature mixture, has significant antimicrobial activity and thus can be employed as an effective antiplaque agent and can be used in the prevention of dental caries.

One of the toothpaste is Theramed (Schwarzkopf & Henkel) which contains sodium fluoride, zinc sulfate and the chemicals particularly used in toothpastes. It has been claimed to provide triple protection against cavities, gingivitis and also plaque. There is no study about its antibacterial effect on the active substances on dental plaque and cariogenic microorganisms in the literature so far.

Toothpastes including natural and herbal contents are becoming very popular nowadays[1]. Parodontax® (GlaxoSmithKline, Middlesex, United Kingdom), one of the herbal dental products, has been met with great interest. The manufacturers claim that Parodontax® inhibits inflammation and gum bleeding, prevents further damage to gums, reduces plaque because of its natural herbs content. It contains sodium bicarbonate, sodium fluoride and herbal contents: peppermint oil, which has analgesic, anti-septic and anti-inflammatory properties; chamomile, which provides anti-inflammatory properties and decreases gingival inflammation; echinacea, which is supposed to stimulate the immune response; sage and rhatany, which have anti-hemorrhagic properties; myrrh, claimed to be a natural anti-septic[4]. Yankell et al.[7] reported that the herbal toothpaste Parodontax® inhibited plaque bacteria in an in vitro experiment.

Recently, a new Tea Tree Oil (TTO) based toothpaste is commercially available. TTO toothpaste (Dr.Müller Pharma s.r.o., Czech Republic) is composed of sodium fluoride and Melaleuca Alternifolia. According to the manufacturer' claim, the paste has antibacterial and antifungal effects due to the Melaleuca Alternifolia content. The essential oil of the Melaleuca Alternifolia, known also as Tea Tree Oil, has been used medicinally for more than 70 years[8]. It also has antibacterial, antiviral and antifungal properties[9]. In vitro studies have confirmed the antimicrobial effect of Tea Tree Oil[8,10]. But there is no such study evaluating antimicrobial effect of TTO toothpaste in the literature so far.

The aim of this study was to compare the in vitro antimicrobial activity of TTO toothpaste (Dr.Müller Pharma s.r.o., Czech Republic), Parodontax® (GlaxoSmithKline, Middlesex, United Kingdom) and Theramed (Schwarzkopf & Henkel, Germany) toothpastes, using Colgate Total® (Colgate-Palmolive, New York City) as a positive control group (2, 3) on *Streptococcus mutans (S. mutans)*, *Lactobacillus acidopphilus (L. acidopphilus)* and *Candida Albicans (C.albicans).*

Materials and Methods

Three different toothpastes were tested in this study; TTO toothpaste (Dr.Müller Pharma s.r.o., Czech Republic), Parodontax® (GlaxoSmithKline, Middlesex, United Kingdom) and Theramed (Schwarzkopf & Henkel, Germany) The composition of these toothpastes are given in Table 1. Colgate Total® (Colgate-Palmolive, New York City) was used as the positive control.

Antibacterial activities of the toothpastes were evaluated against the *Streptococcus mutans* (RSKK 676), *Lactobacillus acidopphilus* (ATCC 11975) and *Candida Albicans* (ATCC 18804) using Agar Diffusion Test (double layer agar-well technique). The strains were obtained from Refik Saydam Central National Institute of Health, Ankara, Turkey and Kirikkale University Faculty of Medicine Department of Microbiology, Kirikkale, Turkey. Standard stock solutions of each toothpaste were prepared. 3 g of each toothpaste were dissolved in 10 ml sterile distilled water to give standard stock solution, respectively. Petri dishes previously sterilized were prepared containing Muller-Hinton agar. Inocula from a 24 hour growth of the test organisms were added in sterile saline, incubated at 37 ºC, and allowed to grow to obtain a turbidity equivalent to the 0.5 McFarland Standard. 5mm diameter, 5mm depth wells were made by removal of agar at equidistant points using sterile pipette tip, and these were filled immediately with 100 µl of the stock solution of toothpastes. After the toothpastes setting, S.mutans, L.acidopphilus and C.albicans were incubated at 37 ºC for 24 hours, under microaerophilic conditions (BD BBL GasPak™ Plus Anaerobic System Envelopes with Palladium Catalyst, Becton, Dickinson and Company, USA) to allow the microorganisms to grow and reagents to diffuse through the culture medium. After incubation, if antimicrobial activity was present on the plates, it was indicated by an inhibition zone surrounding the

wells containing toothpastes. The diameter of each inhibition zone (mm) was measured two times with a millimeter ruler and recorded at 24 hour and 48 hour intervals by only one observer. For each toothpaste tested, the experiment was repeated three

Name of toothpaste	Ingredients as given on package
TTO toothpaste (Dr.Müller Pharma s.r.o., Czech Republic)	Sorbitol,Glycerin, Silica, Aqua, Propylene glycol, Olafur, PEG-8, Sodium lauryl sulfate, Hydroxyethylcellulose, Aroma, Melaleuca Alternifolia (Terpinen-4-ol), Titanium dioxide, Methylparaben, Propylparaben, Saccarin, 0,125 % fluoride.
Parodontax® (GlaxoSmith Kline, Middlesex, United Kingdom)	Sodium bicarbonate, aqua, glycerin, cocamidopropyl betadine, alcohol, krameria triandra extract, echinacea purpurea juice, xanthan gum, chamomilla recutita extract, commiphora myrrha extract, sodium fluoride, sodium saccharin, sodium benzoate, saliva officinalis oil, mentha piperita oil, mentha arvensis oil, limonene, CL 77491
Theramed (Schwarzkopf & Henkel, Germany)	Sorbitol, Aqua, Hydrated Silica, Alcohol, PEG-32, Sodium Lauryl Sulfate, Aroma, Sodium Fluoride, PEG-30 Glyceryl Stearate, Xanthan Gum, Disodium Phosphate, Sodium Saccharin, Cocamidopropyl Bentaine, Zinc Sulfate. Sodium Benzoate, Cl 42090, Contains Sodium Fluoride (1450 ppm F).
Colgate Total ® (Colgate-Palmolive, New York City)	Sodium Fluoride 0.24% (0.14% W/V Fluoride Ion); Triclosan 0.30%; Water; Hydrated Silica; Glycerin; Sorbitol; PVM/MA Copolymer; Sodium Lauryl Sulfate; Cellulose Gum; Flavor; Sodium Hydroxide; Propylene Glycol; Carrageenan; Sodium Saccharin; Titanium Dioxide

Table 1. Used toothpastes.

times in duplicate. From the values obtained, the average value and standart deviation were evaluated (Table 2). Data collected were statistically analyzed by comparing the means of the zones of inhibition for each toothpaste using one-way analysis of variance (ANOVA). Therefore, we used a multiple comparison procedure known as Tukey test and Paired sample t-test to determine the significance of differences among the means at a level of significance of 5% ($P<0.05$).

Results

The mean diameter of inhibition zones (millimeters) of the tested toothpastes and control group against the test microorganisms are shown in Table 2. Only *L.acidopphilus* did not show microbial growth at 24 hours, but at 48 hours microbial growth and well-defined inhibition zones was observed for all test microorganisms. The positive control produced significantly sized inhibition zones for all three microorganisms at 24 hours and 48 hours. ($P<0.05$)

In plates of *S.mutans*, Parodontax® showed almost the same inhibitory effect with Control group at 24 hours. The difference between Parodontax® and control group was not statistically significant. ($P>0.05$) Theramed produced significantly smaller inhibition zones then positive control and Parodontax®.($P<0.05$) At 48 hours Control group produced significantly larger inhibition zones than Parodontax® and Parodontax® produced significantly larger inhibition zones than Theramed.($P<0.05$) TTO toothpaste has shown no inhibitory effect on *S.mutans* at both 24 hours and 48 hours.

All of the tested toothpastes demonstrated inhibitory effect against *L.acidopphilus* at 48 hours. Theramed was more effective than TTO toothpaste and Parodontax® was less effective than TTO toothpaste. ($P<0.05$) Theramed showed the same inhibitory effect with Control group.

In plates of *C.albicans*, TTO toothpaste did not produce inhibition zones. Parodontax® was less effective than Control group, more effective than Theramed at 24 hours. ($P<0.05$) At 48 hours, Parodontax® produced larger inhibition zones than Theramed and Control group but the difference was only significant with the Theramed group ($P<0.05$).

Among the tested 3 toothpastes, the antimicrobial activity at 24 hours and 48 hours were not statistically different. ($P>0.05$). In the Control group, only antimicrobial activity on *C.albicans* at 24 hours was significantly larger than 48 hours ($P<0.05$).

Discussion

The purpose of the present study was to evaluate the antibacterial properties of different toothpastes, with comparable to a control group (Colgate Total ®). *S. Mutans*, *L.acidopphilus* and *C.albicans* were chosen as test microorganisms, because these microorganisms play a major role in dental diseases. *S.mutans* is associated with the initial phase of human dental caries which plays an important role in fermenting carbohydrates that results in acid production, leading to the destruction of tooth tissue[11]. Lactobacillus species are also associated with active caries lesions and

Lactobacillus acidopphilus is the most common among of these[2]. *Candida albicans* is an opportunistic pathogen present in about 50-60% of the healthy human population[12]. It is the most common yeast isolated from the oral cavity which

Toothpastes and Controls	Mean diameter (mm)* and standart deviation (SD) of inhibition zones					
	Streptococcus mutans		Lactobacillus acidopphilus		Candida Albicans	
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
Positive Control (Colgate Total ®)	$28,3^{\S} \pm 2,88$	$27 \pm 1,73$	(-)	$25^{\dagger} \pm 0$	25 ± 0	$21,3^{\ddagger} \pm 1,15$
TTO toothpaste	0	0	(-)	$17,3 \pm 0,57$	0	0
Parodontax®	$23,7^{\S} \pm 1,15$	$23,7 \pm 1,15$	(-)	12 ± 0	$22,3 \pm 0,57$	$22,7^{\ddagger} \pm 0,57$
Theramed	$18,3 \pm 2,88$	$18,7 \pm 1,15$	(-)	$25^{\dagger} \pm 1,15$	$13 \pm 1,73$	$13 \pm 1,73$

Table 2. Toothpastes and corresponding inhibition zones. **(-)** no observed microbial growth. ***** Each value is the mean of 3 measurement
For each time period and each bacterial strain, data with the same superscript letters are not statistically different (p>0.05) and data without any superscript letters are significantly different from all others (p<0.05).

is associated with oral fungal infections[2]. It becomes pathogenic when the host immune defence is undermined such as in HIV infection[12]. Tested toothpastes' ability to inhibit these microorganisms, provides us to evaluate which can be used in different dental diseases correctly. TTO toothpaste has no inhibition properties against *C.albicans*. So, a practitioner may not recommend this toothpaste for a patient who is susceptible to oral fungal infections.

The agar well diffusion testing method was employed to establish the minimum inhibitory concentrations of toothpastes on the tested microorganisms. This method is the most commonly employed technique for evaluation of antimicrobial activity because the variables are easy to control[13]. It has been used by many authors in antimicrobial studies[1,11,13].

Tooth brushing with a toothpaste is the most widely and most effective method of removing dental plaque[4]. In addition to this, Mandel suggested that toothpaste should include antimicrobial contents to enhance plaque control[14]. Fluoride, metal ions, essential oils, amine fluoride/stannous fluoride and triclosan used particularly in toothpastes, have antibacterial effects[15]. One of them triclosan, is a broad spectrum antimicrobial agent and currently used in numerous oral care products. Triclosan is non-ionic, so it is compatible with toothpaste ingredients and retained in the mouth. In order to increase retention in the oral cavity triclosan has been tested in conjunction with additional substances i.e zinc citrate and PWM/MA (polyvinylmethylether maleic acid)[16]. Nabi et al.[17] found a linear increase in the retention of triclosan with increasing concentration of the copolymer PVM-MA**.** Thus, the antimicrobial activity of Colgate Total ® used as a standard[1,2] on tested microorganisms could depend on fluoride and triclosan combined with a PVM/MA copolymer contents. Colgate Total ® produced significantly sized inhibition zones on *S.mutans* at 48 hours, on *C.albicans* at 24 hours in this study.(*P<0.05*) Sodium lauryl sulfate although included in toothpastes formulations for its detergent properties, an antibacterial effect can also be attributed to this detergent18. Theramed showed the same antimicrobial activity with Colgate Total ® against *L.acidopphilus*, it was not statistically different.(*P>0.05*) Because of zinc sulfate (metal ion), fluoride and sodium lauryl sulfate contents, Theramed may have shown antimicrobial activity.

Tea tree oil, has broad-spectrum antimicrobial and anti-inflammatory activity in vitro[12]. The most important active component of essential oil are; terpinen-4-ol and 1,8-cineole. The antimicrobial activity of TTO is attribute mainly to terpinen-4-ol and anti-inflamatuar activity is attribute mainly 1,8-cinelone. Terpinen-4-ol enters microorganism cell membranes and acts against its structural permeability. Its antimicrobial effect is explained by this mechanism[9]. In vitro studies have confirmed, the antimicrobial effect of Tea Tree Oil at %0.5 concentration against various microorganisms such as our tested microorganisms; *S. mutans*, *L. acidopphilus* and *C. Albicans* [18,10]. In the present study, TTO toothpaste with %0.5 Melaleuca Alternifolia concentration showed antimicrobial effect against only *L.acidopphilus*. However, the complexity of toothpastes, with their different constituents (abrasives, detergents, flavourings, preservatives and humectants), may lead to mutual inactivation. For instance, an antimicrobial agent, chlorhexidine, can be inactivated by the detergent sodium lauryl sulphate, which is an ingredient in many toothpastes. So that the effectiveness of a specific single material in a complex toothpaste mixture can be difficult to prove[19]. TTO toothpaste has not shown inhibitory

effect on *S.mutans* and *C.albicans* in this study. It can be explained terpinen-4-ol interaction with other components of the toothpaste formulation. Further studies will be useful for evaluating the antimicrobial properties of TTO containing toothpastes.

Herbal toothpastes contain extracts of several plants. These substances are responsible for different functions as; terpenoids give plants their odors, quinoned and tannis are responsible for plant pigment, etc. Major classes of antimicrobial compounds (phytochemicals) from plant extracts are phenolics, terpenoids and essential oils, alkaloids, lectins and polypeptides, polyacetylenes[20]. According to Palombo's review[21], plant extracts, essential oils and purified phytochemicals have the potential to be preventative or treatment therapy agents for oral diseases. Based on this review and the present study, Parodontax® could be identified as a safe and effective antimicrobial toothpaste.

Conclusion

Compared with Parodontax® and Theramed, TTO toothpaste offered low antibacterial advantages on tested microorganisms. But, further studies will be useful for evaluating the antimicrobial properties of TTO containing toothpastes.

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