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EFFECT OF VINEGARS ON *AGGREGATIBACTER ACTINOMYCETEMCOMITANS* AND *PREVOTELLA INTERMEDIA* IN VITRO

SİRKELERİN IN VITRO ORTAMDA AGREGATIBACTER ACTINOMYCETEMCOMITANS VE PREVOTELLA INTERMEDIA ÜZERİNDEKİ ETKİSİ

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Abstract

Objective: The aim of this study was to evaluate the antibacterial effects of apple and grape vinegars against two of the periodontal pathogens that is *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia.*
Methods: The antibacterial activity of apple and grape vinegars on anaerobic oral pathogens was determined by agar disc diffusion method. CHX gluconate was used as a positive control. Bacterial plantings were repeated three times in the same microorganisms for vinegar and CHX gluconate. The differences between the average results were determined by variance analysis (ANOVA) and the mean values were compared with the Tukey test.
Results: In the *P. intermedia* both apples (2 cm) and grape vinegar (1.9 cm) showed higher antibacterial activity than CHX gluconate (1.6 cm). In *A. actinomycetemcomitans*, 2% CHX gluconate showed higher antibacterial activity, but its antibacterial activity was reduced compared to of 1% CHX gluconate.
Conclusion: Apple and grape vinegars can be used as an alternative to chlorhexidine for antibacterial effect to *A. actinomycetemcomitans* and *P. intermedia.*

Keywords: *Antibacterial activity, oral pathogens, Prevotella intermedia, Aggregatibacter actinomycescomitans, apple and grape vinegar*

Öz

Amaç: Bu çalışmanın amacı, elma ve üzüm sirkelerinin, iki periodontal patojen olan *Aggregatibacter actinomycetemcomitans* ve *Prevotella intermedia*'ya karşı antibakteriyel etkilerini değerlendirmektir.
Yöntem: Elma ve üzüm sirkelerinin anaerobik oral patojenler üzerindeki antibakteriyel aktivitesi agar disk difüzyon yöntemi ile belirlendi. Pozitif kontrol olarak CHX glukonat kullanıldı. Bakteri dikimleri sirke ve CHX glukonat için aynı mikroorganizmalarda üç kez tekrarlandı. Ortalama sonuçlar arasındaki farklar varyans analizi (ANOVA) ile belirlenmiş ve ortalama değerler Tukey testi ile karşılaştırıldı.
Bulgular: *P. intermedia*'da hem elma (2 cm) hem de üzüm sirkesi (1,9 cm), CHX glukonattan (1,6 cm) daha yüksek antibakteriyel aktivite gösterdi. *A.actinomycetemcomitans*'ta, %2 CHX glukonat daha yüksek antibakteriyel aktivite gösterdi, ancak antibakteriyel aktivitesi %1 CHX glukonata kıyasla azaldı.
Sonuç: Elma ve üzüm sirkeleri, *A. actinomycetemcomitans* ve *P. intermedia*'ya antibakteriyel etki için klorheksidine alternatif olarak kullanılabilir.

Anahtar Kelimeler: *Antibakteriyel aktivite, oral patojenler, Prevotella intermedia, Aggregatibacter actinomycescomitans, elma ve üzüm sirkesi*

Introduction

Dental plaque, a biofilm, which usually adheres over the tooth surface, is the common cause of periodontal diseases.[1] The onset or progression of periodontal disease can be controlled by regular plaque control practices.[2] Nowadays, various commercially produced mouthwashes are used as both antimicrobial and antiplaque agents for the above-mentioned purpose.[3] Chlorhexidine (CHX) gluconate, a cationic bisbiguanide, is a broad spectrum antimicrobial agent. CHX gluconate may continue to be effective even several hours after oral rinsing due to the reversible binding properties of the contained bisbiguanide to the surfaces within the mouth. In addition, despite some side effects such as more staining and calculus formation than other mouthwashes, it is also recommended as the most effective anti-plaque agent.[4,5]

Vinegar is a liquid compound which is fermented from various fruits such as apple and grape and is dominated by acetic acid. The history of the vinegar use dates back to the 5000 years ago B.C. Vinegar is produced worldwide and is frequently used in daily consumption in foods to increase flavor. Vinegar has several functional therapeutic features such as antioxidant activity, blood pressure reduction, increased vigor after exercise and weight loss.[6] In addition, many studies have reported antibacterial properties of vinegar against several bacteria.[7,8]

CHX gluconate is considered the gold standard for preventing plaque accumulation, but many studies have shown that CHX gluconate has negative effects on human cells as well as the above-mentioned side effect.[9,10] For this purpose, studies related to various antimicrobial agents remain current. To the best of our knowledge, there is no study on the effect of vinegar on periodontal pathogens. The aim of this study was to evaluate the antibacterial effects of apple and grape vinegars against two of the periodontal pathogens that is *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* and comparing them with CHX gluconate.

Materials and Methods

Strains and Cultivation

Aggregatibacter actinomycetemcomitans (DSM 11123) and *Prevotella intermedia* (DSM 20706) bacterial strains were selected for antimicrobial testing of apple and grape vinegar. These strains were purchased commercially providers in lyophilized form DSMZ, Braunschweig, Germany.

P. intermedia, one of the periodontal pathogens used in the study, was cultured by adding 10% sheep blood, 10% vitamin K and hemin solution to fastidious anaerob agar medium and incubating in an anaerobic cabinet at 37 °C for 5-7 days. *A. actinomycetemcomitans* was cultured into baccarrasin vancomycin agar including 10% horse serum, 10% vitamin K and hemin solution prepared by adding tryptic soy serum and incubated at, 10% CO_2 incubator for 4-5 days.

Vinegar materials

Organic grape and apple vinegars (Kemal Kukrer AS, Turkey) purchased commercially from Ordu province markets were used.

Well diffusion method

The antibacterial activity of apple and grape vinegars on anaerobic oral pathogens was determined by agar disc diffusion method. According to this method, 6 mm diameter and 2 mm deep wells on the bloody agar prepared with 5% defibrinated sheep blood were drilled. In addition, in each petri dish containing the wells with same diameter and same depth, were opened for the control group. Then the prepared bacteria suspensions were dripped on the medium with the help of swabs. After that, 50 µl vinegar was placed in the wells opened on the solid media. Then, *P. intermedia* pathogens were incubated in anaerobic cabinet at 37 °C for 5-7 days and *A. actinomycetemcomitans* were incubated in a 10% CO2 incubator for 24-48 hours. As a positive control 2% CHX gluconate was used. The diameters of inhibition zones against pathogens were measured and bacterial growth was evaluated. Bacterial cultivations were repeated three times in the same microorganisms for vinegar and CHX gluconate. As a result, the inhibition zone diameter was measured three times for each and the mean values were calculated.

Statistical analysis

Statistical analyzes were performed using SPSS 20.0 (IBM, Armonk, NY, USA) statistics program. The differences between the average results were determined by variance analysis (ANOVA) and the mean values were compared with the Tukey test. Statistical significance was accepted as $p<0.05$.

Results

The antimicrobial properties of vinegars were evaluated by comparing the zone diameters of CHX gluconate. Dilution of CHX-g are also given in Table 1.

Table 1. Inhibition zones (cm) of vinegars and chlorohexidine against periodontal pathogenic bacteria *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia*

	Zone Diameter Average (cm) by Well Diffusion Method	
Tested agents	***A. actinomycetemcomitans***	***P. intermedia***
Apple vinegar	1.7±0.20	2.0±0.24
Grape vinegar	1.8±0.24	1.9±0.23
2% CHX	1.9±0.27	1.6±0.18
1% CHX	1.6±0.19	1.2±0.13

In our study, it was determined that pure apple and grape vinegars (1:1) exhibit antibacterial activity against *P. intermedia* and *A. actinomycetemcomitans* periodontal pathogens in agar well diffusion method (Table 1). It has been determined that apple cider vinegar against *P. intermedia* pathogens exhibit stronger antibacterial activity than CHX gluconate which is positive control with 2 cm zone diameter. In fact, grape vinegar showed higher antibacterial activity than CHX gluconate against *P. gingivalis* and *A. actinomycetemcomitans.* No significant difference was found between the diameters of *P. intermedia* and *A. actinomycetemcomitans* and the zone diameters of CHX ($p > 0.05$). The antimicrobial effects of 2% CHX gluconate, grape vinegar, apple vinegar against *A. actinomycetemcomitans* were determined, respectively. Apple vinegar, grape vinegar, and 1% or 2% CHX gluconate indicated antimicrobial activity against *P. intermedia*. In *P.*

intermedia, grape and apple vinegars show a stronger antibacterial effect than CHX gluconate, which is a powerful antimicrobial mouthwash. The antibacterial properties of 1% CHX gluconate compared to the vinegars were relatively low ($p < 0.05$) (Table 1).

Discussion

In order to reduce the number of microorganisms to prevent oral infections, dental disinfection is needed and various gargles are used for this purpose.[11-13] However, mouthwashing solutions may not be reached immediately or always at hand, and instead it is thought that vinegars, which are usually found in every household, can be used. Because the antibacterial property of vinegars has been known for many years. For this purpose, antibacterial activity of apple and grape vinegar against *P. intermedia* and *A. actinomycetemcomitans*, which are the causative agents of periodontal diseases such as periodontitis and gingivitis, were investigated. In our study, the antibacterial property was determined by measuring the inhibition zones formed by CHX, which is a positive control with apple and grape vinegars on anaerobic bacteria. In *A. actinomycetemcomitans*, 2% CHX gluconate and in *P. intermedia* apple vinegar for demonstrated the most antibacterial activity. For, *A. actinomycetemcomitans* 2% CHX gluconate and apple vinegar demonstrated the highest antibacterial effect and this effect was found identicall (P>0.05). When we compare the antibacterial effect of apple and grape vinegars against oral pathogens, grape vinegar for *A. actinomycetemcomitans* is more effective than apple vinegar and vice versa for *P. intermedia*. In generally, the antibacterial effects of apple and grape vinegar were almost the same. The results of our study supports the study done by Kılınç and Yavuz in 2011. The authors did not perform statistical analysis in this study.The researchers investigated the effect of trout fillets stored in apple cider vinegar and grape vinegar on the bacteria growth and shelf life of trout fillets. In their study, they reported that they were effective antimicrobial agents for trout fillets that were dipped in grape and apple vinegars at different times (30 sec., 10 min. and 30 min.). Trout fillet in apple vinegar increased its shelf life by 2 days. The shelf life of the trout fillets, which are kept for 30 sec., 10 min. and 30 min in the grape vinegar, is extended to 10 days. Shelf life was increased by 4 days compared to the control group. In addition, grape vinegar has been found to be more effective than apple vinegar.[14] Similar results were obtained in our study and grape vinegar was more effective than apple vinegar. In Raj et al. study, the effect of vinegar, lime and salt water was shown as they were potential as other home decontamines for toothbrushes. They reported a statistically significant result for decontamination of vinegar group toothbrushes compared to other test and control agents.[15] Again, in previous studies, it was mentioned that the vinegars with acetic acid content of 4-5% were used in dentistry because they were natural product and cheap and effective in removing the tooth stone.[16] A group of researchers evaluated the use of vinegar as a disinfectant of human teeth extracted for dental education. Extracted teeth were placed into nutrient broth and investigated for microbial growth by the evaluation of turbidity at the end of the 48-hour incubation period. In the teeth put into the nutrient broth, vinegars was completely effective in sterilization and it was statistically significant.[17]
In our study, two different statistical evaluations were made. In the first statistic, it is evaluated whether there is a difference between bacteria. In the second statistic, it is evaluated whether the effect is different according to the variety of vinegar. There is no significant difference in terms of bacteria and vinegar varieties. However, there is a significant difference compared to CHX. Table 1 shows this effect by giving the zone diameters.
There may be several reasons why the antibacterial activity of apple and grape vinegar on oral pathogens is different. One of them is the acetic acid content, the most important quality criterion of vinegars. Acetic acid usually lowers the pH of the medium. In addition, it has been found that acetic acid pass through the cell wall to penetrate the cell and to denature the plasma. Since the antimicrobial effect of acetic acid is realized with its non-dissociated molecules, the effect of acetic acid increases as the pH of the environment decreases. Acetic acid has more antimicrobial action against bacteria.[18]

Conclusion

It is found that the antibacterial activity of chlorhexidine gluconate, which is a good mouthwash in dentistry, is not so high compared to apple and grape vinegars. Thus, İs is our opinion that apple and grape vinegar available in markets may be used instead of chlorhexidine gluconate. In order to be used for mouth and teeth cleaning such as mouthwash for vinegars, the procedures of mouthwashes can also be applied exactly. Briefly, the mouth can be rinsed after taking some vinegar in the mouth and waiting for a while (about 30 seconds of gargling time). For those who use the prosthesis, an entire prosthesis can be immersed in a container filled with vinegar.

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Conflict of Interest
None.

Compliance with Ethical Statement
Ethics committee was not taken as it was an *in vitro* study.

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None.

Author Contributions
YMC: Project development, manuscript writing, DY: Project development, manuscript writing, data analysis, ÖM: Data collection, data analysis.

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