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Effectiveness of calcium phosphate and plant extract mixtures as natural dentifrices

Kalsiyum fosfat ve bitki özüt karışımlarının diş macununda etkinlikleri

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Effectiveness of Calcium Phosphate and Plant Extract Mixtures as Natural Dentifrices

Highlights

- *An optimum weight fraction of hydroxyapatite and monetite particles around 1/3 was found to balance abrasiveness and remineralization capacity of the mineral phase*
- *Sintered hydroxyapatite with a density of 65% and hardness of 48 HV served as a reproducible artificial tooth*
- *Brushing the artificial tooth with calcium phosphate pastes significantly inhibited hardness reduction in Lactobacillus casei culture, presumably by effective remineralization*
- *Plant extracts from Aegean flora all inhibited Streptococcus mutans growth within the studied concentration range of 0.008-0.25 g/ml , however fig leaf, juniper fruit and blueberry leaf extracts did not yield minimum bacteriocidal concentrations*
- *Blueberry leaf and geranium leaf extracts were significantly effective against Lactobacillus casei activity at their minimum inhibitory concentrations obtained for Streptoccoccus mutans*

Graphical Abstract

Effective compositions of hydroxyapatite/monetite mixtures as abrasives and plant extracts from Aegean flora were determined based on mechanical and biological tests using two cell line cultures present in the oral environment.

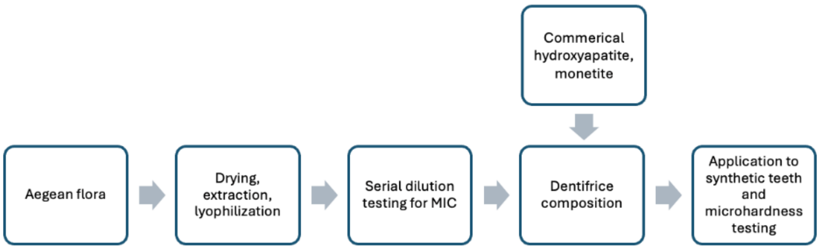

Figure. 1

Aim

Abrasiveness, remineralization and antibacterial functions of an aqueous paste were aimed to be improved by incorporation of various compositions of calcium phosphates and plant extracts.

Design & Methodology

Studied compositions were ranked based on their inhibition capacity for hardness reduction on the surface of artificial teeth immersed in Lactobacillus casei culture. Antibacterial activity of plant extracts were determined by serial dilution tests using Streptococcus mutans culture.

Originality

Different physico-chemical properties of calcium phosphates that are commonly utilized as pure bioceramics result in various abrasiveness and remineralization capacities which are important design criterions for dentifrices. These functions are optimized in this study by studying in situ behavior of mixtures of hydroxyapatite and monetite in a simulated oral environment. The pastes were further improved in terms of antibacterial activity by incorporation of various plant extracts with little economical value.

Findings

A biphasic mixture of hydroxyapatite and monetite is found as a more effective mineral phase compared to pure powders. Geranium and blueberry leaf extracts from Aegean flora were found as highly effective against the cell lines studied.

Conclusion

Dentifrices with natural components were prepared and tested to be effective against the degradation of artificial teeth induced by an artificial oral environment. Specifically monetite/hydroxyapatite powders at a weight fraction around 3 provided an effective combination of abrasion and remineralization. Geranium and blueberry leaf extracts were particularly effective in inhibiting the growth of Lactobacillus casei at concentrations of 0.0166 and 0.066g/ml respectively.

Declaration of Ethical Standards

The author of this article declares that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Effectiveness of Calcium Phosphate and Plant Extract Mixtures as Natural Dentifrices

Araştırma Makalesi / Research Article

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ABSTRACT

This study investigates the effects of calcium phosphates and local plant extracts on the oral flora as naturally available ingredients for dentifrices. Effective compositions of two calcium phosphate minerals (hydroxyapatite and monetite) to serve as abrasive particles and extracts of plants (olive, fig, juniper, mastic, geranium, blueberry leaves, pomegranate peel and juniper fruit) obtained from Aegean region to serve as antibacterial agents in the as prepared natural dentifrices were investigated by mechanical (Vickers microhardness) and biological (minimum inhibitory concentrations from UV-spectrophotometry) tests. Within this framework, surface hardness of synthetic hydroxyapatite disks immersed in Lactobacillus casei cell culture was measured before and after periodic brushing with dentifrices containing calcium phosphates and plant extracts. Also in vitro serial dilution test was applied to plant extracts using Streptococcus mutans cell culture to determine their antibacterial activity. The results point to an optimum composition of 3 part monetite to 1 part hydroxyapatite powder mixtures and blueberry or geranium leaf extracts at their minimum inhibitory concentrations for highest hardness, i.e. lowest caries formation.

Keywords: Dentifrice, hydroxyapatite, monetite, plant extract, hardness test, antibacterial test

Kalsiyum Fosfat ve Bitki Özüt Karışımlarının Diş Macununda Etkinlikleri

ÖZ

Bu çalışmada diş macunu formülasyonlarında kullanılmak üzere doğal kaynaklardan kolaylıkla elde edilebilen kalsiyum fosfatların ve lokal bitki özütlerinin ağız florası üzerindeki etkisi incelenmiştir. Doğal hammaddelerle hazırlanan diş macunlarında aşındırıcı mineral olarak kullanılan iki kalsiyum fosfat fazının (hidroksiapatit ve monetit) optimum karışım kompozisyonu ve Ege bölgesinden çeşitli bitkilerin (zeytin, incir, ardıç, sakız, ıtır, yaban mersini yaprakları, nar kabuğu ve ardıç meyvesi) özütlerinin diş macunu antibakteriyel komponenti olarak etken konsantrasyonları mekanik (Vickers mikrosertlik) ve biyolojik (UV-spektrofotometre ile minimum inhibe edici konsantrasyon tespiti) testlerle incelenmiştir. Bu çerçevede ağız ortamını temsil eden Laktobasilus kasei bakteri ortamı içinde tutulan sentetik hidroksiapatit dişlere taşıyıcı macun vasıtası ile periyodik şekilde uygulanan kompozisyonların süreç sonunda diş sertliklerine etkisi belirlenmiştir. Ayrıca bitki özütlerinin yine ağız ortamında bulunan Streptokok mutans bakterisi üzerinde antibakteriyel etkileri seri seyreltme yöntemiyle belirlenmiştir. Sonuç olarak diş sertliği, dolayısıyla çürüme üzerinde en etkili diş macunları 3'e 1 monetit/hidroksiapatit karışımı ile yaban mersini veya ıtır yaprağı özütleri içeren formülasyonlar olarak bulunmuştur.

Anahtar kelimeler: Diş macunu, hidroksiapatit, monetit, bitki özütü, sertlik testi, antibakteriyel test

1. INTRODUCTION

Consumer sensitivity and awareness are increasing in today's world, especially in the field of health products. The growing demand for natural and biocompatible materials necessitates updating the traditional healthcare methods. For example, dentifrices of the early 1900s, containing simple detergent and mineral compositions gave way to dentifrice with enamel-hardening fluoride and new detergents in the 1950s. Recently, harmful chemicals have been replaced with natural plant extracts and minerals compatible with the human body [1-3].

The two main functions of dentifrice are to abrasively remove bacterial formations on the tooth surface and to neutralize bacteria. The primary component used for the former is abrasive minerals. Removing bacterial plaque and dental calculus from tooth surfaces helps prevent cavities and gum diseases. According to clinical trials, the abrasives in dentifrice accelerate plaque removal on the tooth surface by 50% [4]. Research has shown that particles larger than 50 micrometers are not effective in removing plaque. Increasing particle size reduces the surface area necessary for the paste to adhere, leading to clumping and separation during brushing [5, 6]. Similarly, exceeding the optimal particle ratio results in particles remaining inside the brush or swept around instead of transmitting stresses applied on the paste [7]. In addition to removing bacterial plaque, the minerals in dentifrice can also cause enamel erosion by scratching the tooth's enamel layer. Various mineral types, ranging from the hardest alumina to relatively soft calcium phosphates, are used in dentifrice formulations [8].

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Hydroxyapatite (HA), the hardest calcium phosphate (CaP), constitutes 96% of the dense enamel layer. Using a mixture of CaP phases with different hardness levels in dentifrice helps prevent enamel erosion while maintaining effective plaque removal [9]. In this study, the effective composition of CaP phases, which are also utilized for their dissolution and crystallization potentials in bone cements, was investigated in dentifrice. Specifically, the impact of HA, a bone cement product with the highest hardness and lowest solubility, and monetite (dicalcium phosphate anhydrate, DCPA), a product with low hardness and high solubility [10], on synthetic tooth hardness was examined. The remineralization effect of alternative fluoride salts, which create a hard fluorapatite layer on enamel surfaces has been extensively researched [11]. It is hypothesized that enhancing the solubility of CaP minerals with DCPA additive will lead to similar high remineralization and a safer composition compared to fluorides.

Antimicrobial chemicals, minerals, or plant extracts are also used in dentifrices as therapeutic agents. For instance, triclosan, used in dentifrices effective against gum inflammation, is an antibacterial agent also found in soaps and deodorants. It inhibits plaque bacteria by preventing acid, toxin, and enzyme secretion, thus reducing inflammation [12]. Chemicals like zinc oxide and chlorine dioxide used in mouthwash serve to combat bad breath primarily by reducing the amount of sulfate gases produced by anaerobic bacteria in the oral flora [13]. Furthermore, natural polyphenols are generally known to have therapeutic effects on bacterial groups [14].

Throughout history, various societies have effectively used plant-based products such as miswak, wheat husk, and clove oil for dental care. Plants containing polyphenols, which are effective against bacteria in the oral environment, are potential components of dentifrices [15, 16]. Polyphenols are divided into hydrolysable tannins, phenylpropanoids, and condensed tannins. Condensed tannins, which are the most abundant polyphenols, make up almost half of the dry weight of nearly every plant leaf [17]. While tannins serve as a defense mechanism against herbivores in plants, they are also part of decay and nitrogen cycling [18]. Plant extracts containing polyphenols are traditionally obtained through organic solvent extraction. The most commonly used organic solvent, ethanol, can separate polyphenols and other aromatic compounds from dried plant material [19]. In this study, various plants obtained from the flora of the Aegean region in Türkiye were extracted and delivered in dentifrices to investigate their effectiveness as antibacterial agents against oral bacteria. One of the plants studied, the olive leaf, has been found to have antibacterial and antifungal properties in clinical trials, as well as effects on lowering blood pressure and reducing inflammation [20]. In a related study, it has been discovered that the antioxidant capacity of olive leaf is twice that of green tea and four times that of vitamin C [21]. The antioxidant polyphenols it contains help reduce the harmful effects of free radicals in the body [22]. In addition to the well-known benefits of pomegranate fruit and seed, it has also been found that its peel has antimicrobial and antioxidant properties [23]. Pomegranate peel was found to have the highest antioxidant capacity among 28 types of fruit pulp, seed, and peel [24]. Although many benefits of fig fruit are known, fig leaf has only recently been subject of research. Tests on laboratory animals have proven that fig leaves are beneficial for metabolisms with high sugar and cholesterol levels [25]. The positive effects on health and the high antioxidant value are attributed to the fig leaf's high polyphenol content. In a related study, fig leaf extract has been observed to be effective against Bacillus and Escherichia coli (E. coli) bacteria [26]

Geranium is a potential active ingredient in dentifrice and is commonly found among plants in the Aegean region. Tests have shown that its leaves contain high levels of alcohol and polyphenols. In traditional medicine, geranium essence has been used for inflammation, diabetes, diarrhea, kidney stones, dysentery, and even cancer treatments [27]. Its antibacterial effect has been observed against Bacillus and E. coli bacteria [28]. Juniper, traditionally used for kidney and indigestion issues, has both its fruit and leaf extracts proven to have inhibit various bacteria. In a recent study, it was found that juniper essence from the Aegean region is effective against Bacillus bacteria but not against E. coli [29]. Both the fruit and the leaves of juniper have been the subject of this study. The historical use of mastic resin for treating dental diseases and bad breath is intriguing [30]. Recent studies on extracts obtained from mastic resin and leaves shed light on the reasons behind its efficacy. Both mastic resin and leaves have been found to be effective against Staphylococcus aureus (S. aureus) and E. coli bacteria [31, 32]. Blueberry, also commonly found in the Aegean region, has a high polyphenol content, resulting in its elevated antioxidant capacity [33] and reputation to protect the body against inflammation and bacteria [34].

2. MATERIAL and METHOD

In the first stage of the study, an aqueous suspension with hydrogel additives was prepared to dissolve and transport the active ingredients as a dentifrice. The suspension containing 20% glycerol (Sigma-Aldrich G7757, 99%), 2% sodium alginate (Sigma-Aldrich 180947, 13% H_2O), 1% sodium bicarbonate (Sigma-Aldrich 13433, 99%) and 77% deionized water (Type I obtained from Millipore Milli-Q) by weight was homogenized by mixing for 24 hours with a magnetic stirrer. Glycerol and sodium alginate were added to increase the viscosity and stability of the carrier phase, and sodium bicarbonate was used as a foaming agent to ensure more effective friction of the minerals on the tooth surface. CaP phases, the effectiveness of which was investigated, were added to this mixture at a weight fraction of 20% and applied to the synthetic tooth surface. CaP mixtures were obtained by mixing commercial HA (Sigma-Aldrich C3161,

>96%) and monetite (DCPA, Sigma-Aldrich C7263, >99%) powders in various ratios (0/1, 0.25/0.75, 0.5/0.5, 0.75/0.25 and 1/0) in dry form in 50 ml tubes by the facility of a vortex shaker for 10 minutes. The mixtures were added to the paste in dry form and stirred with a magnetic stirrer for 24 hours. Particle sizes of the CaP powders were determined using a Micromeritics Sedigraph 5100.

The effect of abrasive minerals was determined by applying dentifrices containing no plant extracts to synthetic teeth kept in a growth medium for oral bacteria at regular intervals and by testing the hardness. Synthetic teeth were produced by uniaxially pressing the HA powder to pellets with a diameter of 20 mm and a thickness of 5 mm using a hydraulic press under a pressure of 10 MPa and finally sintering at 1200 C for 1 hour. Archimedes' test (Sartorious BP 2105) was applied to three samples that gave an average density of 65%. Synthetic teeth were kept in a medium for 28 days in which Lactobacillus casei (L. casei) , a type of acid bacteria found in the mouth, was incubated to mimic the oral environment and brushed regularly every day. The medium was regularly refreshed before brushing every 24 hours with a commercial toothbrush. After incubation and 28 days of brushing, hardness values were taken from 10 points on the synthetic teeth surfaces with a Vickers microhardness tester (Tronic HVS-1000) and the averages were calculated. The hardness values of the control samples that were not brushed and kept in water were taken as references in the test.

The plant extracts were obtained from fresh fruits and leaves (olive leaf, pomegranate peel, juniper leaf, juniper berry, mastic leaf, fig leaf, blueberry leaf and geranium leaf) collected from trees and shrubs growing in the Aegean region of Türkiye. After collection, they were dried in the oven at 80 °C for one week, ground in a mortar, and extracted in a 30% water-70% ethanol solution at a weight ratio of 1 part per 20 parts solvent by the facility of a rotary evaporator (Heidolph Laborota 4001). The wet extracts were lyophilized (Labconco, FreeZone) for 24 hours and then stored dry at room temperature.

L. casei cells isolated from milk and frozen at the Biochemistry Laboratories of the Department of Chemical Engineering in İzmir Institute of Technology were activated in test tubes containing MRS medium (Merck 1.10661) and then incubated with synthetic teeth and dentifrice in 6-well dishes containing the same medium at 37 °C. Streptococcus mutans (S. mutans) cells isolated from human saliva were similarly activated in a medium containing tryptic soybean medium (Merck 105459) with 5% sucrose and incubated with various plant extract concentrations in the same medium. The effect of the plant extracts on S. mutans strain was first observed by comparing the turbidity of test tubes incubated at 37 °C for one day, using a UV-spectrometer (Perkin Elmer Lambda 25). Then, the mixtures at three different concentrations in which a change in turbidity was observed were serially diluted down to 10-5 in petri dishes and multiplied according to ISO 20776-1 standard. Colony-forming units were manually counted at the end of 48 hours of incubation. As a result of the counting of bacteria growing in an extract-free medium, approximately 240 million colonies were detected. Extract concentrations reaching one thousandth of this number were identified as the minimum inhibitory concentration (MIC), and extract concentrations reaching one hundred thousandth of this number were identified as the minimum bactericidal concentration (MBC). The plant extract ratios added to dentifrices were determined according to the minimum inhibitory concentrations.

The optimum CaP composition of 1/3 HA to DCPA, determined a priori from microhardness tests was added to the pastes containing plant extracts. The MIC concentrations of the plant extracts were added to deionized water using a vortex shaker for one minute, then filtered to prevent contamination and mixed into a paste with the same weight fractions using magnetic stirrer for 24 hours under sterile conditions. The pastes were applied regularly to synthetic teeth for 28 days following the same protocol.

3. RESULT and DISCUSSION

First of all, the effective mineral composition of dentifrices was determined using high purity commercial powders with particle sizes lower than 50 µm that are expected to be more effective abrasors to remove bacterial plaque according to the literature [6, 35]. Table 1 provides the particle size data of HA and DCPA powders.

Table 1. Particle size of HA and DCPA

Precursor	Formula	Mean Particle Size (µm)	d_{50} (µm)
HA	$Ca_{10}(PO_4)_6(OH)_2$	6.0	3.0
DCPA	$CaHPO_4$	20.1	24.0

High purity commercial HA was also used as the synthetic tooth material. The hardness of HA sintered to 65% density is found around 48 Hv. The active ingredients used in the pastes that provide hardness close to this upper limit are considered effective in the hardness tests. Taking the hardness of synthetic teeth that have not decayed and those that have decayed at the maximum rate as reference, it was seen that CaPs in different proportions in the paste had positive effect on removing bacterial plaque from the tooth surface and thus on tooth decay. As seen in Figure 1, the hardnesses of the synthetic teeth were distributed between the hardness values of the negative and positive control samples. The samples composed of 0.25-0.5 HA / 0.75-0.5 DCPA particles gave the highest hardness, reaching that of sintered HA.

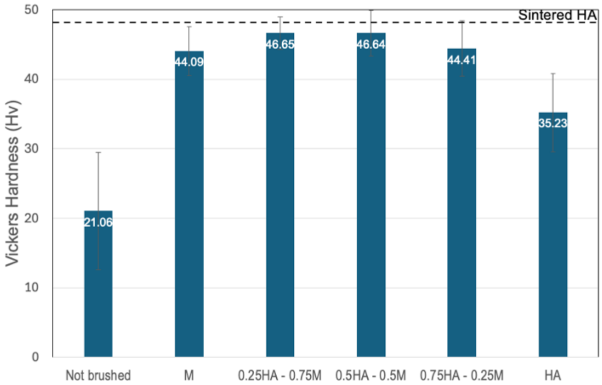

Figure 1. Effect of various ratios of CaP minerals HA and monetite (M) on the hardness of decaying synthetic teeth.

DCPA is a softer CaP phase compared to HA, so it is not possible to scratch tooth enamel, which is composed of approximately 96% HA. It is still capable of scratching the plaque film that has to be removed for remineralization of the dentin. Although HA has higher hardness, DCPA may also significantly contribute to plaque removal due to its mean size more than three times that of HA. It is reported in the literature for CaCO3 particles in the 5-15 micrometers diameter range, that the larger particles are more effective abrasors [36]. It is also understood that the high solubility of DCPA in water contributes to the repair of cracks and cavities in the tooth through remineralization by providing free calcium and phosphate ions to the solution. The solubility of dicalcium phosphate dihydrate (brushite, DCPD), the water-containing form of DCPA, is approximately twice that of DCPA, so it is widely used in remineralizing dentifrices. Synthetic teeth brushed with a paste containing HA, which has a much lower solubility compared to DCPA (calcium and phosphate ion concentrations in neutral water for HA and DCPA are approximately 10-4 and 10-3 [10]), showed lower hardness values, i.e. more decay. In comparison, the hardness of the sample incubated without brushing was found to be very low compared to all samples brushed with different amounts of CaPs. This shows that the remineralization provided by solution chemistry is more effective against tooth decay than the applied mechanical abrasion.

The remineralization of CaP phases has been documented in detail in the literature. For example, DCPD and DCPA cements formed in acidic solutions remineralize into HA as a result of the alkalinization of the aqueous medium over time [37, 38]. On the other hand, it is known that the solubility of HA increases in acidic aqueous solutions and exceeds that of DCPA at pH = 4.2 and below. The difference in remineralization observed between the two CaP phases is thought to be related to the thickener glycerol, hydrogel and sodium bicarbonate acting as a foaming agent in the paste. It is known that sodium bicarbonate neutralizes acidity by dissolving similarly to HA, therefore it may have reduced the remineralization efficiency of HA. Polyelectrolyte hydrogels containing carboxylic acid groups such as sodium alginate expand and increase viscosity by trapping alkaline water due to the negative charge induced on these groups as a result of deprotonation [39]. On the other hand, it is known that the protons in acidic environment form hydrogen bonds between the molecules whereas cations provide the binding of molecules through ionic cross-linking, thus increasing the viscosity with a different mechanism [40]. Therefore, alginate retains less water in an acidic oral environment containing CaP, so that more effective release of ions dissolved in the paste before brushing is possible. DCPA, which is more soluble in neutral and alkaline water may have provided more remineralization as a result. Glycerol molecules added to the paste as a moisturizer and lubricant to facilitate the flow of particles, form weak hydrogen bonds with each other in monomer form, and therefore are only affected by the solution pH [41]. Therefore, glycerol is expected to affect the solubility of CaP phases with a similar mechanism to sodium alginate but to a lesser extent.

The antibacterial activity of 8 different plants collected from the Aegean flora was quantified by the serial dilution method to determine the concentrations to be added to the dentifrice. The effective concentrations given in Table 2 show that although some plant extracts (blueberry, fig leaves, juniper berry) provided MIC at low concentrations, the concentrations required for MBC could not be determined in the studied range (0.008-0.25 g/ml). Olive leaf, juniper leaf, mastic leaf, geranium leaf, pomegranate peel, which have the lowest MBC, are the plants that are most effective on S. mutans. It was determined that pomegranate peel, juniper leaf, mastic leaf, geranium leaf and olive leaf extracts inhibited bacteria down to one in a hundred thousand at different concentrations. It is expected that the extracts tested in this bacterial environment will have different effects on other bacteria found in the oral flora. The effects on another oral cell line, L. casei, were also investigated with hardness tests for the effectiveness of the pastes.

In the second test, the changes in surface hardness of synthetic teeth due to brushing with pastes produced from plant extracts were investigated. The pastes contained the optimum ratio of 0.25-0.75 HA/DCPA mineral mixture, and sodium alginate, glycerol, sodium bicarbonate, water in the same proportions. In these pastes prepared with

Table 2. Antibacterial effect of plant extracts at various concentrations on *S. mutans*

	Fig leaf	Pomegranate peel	Juniper fruit	Juniper leaf	Mastic leaf	Geranium leaf	Blueberry leaf	Olive leaf
MIC (g/ml)	0.0664	0.0332	0.0166	0.0083	0.0083	0.0166	0.0664	0.0083
MBC (g/ml)	>0.2500	0.0664	>0.2500	0.0166	0.0332	0.0664	>0.2500	0.0166

biocompatible raw materials, the only active ingredients that inhibit bacteria were plant extracts. As seen in Figure 2, blueberry leaf, geranium leaf, olive leaf, mastic leaf, and juniper leaf were effective in preventing tooth decay, respectively. Juniper berry and fig leaf provided hardness close to the hardness of the control sample kept in the bacterial environment without brushing. These extracts, whose MBC values on S. mutans could not be determined, had a weak effect on L. casei in the pastes they were added at the MIC ratio. On the other hand, blueberry leaf extract, which did not result in a MBC value for the S. mutans, was more effective on L. casei and provided hardness equivalent to the sample that had not decayed. One of the reasons for this may be that the extract was added to the paste at a higher rate than the others due to its high MIC value. The anti-decay effectiveness of blueberry and geranium leaves added at 0.066 g/ml and 0.0166 g/ml concentrations was found to be almost perfect, reaching the control sample. Olive leaf, juniper leaf and mastic leaf, which were understood to be more potent during in vitro tests due to their low MIC and MBC values, also provided resistance against L. casei in the pastes when they were added at a concentration of 0.008 g/ml. Their effectiveness on this bacterium at different doses are the subject of another study.

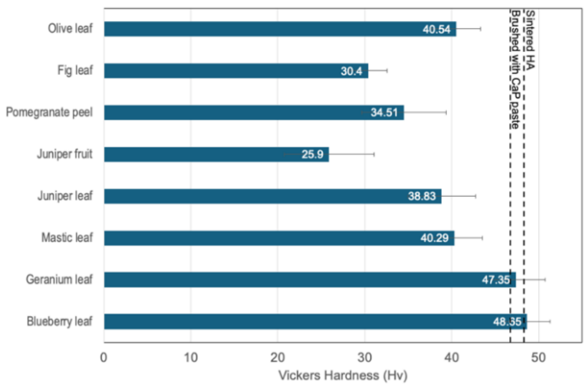

Figure 2. Effect of pastes containing various plant extracts on the hardness of decaying synthetic teeth

In general, all plants examined except juniper berry and fig leaf were found to be effective on oral bacteria. Their effectiveness, which is estimated to vary depending on the types and amounts of polyphenols they contain, increases with concentration. Therefore, applying the MBC value in dentifrices is expected to increase the effectiveness of the dentifrice as well as the cost. As seen in Table 3, where the mass loss of plant extracts during drying and extraction is given, the plant quantities required for effective dentifrice are also related to the efficiency of the extraction processes. While a few grams of some of the plants dried and extracted under the same conditions are sufficient, the production of low-yielding potent extracts such as geranium and olive leaves requires a high amount of fresh plants. On the other hand, the basic costs of these plants, which are mostly left to decay in nature, are basically determined by the energy spent during extraction. They can be suitable raw materials for the production of highly effective, completely natural dentifrices with the use of renewable energy sources.

4. CONCLUSION

The effectiveness of CaP phases and plant extracts, which are considered as natural dentrifice components was determined by mechanical and biological tests. The most effective abrasive and filling mineral function was provided by the 0.75 DCPA / 0.25 HA mixture which resulted in a slight loss (46.7 Hv vs. 48.0 Hv) of surface hardness of artifical teeth incubated with L. casei. The relatively high solubility of DCPA was effective in maintaining synthetic tooth hardness by the remineralization mechanism. The addition of the harder HA particles to abrade dental plaque also prevented the decrease in hardness. Extracts of various plants collected from the Aegean region were added to the dentrifice containing the optimum CaP mixture. The extracts, whose effective MIC and MBC values on S. mutans bacteria were determined by the serial dilution method, were added to the dentifrice at MIC ratios. As a result of

Table 3. Effective MIC concentrations of plant extracts on *S. mutans* and the amount of fresh plants in the paste calculated based on production yields

	Fig leaf	Pomegranate peel	Juniper fruit	Juniper leaf	Mastic leaf	Geranium leaf	Blueberry leaf	Olive leaf
Drying yield (dry weight/fresh weight)	0.2500	0.3500	0.3100	0.3900	0.5200	0.2600	0.4600	0.5500
Extraction yield (extract weight/dry weight)	0.1010	0.2590	0.8300	0.0930	0.4620	0.0750	0.0520	0.0126
Total yield (extract weight/fresh weight)	0.0252	0.0907	0.2572	0.0362	0.2404	0.0194	0.0239	0.0069
Fresh plant for 100 ml dentifrice (g)	262.0	72.8	3.1	45.8	13.7	340.4	276.7	240.4

the application of the obtained dentifrices to synthetic teeth incubated in L. casei medium for 28 days, the extracts that maintained tooth hardness were those obtained from geranium and blueberry leaves, at concentrations of 0.0166 and 0.066g/ml respectively. These extracts demonstrated synergistic interaction with the CaP mixture such that they completely prevented any reduction in surface hardness due to caries and even provided slight enhancement. It was also determined that the tested extracts significantly reduced the decay caused by L. casei at the MIC concentration on S. mutans, except for juniper berry, fig leaf and pomegranate peel. Juniper leaf, mastic leaf, geranium leaf, blueberry leaf and olive leaf, which have little economic value, are valuable natural raw materials for the production of effective dentifrices.

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DECLARATION OF ETHICAL STANDARDS

The author of this article declares that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

CONFLICT OF INTEREST

The author of this article declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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