Research Paper Dental Science

Remineralization Potential of Herbal Mixtures: An *In Situ Study*

Bilgin G Department of Restorative Dentistry (at the of time study), Marmara University, İstanbul, Türkiye

Yanıkoğlu F Department of Restorative Dentistry, Marmara University, İstanbul, Türkiye

Tagtekin D Department of Restorative Dentistry, Marmara University, İstanbul, Türkiye

ABSTRACT

Aim: The aim of this in situ study was to evaluate the effectiveness of two herbal preparations, ginger and rosemary, on remineralization of initial enamel caries lesions and assessment by the FluoreCam detection device.

Methods: This was a single-site, randomized, four-way crossover study involving a total of 10 healthy participants with 3-week test periods. Demineralized human enamel specimens were measured for baseline surface microhardness and light fluorescence with the FluoreCam and QLF. Thirty specimens in each of four groups were used with the following treatments applied three times daily after brushing their teeth with sodium fluoride (NaF) toothpaste (Ipana, Kalsi-Dent, P&G, GmbH, Germany). The groups; 1) ginger + honey + chocolate (experimental, Bind Chocolate, İstanbul, Turkey), 2) rosemary + ginger + honey + chocolate (experimental, Bind Chocolate, İstanbul, Turkey), 3) no treatment, only NaF toothpaste; control group, and 4) hydroxyapatite and fluoride agent (ReminPro, Voco, Cuxhaven, Germany). Each test period lasted 21 days. Post-treatment data were obtained by measurements of surface microhardness and fluorescence. Statistical analyses of the data included ANOVA tests with Tukey's HSD test and Student Newman-Keuls analysis.

Results: Significant differences between treatments were observed in microhardness. Compared with the positive control group (NaF dentifrice), significantly greater remineralization was observed with the ReminPro and ginger-honey-chocolate treatments. With fluorescence assessments (ΔF), using QLF, significantly greater remineralization was observed in all three treatment groups, with the greatest remineralization observed with the two herbal treatments. No significant treatment difference was observed with the FluoreCam.

Conclusions: Enhanced remineralization was observed with all of the treatment systems, with ginger-honey-chocolate and ReminPro being most effective.

KEYWORDS remineralization, ginger, rosemary, chocolate, honey, fluorescence, white spot lesion, microhardness

INTRODUCTION

The first visible sign of tooth caries, the white-spot lesion (WSL), has been defined as "subsurface enamel porosity from carious demineralization" that is manifested clinically by a milky white opacity [1]. This subsurface porosity is caused by an imbalance between the dynamic biological processes of de- and remineralization [2,3]. Demineralization and remineralization processes depend on various different factors; including calcium, phosphate and fluoride amount of saliva and plaque, additionally buffering capacity of saliva, hygine and other factors… Even a few mineral loss means the start of demineralization from dental structure. In the literature we found the article by White DJ (1987)[4] artificial carious lesion produced as 35-50 micron depth. Leavind enamel specimens into demineralizing solution described by White DJ for 72 hours at 37° C. In the minimally invasive dentistry paradigm, incipient enamel carious lesions should be treated with non-invasive remineralization strategies wherever possible, instead of surgical intervention [5]. For this purpose, topical gels, varnishes, mouthwashes, and dentifrices that contain fluoride are used by dentists for the treatment of WSLs. With the concentration of 1100-1400 ppm in dentifrices, while topical gels (≈10 000 ppm F) and varnishes (≈20 000 ppm F), fluoride is the best only remineralizing agent for chemical treatment of initial carious lesions.

Fluoride is a proven agent for caries prophylaxis but excessive use of fluoride may cause dental fluorosis if ingested by very young children, and the use of bactericides or antibacterial agents may cause various side-effects, such as vomiting, diarrhea, and tooth staining, with increased resistance to these chemicals. For financial reasons, in developing countries, there is also a need for alternative prevention and treatment options that are safe, effective, and economical. Thus, instead of using artificial antibiotics and bactericides, it has been proposed that various medicinal plant extracts that have effects on bacteria causing tooth decay be used [6].

Among natural food sources with antimicrobial activities, ginger rhizome (*Zingiber officinale* Roscoe, Zingiberacae) and rosemary (*Rosmarinus officinalis* L., Lamiaceae) have been used as food spices and medicinal plants for centuries. Moreover, they are natural materials, showing no toxicity, and are considered 'generally recognized as safe' (GRAS) by the US Food and Drug Administration (FDA). In particular, their pungent oil components harbor a series of polyphenolic ketones with many pharmacological activities. Their antifungal and antimicrobial effects on oral cavity pathogens have been reported in many studies [7-10]. However, there is no reported study about the effects of these herbal medicaments on remineralization of initial enamel caries.

Another regimen used in many ancient cultures for both nutritional and medicinal purposes is honey. The belief that honey is a nutrient, a drug, and an ointment has carried into present times. Honey is a super-saturated sugar solution, with low water activity that does not support the growth of bacteria [11]. The average pH value of honey is 3.9, so it is acidic and can

inhibit the growth of pathogens because most thrive at pH 4.0-4.5 [11]. However, dilution of honey, for example by saliva, will increase the pH and reduce this effect. Dilution also results in a 2500-50,000 times increase in enzyme activity and the action of the enzyme glucose oxidase and the production of hydrogen peroxide, which is an oxidizing agent, will increase. In honey, hydrogen peroxide is present at a very low level, yet it is still an effective antibacterial agent and compatible with cellular preservation [13]. There are a few reports about the antibacterial effect of honey on oral pathogens[14-17] but none about the effect of honey on remineralization of initial enamel caries. According to the results of the research by Patel RV et al.[16] a paste made by lending ginger and honey was found highly effective on inhibition of S.mutans, L.acidoğhilus, A.viscosus, P.aeroginosa, V.alcaligens and S.aureus.

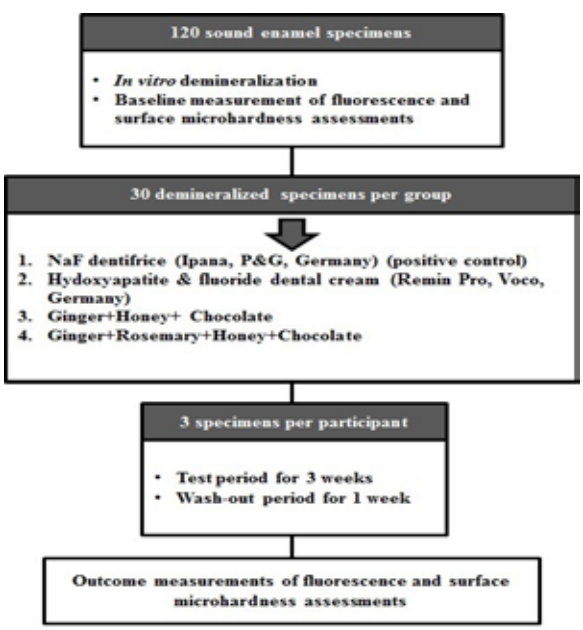
The fluorescence systems do measure the mineral loss and gain in the lesion area as mm^2. These systems compare the mineral differences of the lesion area from its base either sound surface on demineralized surface quantitatively. This way white spot lesions and any remineralizations in these lesions can be detected and recorded non destructively.

The aim of this study was to evaluate the remineralization potential of herbals: ginger, rosemary, and honey. Moreover, we evaluated the efficiency of a new detection device, the FluoreCam, on demineralized and remineralized human enamel in terms of microhardness and QLF systems.

MATERIALS AND METHODS

Study Design. This *in situ* investigation was a single-site, randomized, four-way crossover study involving 10 participants. The study design is shown in Figure 1. This study commenced following the approval from the ethical committee, and it was presented as the thesis of Dt. Gülçin Bilgin [18].

Figure 1. Illustration of the study experimental design.



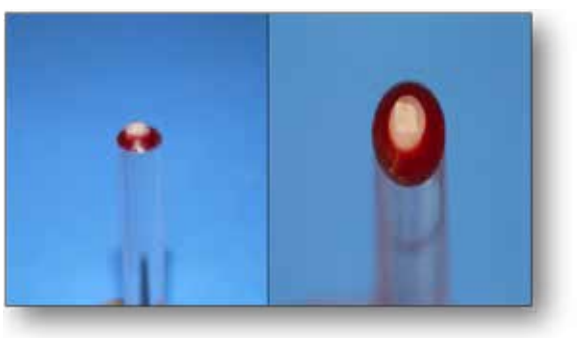
Thirty specimens in each of four groups were used with the following treatments applied three times daily after brushing their teeth with sodium fluoride (NaF) toothpaste (Ipana, KalsiDent, P&G, GmbH, Germany). The groups; 1) ginger + honey + chocolate (experimental, Bind Chocolate, İstanbul, Turkey), 2) rosemary + ginger + honey + chocolate (experimental, Bind Chocolate, İstanbul, Turkey), 3) no treatment, only NaF toothpaste; control group, and 4) hydroxyapatite and fluoride agent (ReminPro, Voco, Cuxhaven, Germany). Each test period lasted 21 days.

Subject Recruitment. Ten healthy adults with no systemic diseases were recruited for this study. Approval was obtained from the ethical committee of The Marmara University Institute of Health Sciences Clinical Research Preliminary Evaluation Board. All subjects provided written informed consent. This investigation was conducted in accordance with the Declaration of Helsinki (World Medical Association).

The subjects generally belonged to the same socio-economic class. All participants were in good health and not taking any medication that might affect the composition of their saliva. The study population was composed of equal numbers of men and women, ranging in age from 30 to 35 years. An intra-oral examination confirmed that each had at least 22 natural teeth with no current caries activity, periodontal disease, or other oral pathology. A stimulated salivary flow rate of > 0.7 mL/min was also required for participation.

Enamel Specimens and Preparation of Subsurface Lesions. In total, 120 human enamel specimens (10 subjects, 3 specimens per patient per test period, 4 test periods) were used. Each group had 30 specimens. Extracted teeth that had been obtained from oral surgeons were used; the teeth were stored in 0.10% thymol solution immediately after extraction and maintained in this solution prior to use. The sound enamel specimens were 3 mm in diameter and 1.6-2.0 mm in thickness from the enamel surface. These enamel cores were mounted on acrylic rods (Figure 2).

Figure 2. Enamel cores were mounted on acrlic rods.



Surfaces of the specimens were polished with a 600-grit grinding disk and with a slurry of 0.05 µm gamma alumina polishing gel. Artificial subsurface carious lesions were formed on each enamel specimen by placing the specimens individually for 72 h at 37°C in 7.0 mL of a demineralizing solution containing 0.1 molar lactic acid and 0.2% Carbopol 907, 50% saturated with hydroxyapatite and adjusted to pH 5.0 using NaOH [4].

Intraoral Appliance. All specimens were mounted on a mandibular removable acrylic appliance. The appliance had two bilateral troughs with a window on the buccal surface to house enamel specimens. In each appliance, three enamel specimens were retained with temporary filling material (Clip, Voco, Germany; Figure 3).

Figure 3. Images of intraoral appliance.

The participants wore the appliance intraorally for 3 weeks continuously, except when eating; drinking water was permitted. With their appliances in place, they brushed their teeth with the same toothpaste twice per day (morning and evening), (Ipana Kalsident, Procter & Gamble, Turkey) during each treatment period. They were careful not to touch the specimens' surfaces during brushing. The test materials were applied immediately following brushing with fluoride toothpaste. ReminPro was applied as a pea-size amount by finger to the specimens. The chocolates were chewed in the molar

region and stayed there approximately 3 min. Then, the materials were distributed throughout the mouth with the tongue. Participants were not allowed to eat or drink for 30 min following the treatments. The appliance was kept fully hydrated by immersion in distilled water in a plastic box when not in use. After every week, participants were asked to visit to check their general health, compliance, and the fitting of the appliance and specimens. All subjects complied well with the protocol. No adverse reaction related to the appliance or treatment materials was observed.

Treatment Materials. Ipana is a standard fluoridated dentifrice (NaF, 1450 ppm). ReminPro is a dental cream that contains hydroxyapatite, fluoride (1450 ppm), and xylitol. Ginger and rosemary were in powder form and mixed with honey (8 mg/mL). The mixtures then were covered with chocolate, which was bitter (15% sugar). The chocolate was specially produced by Bind Chocolate (Tatlı Çikolata San. ve Tic. A.Ş., Tekirdağ, Turkey).

Analysis of Remineralization. The mineral density of the specimens was determined using a digital microhardness tester (Leco LM247AT, Leco Corp., MI, USA) with a load of 200 g for 15 s in four areas. Assessments of the mineral content of the demineralized area of each specimen were obtained before and after each test period using both the FluoreCam (Therametric Technologies, Inc., Noblesville, IN, USA) and QLF (Inspektor Pro, Inspektor Research Systems, Amsterdam, The Netherlands) systems. The parameters assessed included % fluorescence loss, area (mm^2) and lesion volume (mm^2 %).

Statistical Analysis. To compare the remineralization effects of the treatment materials used in each group, repeated-measures ANOVA tests were conducted, with Tukey's multiple comparison tests for *post hoc* analysis. All statistical analyses were carried out using the SPSS software (ver. 15.0 for Windows; SPSS Inc., Chicago, IL). P values < 0.05 were considered to indicate statistical significance.

RESULTS

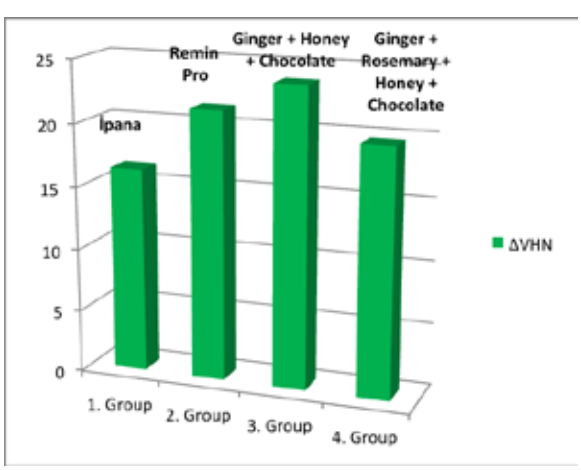
Table 1 presents the fluorescence data. Over time, all fluorescence values (ΔF and ΔQ) in all of the treatment groups showed enhanced remineralization. Although, the increases (ΔQ) observed in the herbal treatment groups were greater than with Ipana and ReminPro in QLF measurements, they were not statistically significant ($p > 0.05$). Moreover, no significant difference was observed in FluoreCam values among the groups using ΔF or ΔQ measurements ($p > 0.05$).

However, the surface microhardness increases (Table 1, Figure 4) showed significant differences between all supplement-treated groups The additional dental cream treatment, ReminPro, and the herbal mixture (ginger + honey + chocolate) treatment showed greater remineralization and they were significantly different from each other ($p < 0.05$). The other herbal treatment (ginger + rosemary + honey + chocolate) was more effective than fluoride dentifrice alone and it was also significantly different ($p < 0.05$).

Table 1. Obtained data from QLF, Fluorecam and Microhardness measurements.

	FluoreCam		Inspector (QLF)		VHN
Group	Delta F Mean ± SD	Delta Q Mean ± SD	Delta F Mean ± SD	Delta Q Mean ± SD	Delta VHN Mean ± SD
1 (n=30)	2,02 ± 1,12	14,06 ± 2,89	0,91 ± 0,16	8,01 ± 4,30	16,36 ± 0,90
2 (n=30)	3,26 ± 1,09	14,65 ± 2,79	1,62 ± 0,18	6,64 ± 4,51	21,40 ± 1,66
3 (n=30)	0,63 ± 0,9	7,76 ± 2,69	3,44 ± 0,34	13,83 ± 5,71	23,72 ± 1,64
4 (n=30)	0,17 ± 1,10	5,88 ± 2,88	4,33 ± 0,28	16,74 ± 7,37	19,63 ± 0,91
		p>0.05		p>0.05	p≤0.05
					Tukey HSD: Differentgroups: 1 and 4; 2 and 3

Figure 4. Remineralization datas of the groups measured by Microhardness



DISCUSSION

The major interactions of the enamel with the oral environment occur on the surface layer of the sample [19]. The remineralizing agents applied on the surface of enamel specimens were evaluated by fluorescence (QLF, FluoreCam) and surface microhardness test methods. These methods are simple, fast, and easy to measure as non-destructive methods and are capable of reflecting mineral changes that have occurred due to treatments. They also allow repeated measurements of the same specimen over a given period of time if required by the study design. Enamel fluorescence assessments using QLF have shown great promise as an early caries detection method. Based on data available, it is suitable for *in vivo* monitoring of mineral changes, as well as for caries prevention programs, although the cost of the equipment is significant and image analyses are time-consuming [20]. Also, there are certain factors that may influence its success, such as dehydration [21] and angulation [22, 23]. The FluoreCam instrument uses the same basic principles to assess enamel fluorescence as QLF. However, the analyses of the suspected demineralization or remineralization area are performed automatically by the FluoreCam software. Although we did not observe any statistical differences in fluorescence assessments induced by the different treatment regimens in this study, due to the large variances, the fluorescence values (ΔF) in the herbal treatment groups changed numerically, suggesting greater remineralization than was observed with only the fluoride dentifrice in QLF measurements (Table 1).

ReminPro is a relatively new agent that is marketed for enhancing remineralization. In this *in situ* study, we found that ReminPro provided additional remineralization beyond that obtained with the fluoride dentifrice, as assessed by both microhardness and fluorescence assessment methods. Probably both ingredients in this treatment agent, fluoride and hydroxyapatite, enhanced the remineralization by infiltrating through the lesion body and increasing remineralization of the lesion.

Brushing the teeth with fluoride toothpaste is a must for an *in situ* study, for ethical reasons. Thus, all volunteers used NaF

toothpaste in all test groups twice per day. Fluoride is retained in enamel lesions in the form of a fluorapatite, and on the enamel surfaces as CaF_2, which may act as a fluoride reservoir; thus, there was a significant amount of remineralization from the fluoride dentifrice in each study group in addition to the added remineralization effects of the test materials.

Recently, many phytochemicals, including antibacterial agents, have been identified from edible plants. There are also numerous reports on components of plants that have revealed antibacterial activities against *Streptococcus mutans*, which is widely known as a cause of dental caries. Ohara et al. [7] investigated the antibacterial activities of 81 edible plants on *S. mutans* in polarity-differing solvents (hexane and ethyl acetate) and ginger was found to be effective (MIC 23 mg/g and 8 mg/g). Moreover, after boiling for 10 min at 100°C or after storage for 1 week at 4°C, ginger retained its antibacterial activity [7]. Gregio et al. [24] found that in glycolic or hydroalcoholic solvents, ginger was effective against *S. mutans* at a MIC of 5 mg/mL.

Streptococcus mutans produces glucosyltransferases and synthesizes an adherent and water-insoluble glucan from sucrose, which allows the organism to adhere firmly to the tooth surface. Cacao beans, which are a main ingredient of chocolate, contain some polyphenols that exhibit anti-glucosyltransferase activity. Ooshima et al.[25] reported that cacao bean husks have an anticariogenic effect on *S. mutans* and *S. sobrinus* in rats, demonstrating that the extract could become a novel anticaries substance, as a mild chemo-prophylactic agent. They found that the husk extract might be able to change a cariogenic flora into a non-cariogenic flora without destroying the ecological balance within the oral cavity, because it markedly reduced the growth rate of *S. mutans*, but did not affect other oral streptococci so strongly [25]. Osawa et al. [26] demonstrated that a 50% ethanol extract of cacao bean husk was superior to the 30% ethanol extract they had used in their previous study. They found that the cacao bean husk had two types of cariostatic substances. Higher-molecular weight polyphenolic compounds and unsaturated free fatty acids, such as oleic and linoleic acids, were isolated from the cacao bean husk. The former showed anti-glucosyltransferase activities and the latter showed antibacterial activity against *S. mutans* [26]. Percival et al. [27] reported that cacao polyphenols can inhibit biofilm formation and acid production by *S. mutans*. In our study, we used chocolate as a carrier for the ginger and honey mixture, and also as a part of the treatment material.

Honey has potent broad-spectrum antibacterial activity and studies have demonstrated that manuka honey has high antibacterial activity and is likely to be non-cariogenic. Patel et al. [15] reported that ginger and honey were more effective than gentamycin against *S. mutans*. They found the MIC of ginger to be 31.25 mg/mL, and that of the mixture of honey 1:2 (%,v/v) and ginger to be 15.63 mg/mL. Our study was consistent with these reports in demonstrating that ginger + honey (8 mg/mL) was a strong remineralizing agent. The high remineralization obtained was probably due to the antimicrobial properties and the high fluoride content (79 ppm) of ginger (Therametric Co. laboratory results). Additionally, the pH of the ginger and honey mixture was essentially neutral (pH 6.35). Even though NaF toothpaste had much more fluoride (1450 ppm), the amount of remineralization increased further with the ginger + honey + chocolate treatment (Table 1). Under the conditions of the study, we could not distinguish whether a larger remineralization effect resulted from the ginger, but it seems that there might be some enhancing effect on initial lesions. However, honey with its low pH may activate the release of fluoride from ginger at the time of application, resulting in higher remineralization.

Tsai et al. [28] demonstrated an inhibitory effect of rosemary on *S. sobrinus*. They found the minimum inhibitory concentrations of aqueous and methanolic rosemary extracts against *S. sobrinus* were 16 and 4 mg/mL. Dalirsani et al. [29] compared rosemary methanolic extract (30 g/100 mL) with chlorhexidine and found that rosemary has inhibitory effects on *S. mutans*. We found that the rosemary-containing treatment mixture was effective in enhancing the remineralization process of enamel but it was not as effective as the ginger + honey mixture alone. In this *in situ* investigation, all test materials enhanced remineralization as NaF toothpaste did as control group. QLF and FluoreCam systems showed remineralization in all groups. All groups were similar in remineralization including NaF toothpaste positive control group in both fluorescence systems. All groups showed remineralization in microhardness test method. Test groups showed higher microhardness values compared to NaF toothpaste positive control group. Herbal mixture (ginger+ honey+ chocolate) and ReminPro groups showed higher microhardness value in this study. Further research is still needed to check the accuracy of FlureCam system.

The daily application of chocolate containing ginger and honey with or without rosemary resulted in enhanced remineralization beyond that provided by the daily use of a fluoride dentifrice. Because more natural products are preferred today, the herbals, ginger and rosemary, may be preferable for prevention purposes on initial remineralization of enamel lesions beyond the daily use of NaF toothpaste, at least under the conditions of this *in situ* study. With these promising findings, we suggest further investigations of the potential benefits of herbal treatments for dental health.

Acknowledgements:
We would like to thank to Dr. Stephen J. Moss, Dr. George Stookey, Dr. Bruce Schemehorn and Dr. Osman Hayran for his valuable contribution.. We are thankful to the volunteers for their participation to the study. This study was funded by BAP-KO of Marmara University (SAG-D-200611-0207)

REFERENCES

1- Summitt JB, Robbins JW, Hilton TJ, Schwartz RS: Fundamentals of Operative Dentistry: A Contemporary Approach. Hanover Park, IL, Quintessence Publishing, 2006.
2- Featherstone, JD: The science and practice of caries prevention. J Am Dent Assoc 2000;131(7):887-899.
3- Kidd EA, Fejerskov O: What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J Dent Res 2004;83 Spec No C: C35-38.
4- White DJ: Use of synthetic polymer gels for artificial carious lesion preparation. Caries Res 211987;(3):228-242.
5- Mount GJ: A new paradigm for operative dentistry. Aust Dent 2007;52(4): 264-270; quiz 342.
6- Palombo EA: Traditional Medicinal Plant Extracts and Natural Products with Activity against Oral Bacteria: Potential Application in the Prevention and Treatment of Oral Diseases. Evid Based Complement Alternat Med 2011;680354.
7- Ohara A, Saito F, Matsuhisa T: Screening of Antibacterial Activities of Edible Plants against Streptococcus mutans. FSTR Food Science and Technology Research 2008;14(2):190-193.
8- Park M, Bae J, Lee DS: Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. Phytother Res 2008;22(11):1446-1449.
9- Bernardes WA, Lucarini R, Tozatti M, Souza M, rade Silva M, da Silva Filho AA, Martins CHG, Miller Crotti, AE , Pauletti PM, Groppo M, Cunha WR: Antimicrobial activity of Rosmarinus officinalis against oral pathogens: relevance of carnosic acid and carnosol. Chem Biodivers 2010;7(7):1835-1840.
10- Smullen J, Finney M, Storey DM, Foster HA: Prevention of artificial dental plaque formation in vitro by plant extracts. J Appl Microbiol 2012;113(4):964-973.
11- Lusby PE, Coombes A, Wilkinson JM: Honey: a potent agent for wound healing? J Wound Ostomy Continence Nurs 2002;29(6):295-300.
12- Bansal V, Medhi B, Pandhi P: Honey-a remedy rediscovered and its therapeutic utility. Kathmandu Univ Med J (KUMJ) 2005;3(11):305-309.
13- Stephen-Haynes J: Evaluation of a honey-impregnated tulle dressing in primary care. Br J Community Nurs Suppl: 2004;S21-27.
14- Basson NJ, du Toit IJ, Grobler SR: Antibacterial action of honey on oral streptococci. J Dent Assoc S Afr 1994;49(7):339-341.
15- Ghabanchi J, Daghigh AM, Bazargani A, Balady FS, Dad AS: In vitro assessment of anti-Streptococcus mutans potential of honey. Iran. Red Crescent Med.J. Iranian Red Crescent Medical Journal 2010:12(1):61-64.
16- Patel RV, Thaker VT, Patel VK: Antimicrobial activity of ginger and honey on

isolates of extracted carious teeth during orthodontic treatment. APJTB Asian Pacific Journal of Tropical Biomedicine 2011;1(1):558-561.
17- Nassar HM, Li M, Gregory RL: Effect of honey on Streptococcus mutans growth and biofilm formation. Appl Environ Microbiol 2012;78(2):536-540.
18- Bilgin G. Remineralization Potential of Various Materials and Assessment by a New Caries Detection Device. Thesis, Marmara University, İstanbul, Turkiye, December, 2012.
19- Argenta RM, Tabchoury CP, Cury JA: A modified pH-cycling model to evaluate fluoride effect on enamel demineralization. Pesqui Odontol Bras 2003;17(3):241-246.
20- Iijima Y: Early detection of white spot lesions with digital camera and remineralization therapy. Aust Dent 2008;J 53(3):274-280.
21- Pretty IA, Edgar WM, Higham SM: The effect of dehydration on quantitative light-induced fluorescence analysis of early enamel demineralization. J Oral Rehabil 2004;31(2):179-184.
22- Buchalla W, Lennon AM, van der Veen MH, Stookey GK: Optimal camera and illumination angulations for detection of interproximal caries using quantitative light-induced fluorescence. Caries Res 2002;36(5):320-326.
23- Ando M, Eckert GJ, Stookey GK, Zero DT: Effect of imaging geometry on evaluating natural white-spot lesions using quantitative light-induced fluorescence. Caries Res 2004;38(1):39-44.
24- Grégio, AMT,. Fortes ESM, et al.:Antimicrobial activity from Zingiber officinalle on oral cavity pathogens. Estud Biol 2006;28(62):61-66.
25- Ooshima T, Osaka Y, Sasaki H, Osawa K, Yasuda H, Matsumura M, Sobue S, Matsumoto M: Caries inhibitory activity of cacao bean husk extract in in-vitro and animal experiments. Arch Oral Biol 2000;45(8): 639-645.
26- Osawa K, Miyazaki K, Shimura S, Okuda J, Matsumoto M, Oosima T: Identification of cariostatic substances in the cacao bean husk: their anti-glucosyltransferase and antibacterial activities. J Dent Res 2001;80(11):2000-2004.
27- Percival RS, Devine DA, Duggal MS, Chartron S, Marsh PD: The effect of cocoa polyphenols on the growth, metabolism, and biofilm formation by Streptococcus mutans and Streptococcus sanguinis. Eur J Oral Sci 2006;114(4):343-348.
28- Tsai PJ, Tsai TH, Ho SC: In vitro inhibitory effects of rosemary extracts on growth and glucosyltransferase activity of Streptococcus sobrinus. Food Chemistry 2007;105(1):311-316.
29- Dalirsani, Z., M. Aghazadeh, Adibpour M, Amirchaghmaghi M, Pakfetrat A, Mosannen Mozaffari P, Mehdipour M, Taghavi Zenooz A: In vitro Comparison of the Antimicrobial Activity of Ten Herbal Extracts Against Streptococcus mutans with Chlorhexidine. Journal of Applied Sciences; 2011;11(5):878-882.

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see:
http://www.textcheck.com/certificate/Lg1kfh