Introduction

The oral environment is colonized by many microorganisms, which coexist in balance with the host under healthy conditions. Toothbrushing with fluoridated dentifrices is the main method for removal or control of dental biofilm, and consequently the most effective means of preventing dental caries and periodontal disease [1,2].

However, accumulation of biofilm can lead to an imbalance in the oral environment, changing the microbial profile and increasing the risk of pathologies. Treatment with orthodontic fixed appliances (for example) can lead to greater accumulation of biofilm, which can cause dysbiosis by altering the salivary count of Lactobacillus spp. in patients with fixed orthodontic appliances [3-5].

The likelihood of developing carious lesions and periodontal disease during orthodontic treatment is increased due to the prolonged effect of accumulation and retention of dental biofilm, as the appliances make conventional oral hygiene for plaque removal more difficult [6]. In such cases, different formulations of chemical control agents such as toothpaste and mouthwashes can be used in addition to mechanical biofilm control [7-9]. The use of natural products has become a trend in the last few decades due to their positive pharmacological effects in the oral cavity, and a number of natural products have been incorporated into several formulations [10-12].

Brazilian red propolis (BRP) was the 13th type of propolis reported in the literature, containing isoflavonoids, pterocarpan, chalcones, flavonoids, prenylated benzophenones, terpenes and tannins. Due to its especially high amounts of flavonoids and isoflavonoids, it exerts antibacterial/anticaries activities mainly against S. mutans and Lactobacillus spp., as well as antifungal, anti-inflammatory, immunomodulatory, antioxidant, and antiproliferative activities, among others [13-17]. It has been used widely in popular medicine since ancient times, and is a promising agent for topical products in view of its diverse pharmacological properties [13,14].

Propolis can be classified according to its physicochemical properties (color, texture, or chemical composition). Brazilian red propolis (BRP) obtained from the literature, containing isoflavonoids, pterocarpans, chalcones, flavonoids, prenylated benzophenones, terpenes and tannins. Due to its especially high amounts of flavonoids and isoflavonoids, it exerts antibacterial/anticaries activities mainly against S. mutans and Lactobacillus spp., as well as antifungal, anti-inflammatory, immunomodulatory, antioxidant, and antiproliferative activities, among others [15-17]. Due to the biological characteristics of BRP, the region from where it originates has received geographical recognition by the National Institute of Industrial Property in Brazil (INPI-BR) [14,15,17].

Tooth decay and gingival disease can progress slowly during life and must be controlled through non-invasive interventions. For this reason, a toothpaste containing BRP has been developed, and filed as a patent under protocol BR1020170110974 at INPI-BR. This dentifrice has shown clinical and microbiological efficacy in orthodontic patients with gingivitis, reducing both gingival bleeding and S. mutans [17]. In seeking more scientific evidence to justify the use of this product, the objective of the present study was to evaluate the visible plaque index (VPI) and the salivary count of Lactobacillus spp. in patients with fixed orthodontic appliances.

Materials and Methods

Study design and ethical aspects

This was a longitudinal, parallel, randomized, double-blind, controlled study compliant with the CONSORT checklist. This study was approved by the Ethics Committee of the Federal University of Ceará (approval number 4.158.225), respecting Brazilian standards for clinical studies and the ethical principles of the Declaration of Helsinki. The study was recorded in the Brazilian Registry of Clinical Trials (REBEC-registration RBR-9hszh). The BRP extract was collected in the city of Marechal Deodoro, Alagoas State, Brazil (south latitude 9º44.555', west latitude 35º52.080', altitude of 18.1 m asl), a region with geographical recognition by INPI-Brazil.

The BRP extract was obtained by maceration in 80% ethanol to yield 13th type of propolis reported in the literature, containing isoflavonoids, pterocarpans, chalcones, flavonoids, prenylated benzophenones, terpenes and tannins. Due to its especially high amounts of flavonoids and isoflavonoids, it exerts antibacterial/anticaries activities mainly against S. mutans and Lactobacillus spp., as well as antifungal, anti-inflammatory, immunomodulatory, antioxidant, and antiproliferative activities, among others [13-17]. In seeking more scientific evidence to justify the use of this product, the objective of the present study was to evaluate the visible plaque index (VPI) and the salivary count of Lactobacillus spp. in patients with fixed orthodontic appliances.

Abstract

Purpose: The objective was to evaluate the efficacy of a dentifrice containing Brazilian Red Propolis (BRP) against salivary Lactobacillus spp. and plaque formation.

Methods: This was a randomized, double-blind clinical trial. Forty-two participants were randomized into two groups according to the dentifrice employed: G1 (fluoridated BRP dentifrice) and G2 (fluoridated common dentifrice). Saliva was collected and the visible plaque index (VPI) was recorded at the baseline (D0) and 4 weeks after day 0 (D28). Microbiological analysis was performed using two dilutions. Lactobacillus spp. isolates were identified and their abundance was expressed as log (CFU/mL).

Results: For the first dilution, the counts of Lactobacillus spp. in G1 was 1.15 ± 0.41 at D0 and 0.68 ± 0.15 at D28 (P < 0.05) and in G2 it was 1.33 ± 0.52 at D0 and 1.84 ± 0.39 at D28 (P < 0.05). For the second dilution, the corresponding values in G1 and G2 were 0.87 ± 0.34 and 0.64 ± 0.37, respectively (P = 0.1547), and 1.54 ± 0.47 and 1.62 ± 0.37, respectively (P = 0.9999). The corresponding VPI values for G1 and G2 were 38.10 ± 17.95 and 20.60 ± 16.44, respectively (P < 0.05), and 38.38 ± 19.65 and 27.40 ± 14.63, respectively (P = 0.03).

Conclusion: The dentifrice containing BRP showed antimicrobial activity against Lactobacillus spp. and decreased the VPI for up to 4 weeks.

Keywords: clinical trial, dentifrice, lactobacillus, propolis, visible plaque index

A double-blind randomized clinical trial of Brazilian red propolis dentifrice efficacy in orthodontic patients

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then transferred to a separation funnel, and hexane (500 mL) was added to eliminate any remaining grease and wax. The hexane layer was removed with a separation funnel, and then ethyl acetate solvent was added in two liquid-liquid extraction steps to obtain an ethyl acetate extract enriched with flavonoids and isoflavonoids from BRP, free of grease and wax.

Identification of the constituents was carried out by High Performance Liquid Chromatography (HPLC, Shimadzu, Kyoto, Japan) with a C18-20A controller, LC-20AT quaternary pump, SPD-M 20A diode array detector, and Shimadzu LC version 1.21 SP1 software. For running the analysis, a Shimadzu Shim-Pack CLC-ODS (M) column was used (4.6 mm × 250 mm; particle diameter 5 μm: pore diameter 100 Å). For evaluation of phenolic derivatives, the eluent solution consisted of methanol and acidic water with formic acid (0.1% w/w), 20-95%, and was run for 77 min at a flow rate of 0.8 mL/min. Detection was performed at 275 nm. PEE, PWE, PMM, and PSDE (n = 3) were individually diluted with methanol/water and homogenized in an ultrasonic bath. The volume was then acidified to pH 2.70 with formic acid. After filtration (0.45 μm), 20 μL was injected into the HPLC system [18]. Under these chromatographic conditions the main chemical constituents in the propolis extract — quercetin, vestitol and neovestitol— were identified. Identification was carried out by comparing the chromatographic profile of the BRP samples with these chemical markers subjected to the same analytical conditions. When there was coincidence between the retention times, the absorption spectrum was compared between the sample and the reference, seeking to establish similarity.

The dentifrices were produced using calcium carbonate (CaCO₃) and 1,500 ppm sodium monofluorophosphate (MFP) in the pharmacotechnical laboratory of the Pharmacy Department at the Federal University of Ceará. For the PVB dentifrice, a concentration of 1% was used, as reported previously [17]. The dentifrices were prepared in a standardized way, in terms of taste, color and odor. The samples were stored in tubes and encoded alphabetically to guarantee double blinding.

Clinical trial
The study participants were selected in the city of Aracati-CE, Brazil. This city does not provide fluoridation in the public water supply. The participants were selected by the lead researcher. The intraoral clinical examination to assess and determine the VPI of the participants at the beginning and end of the study was the responsibility of two previously calibrated examiners who were unaware which toothpaste had been delivered to the participants.

The inclusion criteria adopted in the subject recruitment process were: age between 12 and 18 years; free of carious lesions (ICDAS II 0); absence of any salivary or dental biofilm accumulation; and being right-handed. Subjects were excluded if any of the following applied: systemic changes related to the periodontal health-disease process; antimicrobial therapy (treatment with antibiotics and/or anti-inflammatory agents) up to six months before the study; users of legal/illegal drugs; use of prostheses; presence of fewer than 10 dental elements per dental arch; a history of allergies (asthma, hives, rhinitis, sinusitis); a history of hypersensitivity to drugs, food or other factors; a history of any salivary dysfunction; a history of any salivary dysfunction; and use of any salivary suppression agents such as medications, anticholinergic, or antispasmodic drugs.

Saliva collection and microbiological analysis
The saliva of each patient was collected at the baseline (D0) and at 28 days from the beginning of the study (D28). Patients chewed a fragment (3 × 3 cm) of Parafilm (Laboratory Film, Chicago, IL, USA) for 60 s to stimulate saliva production and release bacteria from the dental biofilm. This was collected using a disposable pipette and stored in sterile microcentrifuge tubes for subsequent analysis. The samples were transported to the laboratory in a Styrofoam container with ice, and analyzed within 2 h after collection. A 0.1-mL volume of each sample was transferred to a sterile hemolysis tube containing 0.9 mL of saline. This process was repeated twice, thus establishing 1:10 and 1:100 dilutions. A volume corresponding to 10 μL of each dilution was spread on Rogosa agar culture medium in triplicate to reveal any salivary Lactobacillus spp. The plates were incubated at 37°C for 48 h in microaerophilic jars and placed in an oven. Colonies with morphological rod-shaped characteristics were then counted. The bacterial count was expressed as CFU/mL of saliva.

Data analysis
Comparisons between the two treatment groups in relation to the absolute variation in the amount of Lactobacillus spp. on D0 and D28 were performed using Student’s t-test for unpaired variables. Analysis of covariance (ANCOVA) complemented by the Bonferroni multiple comparison test was used to perform the same comparisons, considering any adjustment for covariates that would influence the absolute variation in the amount of Lactobacillus spp., and taking the treatments (common dentifrice and BRP dentifrice) as the main effect and the amount of Lactobacillus spp. measured on D0 as the covariable.

Comparisons between the two groups regarding VPI at different times were performed using the Bonferroni multiple comparison test. Two-tailed tests were used in all analyses with the significance level set at 0.05 (5%), thus considering a P-value of <0.05 to be statistically significant. The GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA) software programs were used for the statistical procedures.

Results
In this study, 42 participants were randomized into two groups (Fig. 1). All of the participants completed the study.

Figure 2 shows data corresponding to the mean and standard deviation of Lactobacillus spp. measurements performed on the saliva samples of each group on D0 and D28, using a 1:10 dilution of saliva. The groups showed a significant difference on day 28 (P < 0.05). In the group treated with the BRP dentifrice there was a reduction in the count of Lactobacillus spp. relative to D0 (P < 0.05), while in the group treated with common dentifrice there was an increase (P < 0.05).

Figure 3 shows data corresponding to the mean and standard deviation of Lactobacillus spp. measurements performed on saliva samples from each group on D0 and D28 using a 1:100 dilution of saliva. The group treated with the BRP dentifrice showed statistically significant differences from the group treated with common dentifrice on D0 (P < 0.05) and on D28.

Table 1 shows the absolute variation in amounts of Lactobacillus spp. measured on D0 and D28 considering the 1:10 and 1:100 dilutions, with covariate adjustment. For the first dilution (1:10), there were significant differences between the two groups in relation to the absolute variation in the amount of Lactobacillus spp. There was a reduction in the amount of Lactobacillus spp. in the group treated with BRP dentifrice, whereas through the same instructor, with brushing and the number of brushings per day being standardized (daily brushings for 2 min after breakfast, after lunch and before bedtime).

The participants were divided into two groups: G1—BRP fluoridated dentifrice (1,500 MFP + 1% BRP extract) and G2—common fluoridated dentifrice (1,500 MFP). They were consulted at two points during the 28 days of the experiment. Saliva collection, toothpaste provision for four weeks of use, and instructions on oral hygiene were initially performed on the first visit (Day 0) together with a clinical consultation. On the second occasion, a clinical examination and final saliva collection were performed (Day 28).
an increase was evident in the group treated with common dentifrice ($P < 0.05$). Considering the 1:100 dilution, a similar trend was verified, i.e. a greater reduction than the previous dilution in the BRP group and a greater increase in the common dentifrice group ($P < 0.05$).

Table 2 shows the mean values for the VPI among the participants. A significant reduction in the VPI was observed for both groups.

**Discussion**

The results of the present study demonstrated that toothbrushing with a BRP dentifrice was more effective for reducing the amount of salivary *Lactobacillus* spp. and controlling dental biofilm over 28 days of treatment.

The use of orthodontic appliances increases the risk of developing caries and gingivitis, as these devices retain plaque and both conditions are bio-
Bright Toothgel (Forever Living Products) was more effective than Colgate Total dentifrice (Colgate-Palmolive Company, Sanand, India Ltd.) and Meswak dentifrice (Dabur, New Delhi, India Ltd.) in controlling plaque formation in a crossover study. Mohsin et al. [40] observed that Probe dentifrice (Seoul Propolis, Daecjon, South Korea) was effective in reducing S. mutans in children with mixed dentition during 4 weeks of treatment.

Propolis incorporated into dentifrices favors reduction of dental plaque [34,35,37-40]. In the present study, there was a significant reduction of the VPI in the group treated with common dentifrice (P = 0.03) and the group treated with BRP dentifrice (P < 0.05); however, the best result was observed in the BRP group. The fact that both treatments resulted in a reduction of VPI may have been due to the oral hygiene instructions offered and the standardized brushing technique taught.

The aforementioned results indicate that use of propolis-containing products in vivo helps control biofilm formation, and achieves antibacterial and anti-inflammatory effects, as well as having regenerative properties. Unfortunately, most studies did not specify the type of propolis used.

The present dentifrice had shown similar results in a clinical trial employing the same treatment period and similar methodology, being effective in reducing both the rate of gingival bleeding and the numbers of Gram-negative bacteria and S. mutans [17]. In the present study, toothbrushing with the PVB dentifrice was effective for reducing the plaque index (P < 0.05) and salivary Lactobacillus spp. count (P < 0.05), providing more scientific evidence to justify the use of the product.

A large number of previous studies have evaluated the biological effects of formulations containing natural products and molecules isolated from them. However, most such studies were performed in vitro, and only a small proportion were clinical trials at different stages, which accounts for the fact that only a small number of such products have become commercially available so far [11].

In conclusion, the present data indicate that a BRP-containing dentifrice showed antimicrobial activity against salivary Lactobacillus spp. and decreased the VPI within 4 weeks in orthodontic patients. This period was considered appropriate for the type of study. Although the results are encouraging, further clinical, randomized and controlled trials will be required to assess the long-term use of the BRP dentifrice and its role in preventing tooth decay.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

References

1. Akca AE, Akca G, Topcu FT, Macit E, Pikdokçu L, Özen İŞ (2016) The comparative evaluation of the antimicrobial effect of propolis with chlorhexidine against oral pathogens: an in vitro study. Biomed Res Int 2016, 3627463.
2. Marsh PD, Zaura E (2017) Dental biofilm: ecological interactions in health and disease. J Clin Periodontol 44, 512-522.
3. Arab S, Malekshah SN, Mehrizi EA, Khanghah AE, Naehr E, Imani MM (2016) Effect of fixed orthodontic treatment on salivary flow, pH and microbial count. J Dent (Tehran) 13, 18-22.
4. Al-Melh MA, Bhaward RG, Pauline EM, Karched M (2020) Real-time polymerase chain reaction quantification of the salivary levels of cariogenic bacteria in patients with orthodontic fixed appliances. Clin Exp Dent Res 6, 328-335.
5. Mummolo S, Tieri M, Nota A, Caruso S, Darvizeh A, Albani F et al. (2020) Salivary concentrations of Streptococcus mutans and Lactobacillus spp. in children with mixed dentition during 4 weeks of orthodontic treatment. An observational study comparing fixed and removable orthodontic appliances. Clin Exp Dent Res 6, 181-187.
6. Topaloglu-Ak A, Ertrugul F, Eden E, Ates M, Balhat H (2011) Effect of orthodontic appliances on oral microbiota—6 month follow-up. J Clin Pediatr Dent 35, 433-436.
7. Tlili ET, Navarro PV, Tijhuis JM, Mariscano JA, Hennegues JP, Janoug G, Lauris JR et al. (2009) Effectiveness of 0.50% and 0.75% chlorhexidine dentifrices in orthodontic patients: a double-blind and randomized controlled trial. Am J Orthod Dent Orthop 136, 651-656.
8. Alp S, Baka ZM (2018) Effects of probiotics on salivary Streptococcus mutans and Lactobacillus levels in orthodontic patients. Am J Orthod Dent Orthop 154, 517-523.
9. Deghmani M, Abtahi M, Hasanzadeh N, Farahzad Z, Noori M, Noori M (2019) Effect of probiotics mouthwash on plaque and gingival indices over fixed orthodontic patients. J Clin Exp Dent 11, e244-e249.
10. Lobo PLD, Fonteles CSR, Marques LARV, Jamacaru FVF, Fonseca SG da C, Carvalho CBM et al. (2014) The efficacy of three formulations of Lippia sidoides Cham. Essential
oil in the reduction of salivary Streptococcus mutants in children with caries: a randomized, double-blind, controlled study. Phytomedicine 21, 1043-1047.

11. Freires IA, Sorsal PN (2016) How natural product research has contributed to oral care product development? A critical view. Pharm Res 33, 1311-1317.

12. Valadas LAR, Gurgel MF, Morojo JM, Fonseca SGDC, Fontelles CSR, de Carvalho CBM et al. (2019) Dose-response evaluation of a copaiba-containing varnish against streptococci mutants in vivo. Sisalh Pharm J 27, 363-367.

13. Furtado Junior JHC, Valadas LAR, Mendoza KS, de Oliveira Filho RD, Gadelha LMU, Fiallos NM et al. (2018) Propolis and its dental applications: a technological perspective. Recent Pat Biotechnol 12, 288-296.

14. Rodrigues Neto EM, Valadas LAR, Lobo PLD, Fernandes AMB, Fonseca SG da C, Fechine FV et al. (2020) Dose-response evaluation of propolis dental varnish in children: a randomized control study. Recent Pat Biotechnol 14, 41-48.

15. Freires IA, de Alencar SM, Rosalen PL (2016) A pharmacological perspective on the use of Brazilian red propolis and its isolated compounds against human diseases. Eur J Med Chem 110, 267-279.

16. Carvalho C de, Fernandes WHC, Moutinho TBF, Souza DM de, Marcucci MC, D’Alonpo PHP (2019) Evidence-based studies and perspectives of the use of Brazilian green and red propolis in dentistry. Eur J Dent 13, 459-465.

17. Furtado Junior JHC, Valadas LAR, Fonseca SG da C, Lobo PLD, Calixto LHM, Lima AGF et al. (2020) Clinical and microbiological evaluation of Brazilian red propolis containing-dentifrice in orthodontic patients: a randomized clinical trial. Evid Based Complement Alternat Med 2020, 1-7.

18. Berretta AA, de Castro PA, Cavalheiro AH, Fortes VS, Bom VP, Nascimento AP et al. (2013) Evaluation of Mucocadhesive Gels with Propolis (EPP-APF) in Preclinical Treatment of Candidiasis Vulvovaginal Infection. Evid Based Complement Alternat Med 2013, 207984.

19. Turkoz C, Canigur Bavbek N, Kale Varlik S, Akca G (2012) Influence of thermoplastic retainers on Streptococcus mutants and Lactobacillus adhesion. Am J Orthod Dentofacial Orthop 141, 598-603.

20. Munda A, Elhamzaoui S, Mansari AE, Souly K, Farissi F, Zouhdi M et al. (2018) Evaluation of changes in cariogenic bacteria in a young Moroccan population with fixed orthodontic appliances. Int J Dent 2018, 1-4.

21. Sanz M, Serrano J, Iniesta M, Santa Cruz I, Herrera D (2013) Antiplaque and antigingivitis activity. Nat Prod Res 31, 1318-1324.

22. Jafer M, Patil S, Hosmani J, Bhandi SH, Chalisserry EP, Anil S (2016) Plaque control strategies in the prevention of biofilm-associated oral diseases. J Contemp Dent Pract 17, 337-343.

23. Valkenburg C, Else Slot D, Van der Weijden GF (2020) What is the effect of active ingredients in dentifrices on inhibiting the regrowth of overnight plaque? A systematic review. Int J Dent Hyg 18, 128-141.

24. Bueno-Silva B, Kawamoto D, Ando-Sugimoto ES, Casarin RCV, Alencar SM, Rosalen PL et al. (2017) Brazilian red propolis effects on periodontal macrophage activity: nitric oxide, cell viability, pro-inflammatory cytokines and gene expression. J Ethnopharmacol 207,100-107.

25. Ren Y, Jongsma MA, Mei L, van der Mei HC, Buscher HJ (2014) Orthodontic treatment with fixed appliances and biofilm formation—a potential public health threat? Clin Oral Investig 18, 1711-1718.

26. Jacob A, Parolia A, Pau A, Davamani Amalraj F (2015) The effects of Malaysian propolis and Brazilian red propolis on connective tissue fibroblasts in the wound healing process. BMC Complement Altern Med 15, 294.

27. Bueno-Silva B, Kawamoto D, Ando-Sugimoto ES, Casarin RCV, Alencar SM, Rosalen PL et al. (2017) Brazilian red propolis effects on periodontal macrophage activity: nitric oxide, cell viability, pro-inflammatory cytokines and gene expression. J Ethnopharmacol 207,100-107.

28. Ren Y, Jongsma MA, Mei L, van der Mei HC, Buscher HJ (2014) Orthodontic treatment with fixed appliances and biofilm formation—a potential public health threat? Clin Oral Investig 18, 1711-1718.

29. Figuero E, Nobrega DF, Garcia-Gargallo M, Tenuta LM, Herrera D, Carvalho JC (2017) Mechanical and chemical plaque control in the simultaneous management of gingivitis and caries: a systematic review. J Clin Periodontol 44, S116-S134.

30. Santiago KB, Piana GM, Conti BJ, Cardoso EO, Andrade BFM, Zanutto MR et al. (2017) Microbiological control and antibacterial activity of a propolis-containing mouthwash and control of dental plaque in humans. Nat Prod Res 32, 1-5.

31. Kiran SD, Ghiya K, Makwani D, Bhatt R, Patel M, Srivastava M (2018) Comparison of plaque removal efficacy of a novel flossing agent with the conventional floss: a clinical study. Int J Pediatr Dent 11, 474-478.

32. Anaata Netto C, Marcucci MC, Paulino N, Anido-Anido A, Amore R, Mendonça S et al. (2015) Effects of typified propolis on mutants streptococci and lactobacilli: a randomized clinical trial. Braz Dent Sci 16, 31-36.

33. Silva MA, Valadas LAR, Girão Junior JF, Oliveira GAL, Fiallos ACM, Rodrigues Neto EM et al. (2019) Perception and adverse effects of patients after using propolis-containing dentifrice. J Young Pharm 11, 421-423.

34. Skaba D, Morawiec T, Tanasiewicz M, Mertas A, Bobela E, Szliszka E et al. (2013) Influence of the toothpaste with Brazilian ethanolic extract propolis on the oral cavity health. Evid Based Complement Alternat Med 2013, 215391.

35. Pechyeva S, Apostolova E, Gardjova P, Pechyev Z, Kokova V, Angelov A et al. (2019) Effect of Bulgarian propolis on the oral microflora in adolescents with plaque-induced gingivitis. Rev Bras Farmaco 29, 271-277.

36. Morawiec T, Dziedzic A, Niedzielska I, Mertas A, Tanasiewicz M, Skaba D et al. (2013) The biological activity of propolis-containing toothpaste on oral health environment in patients who underwent implant-supported prosthodontic rehabilitation. Evid Based Complement Alternat Med 2013, 704947.

37. Machorowska-Pieniążek A, Morawiec T, Tanasiewicz M, Dziedzic A, Król W (2013) Influence of propolis on hygiene, gingival condition, and oral microflora in patients with fixed appliances and fixed orthodontic appliances. Evid Based Complement Alternat Med 2013, 183915.

38. Biria M, Rezvani Y, Haeri A, Parhiz Z, Eslamiarabadi N, Eftekhar L (2019) Evaluation of antiplaque efficacy of a propolis-based herbal toothpaste: a single-blind parallel clinical trial. J Islam Dent Assoc Iran 31, 126-131.

39. Bhat N, Bapat S, Asava K, Täk M, Chatturvedi P, Gupta VV, George PP (2015) The antiplaque efficacy of propolis-based herbal toothpaste: a crossover clinical study. J Nat Sci Biol Med 6, 364-368.

40. Mohsin S, Manohar B, Rajesh S, Asif Y (2015). The effects of a dentifrice containing propolis on mutants streptococci: a clinico-microbiological study. Ethiop J Health Sci 25, 5-16.