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Antimicrobial potential of essential oils mouthrinses with and without alcohol: a randomized clinical trial

Matheus Grunevald[1], Romulo Cantarelli[2], Harry Juan Rivera Oballe[2], Thais de Cássia Negrini[3], Rodrigo Alex Arthur[4], Eduardo Liberato da Silva[5], Tiago Luís Herpich[1], Francisco Wilker Mustafa Gomes Muniz[6,*]

[1.] Student fellow, Federal University Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

[2.] Student fellow, Post-Graduate Program in Dentistry, Federal University Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

[3.] Post-Doctoral Student fellow, Post-Graduate Program in Bioscience and Biotechnology applied to Pharmacy, Department of Clinical Analysis, São Paulo State University, Araraquara, Brazil.

[4.] Professor of Oral Microbiology and Biochemistry and Cariology, Department of Preventive and Community Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul Brazil.

[5.] Master degree student, Post-Graduate Program in Dentistry, Federal University Rio Grande do Sul, Porto Alegre, Rio Grande do Sul Brazil.

[6.] Professor of Periodontology, Department of Semiology and Clinic, Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil.

***Corresponding author:**
Francisco Wilker Mustafa Gomes Muniz. Federal University of Pelotas. Rua Gonçalves Chaves, 457, Pelotas/RS, Brazil, ZIP Code: 96015-560. E-mail: wilkermustafa@gmail.com. Telephone: +55 53 9 91253611.

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Aim: This study aimed to compare the microbiological potential and gustatory perception of essential oils (EO) mouthrinses containing and not containing alcohol. **Methods:** Twenty healthy adult volunteers rinsed with 10mL of the following test solutions: EO with alcohol, EO without alcohol, or a control solution (saline solution with mint essence). A washout period of at least seven days was adopted after a single-use protocol of the respective solution. All participants used all three tested substances. Antimicrobial potential was assessed by counting salivary total viable bacteria both before and after each rinse. Gustatory perception was evaluated using the Visual Analogue Scale (VAS). Multiple comparisons were performed with the Wilcoxon test, using Bonferroni correction. **Results:** Both EO solutions presented a higher antimicrobial potential in comparison to the control solution ($p<0.017$). However, no significant difference in antimicrobial potential was observed between EO containing or not containing alcohol ($p=0.218$). VAS of EO with alcohol (median: 2.7) was similar to control solution (median: 1.6) ($p=0.287$). A better gustatory perception was observed of the EO without alcohol (median 7.6) when compared to the control solution ($p<0.0001$). When EO groups were compared, EO without alcohol also demonstrated a significantly better gustatory perception ($p=0.001$). **Conclusion:** Mouthrinse containing EO without alcohol presented a better taste perception when compared to the EO with alcohol, but no difference was observed in the antimicrobial potential of both EO solutions after a single rinse protocol.

Keywords: Oils volatile. Bacteria. Mouthwashes. Alcohols. Taste perception.

Introduction

The control of supragingival biofilm is essential for preventing the development of several oral diseases, including gingivitis, periodontitis, and caries. To accomplish this, the most common strategy is the mechanical removal of biofilm with toothbrushes[1]. However, the sole use of toothbrushes may not be sufficient to maintain healthy conditions in all individuals. Patients with motor or cognitive problems, lack of motivation, those undergoing post-surgical phases, and those with orthodontic devices may require the use of antimicrobial-containing mouthrinses in order to achieve effective biofilm control[2,3].

Several antimicrobial substances are available on the general market, among which essential oils (EO) present the most favorable results for gingivitis and dental plaque control in the long term. The literature constantly demonstrates better results using EO mouthrinse as an adjunct to the mechanical control of biofilm when compared to other oral hygiene regimens[4,5]. One study showed that EO presented 36.1% and 24.1% higher antiplaque and antigingivitis effects, respectively, when compared to a placebo solution[5].

Alcohol is present in the composition of several mouthrinses as a vehicle solution, despite the fact that it does not demonstrate important effects on gingivitis or plaque control[6]. Moreover, the flavor of mouthrinses is critical, as flavor may interfere with patients' adherence to treatment, especially when prescribed for long-term use[7]. Alcohol is likely responsible for the notoriously strong flavors of such solutions, which may be unpleasant for most individuals. As a result, over the last few years the industry has developed mouthrinses without alcohol. Recently, a randomized clinical trial demonstrated no significant difference in the antiplaque and antigingivitis effects of EO with or without alcohol[8]. However, at the moment, no published study has evaluated the impact of alcohol on the gustatory perception of mouthrinses containing EO. Therefore, the aim of the present study is to compare the microbiological potential and gustatory perception of EO mouthrinses containing and not containing alcohol.

Materials and Methods

Ethical aspects and study design

This is a crossover, randomized, double-blind, clinical trial that followed the Consolidated Standards of Reporting Trials (CONSORT) statement. The study was approved by the Ethical Committee of the Associação dos Funcionários do Estado do Rio Grande do Sul (protocol #1.020.949). All volunteers signed an informed consent form prior to the beginning of the study.

Sample selection

Twenty participants were included in this study (17 women and three men). This study was conducted between April and June of 2015, at the Dental Faculty of the Federal University of Rio Grande do Sul. All participants answered a questionnaire and were

clinically examined to verify the following eligibility criteria: at least 18 years of age, nonsmokers, with at least 24 natural teeth.

The study criteria excluded individuals with a presence or history of periodontitis, active dental caries, pregnant or lactating women, users of removable partial dentures, those with fixed dental prostheses or orthodontic appliances, both alcohol abstainers and alcoholics, and those who had used antibiotics within three months prior to the start of the study.

Interventions and gustatory perception assessment

All participants used all three tested substances, with a washout period of at least seven days between tests. The solutions were as follows:

- EO+ group: Essential oils in an alcoholic solution (Listerine®, Johnson & Johnson, São Paulo, Brazil). Ingredients: Aqua, Sorbitol, Alcohol, Poloxamer 407, Benzoic Acid, Sodium Saccharin, Eucalyptol, Aroma (d-limonene), Thymol, Methyl Salicylate, Sodium Benzoate, Menthol and CI 42053.
- EO- group: Essential oils in an aqueous solution (Listerine Zero®, Johnson & Johnson, São Paulo, Brazil). Ingredients: Aqua, Sorbitol, Propylene Glycol, Sodium Lauryl Sulfate, Poloxamer 407, Eucalyptol, Benzoic Acid, Aroma (d-limonene), Thymol, Methyl Salicylate, Sodium Benzoate, Menthol and CI 42053.
- Control group: Saline solution with mint essence.

All participants were instructed not to drink, eat or perform any control of biofilm, either chemical or mechanical, within one hour of the experimental procedure. Each participant rinsed for one minute using 10 mL of the predetermined solution. In order to assure blindness, all mouthrinses were stored in opaque bottles and coded accordingly by an external researcher not involved in any other study process.

The order in which participants used the mouthrinses was randomly determined by a researcher not involved in data collection (FWMGM), using a randomizing website (randomization.com). The sequence was kept in an opaque envelope until the end of all experimental procedures to assure allocation concealment. During this period, only the researchers responsible for the randomization had contact with these envelopes. The participants were not aware of which solutions they used at any experimental period.

Immediately after the rinse was performed, participants' gustatory perceptions were evaluated using a Visual Analogue Scale (VAS). The VAS was composed of a straight line of 10cm. Markers on the left indicated the most unpleasant taste, while markers on the right indicated the most pleasant taste. The participants were instructed to mark at any point on the line based on this scale. Using a ruler, the distance between the beginning of the line and the participant's mark was measured by one researcher who was blind to the group allocation (RC).

Microbiological analysis

In order to assess the antimicrobial potential of the test solutions, stimulated saliva was collected from all participants both before and after the use of each solution.

All participants were asked to chew a piece of unflavored and inert gum to stimulate salivary flow. Saliva produced during the first minute was spat out and discarded. Participants chewed the gum for an additional five minutes, with the saliva produced during this period of time collected in proper sterile and coded bottles. All pre- and post-rinse codified saliva samples were stored on ice and processed within two hours of their collection. Pre- and post-rinse saliva samples were kept on ice. Saliva samples were serially diluted in sterile 0.89% NaCl solution, and aliquots of 25 µl of each dilution were plated using the drop technique on the surface of Brain Heart Infusion Agar (BHI) supplemented with 5% sheep blood. Plates were incubated aerobically at 37°C for 48 hours[9]. Saliva was collected and analyzed only once. Colony-forming units (CFU) were counted on each drop by one blinded researcher (RC) under a stereomicroscope, and were expressed as CFU/ml of saliva according to the formula described below:

$$\text{CFU/ml saliva} = (\text{CFU} \times 1000/25) \times 10^{f} \quad (1)$$

^{f} = dilution factor from serially diluted samples.

Sample size calculation

The primary outcome of the present study was the microbiological potential. Therefore, the sample size estimation was based on data from a previously published study[10]. It considered a mean (±standard deviation) of aerobic bacteria levels in the essential oil and placebo groups of 11.35±13.11 and 56.41±38.72, respectively. When it was considered a power of 90%, an alpha of 5%, and a total of 16 individuals were necessary. An attrition rate of 20% was expected, totaling 20 participants.

Statistical analysis

For each individual, the percentage of reduction in salivary total bacterial viability was calculated considering pre- and post-rinse saliva samples. The differences in the antimicrobial potential and gustatory perception were analyzed using the Friedman test, as a non-normal distribution was detected in both outcomes. We analyzed data distribution using the Shapiro-Wilk test. As a p-value <0.001 was detected in the Shapiro-Wilk test, multiple pair-wise comparisons were performed using the Wilcoxon test. A Bonferroni correction was established, and the new p-value for statistical significance was <0.017.

Results

Twenty-four individuals were recruited for the present study, among whom four were excluded because they did not fit the inclusion criteria. Reasons for exclusion are reported in Figure 1. Among the included individuals, the response rates were 100% for all follow-up periods. Furthermore, no adverse events were reported throughout the study.

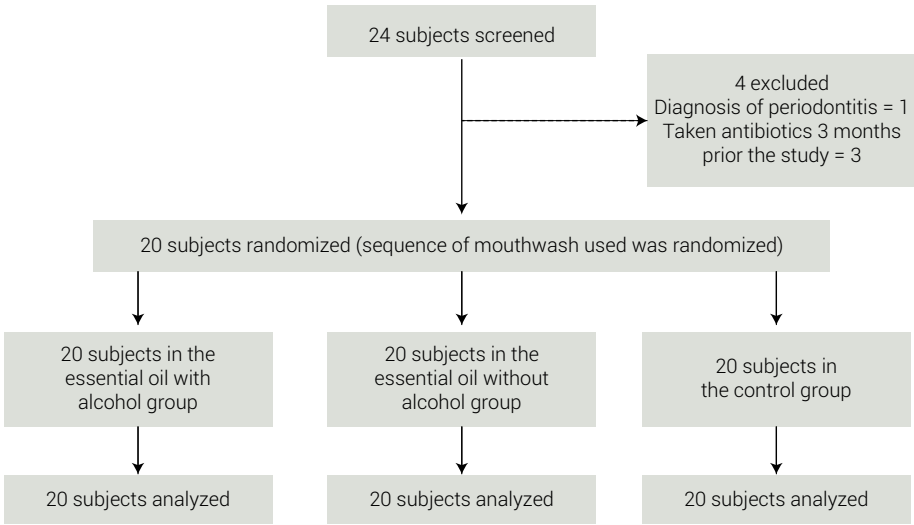

Figure 1. Flowchart of the participants in the study.

During microbiological analysis, no differences were found regarding counts of salivary viable bacteria among groups by the pre-rinsing analysis (p=0.387). Table 1 shows the mean percentage reduction of the total viable bacteria of all three groups. By comparing the percentage of reduction in counts of viable bacteria before and after each rinse, a significant reduction was found (p<0.05), with 60.33±29.33, 62.61±39.25 and 4.16±156.55 in the EO with alcohol, EO without alcohol and control groups, respectively. The antimicrobial potential of both EO mouthrinses was significantly higher than that of the control group (p=0.007 for EO with alcohol and p=0.005 for EO without alcohol). However, when both EO groups were compared, no significant difference was demonstrated (p=0.218).

Table 1. Mean±Standard deviation values for each time point and groups of the colony forming-units of total aerobes.

Group	Mean±SD			P-value	
	Before rinsing	After rinsing	Mean percent reduction	Within groups	Between groups (percentage reduction)
EO +	$1.87x10^8±1.62x10^8$	$0.71x10^8±1.06x10^8$	60.33±29.33	<0.001#	0.218Ω 0.007µ 0.005α
EO -	$3.73x10^8±12.5x10^8$	$0.52x10^8±1.31x10^8$	62.61±39.25	<0.001#	
Control	$6.46x10^8±11.66x10^8$	$5.29x10^8±12.99x10^8$	4.16±156.55	0.029#	
P-value between groups	0.387*	0.074*	0.017*		

Legend: SD: standard deviation; *Friedman test; #Wilcoxon test for the comparison within groups; Ω Wilcoxon test for the comparison between EO+ and EO- groups; µWilcoxon test for the comparison between control and EO+ groups; α Wilcoxon test for the comparison between control and EO- groups.

The gustatory perceptions of all tested solutions are reported in Table 2. These results demonstrated a statistically significant difference in gustatory perception among groups. The control group reported the worst flavor (median 1.6), the EO- group reported the most pleasant gustatory perception (median 7.6), and the EO+ group pre-

sented an intermediate perception (median 2.7). When groups EO+ and control were compared, no statistically significant difference was detected (*P*=0.287). However, in the comparison between EO- and control groups, a statistically significance difference was detected, showing a better gustatory preference for the EO- mouthrinse (*P*<0.001). Regarding the comparison between EO groups, a significantly better gustatory preference was observed for the EO- mouthrinse (*P*=0.001).

Table 2. Gustatory perception after a single rinse with essential oils with alcohol, without alcohol, and control substance.

	Control	EO+	EO-	P-value
Median (min./max.)	1.6 (0.0 – 8.4)	2.7 (0.1 – 10.0)	7.6 (0.5 – 9.9)	<0.001* α 0.001 Ω 0.301#
Mean±SD	2.81±2.72	3.55±2.95	6.90±2.25	

Legend: EO+: essential oil with alcohol; EO-: essential oil without alcohol; *Friedman test; #Wilcoxon test for the comparison between control and EO+ groups; α Wilcoxon test for the comparison between control and EO- groups; Ω Wilcoxon test for the comparison between EO+ and EO- groups.

Discussion

The present study aimed to compare the microbiological potential and gustatory perceptions of EO mouthrinses containing and not containing alcohol. Overall, it was observed that both solutions presented a significantly higher antimicrobial potential when compared to a negative control solution. However, both EO solutions presented similar antimicrobial potential. Regarding the gustatory potential, participants reported the EO without alcohol as having the most pleasant taste.

The chemical control of supragingival biofilm may be performed with mouthrinses. Among these, chlorhexidine is considered the gold standard substance, as it demonstrates a good antiplaque effect and long substantivity; however, several adverse events may be detected after long periods of use[11-13]. Other mouthrinses also demonstrate antiplaque and antigingivitis effects with fewer reported adverse events, and these substances may be used for longer periods[14]. However, a strong, unpleasant flavor and a burning sensation have been reported by the patients who use these mouthrinses.

Among other available mouthrinses, EO may be the most important one for patient outcomes[5]. In low concentrations, EO may inactivate bacterial enzymes, interfering with growth velocity and biofilm maturation. Additionally, its utilization may be an encouraging factor to enhance patient adherence to this method of supragingival biofilm control[15]. Traditionally, mouthrinses containing EO also contain alcohol in their composition. The alcohol is used to dilute or solubilize the oils, and it is also used to extend the product's expire date. One study has shown that alcohol may present some antimicrobial effects[16], but a systematic review showed that an alcoholic vehicle demonstrated very limited effects in terms of antiplaque and antigingivitis efficacy[6]. In addition, short-[17] and long-term[8] clinical trials have demonstrated no statically significant difference in the antiplaque and/or antigingivitis efficacy of EO with or without alcohol.

These results are in accordance with the present study, which demonstrated no significant difference in the antimicrobial potential of the two EO solutions. However, fur-

ther long-term clinical trials are necessary to determine the clinical efficacy of both EO mouthrinses. The mechanical process of chewing an inert piece of gum detaches microorganisms from mucosal and tooth sites. Using this method, microbial diversity found on stimulated saliva is representative of other oral niches. Moreover, stimulated saliva has the advantage of carrying a greater microbial diversity than unstimulated saliva[18]. Considering that one of the outcomes of this study was to assess the antimicrobial effects of EO-containing mouthrinses, it was decided that this study would use stimulated saliva to evaluate this effect on a broad load and diversity of microorganisms (which could not be found by using unstimulated saliva).

The presence of alcohol can also negatively affect the gustatory perception of some individuals due to the strong flavor[4], although a previously published study demonstrated that alcohol was not capable of interfering in the taste perception of chlorhexidine solutions[9]. The literature reports that the flavor of mouthrinses is an important factor in their usage, and it is part of the criteria used by patients when choosing a mouthrinse[7]. It must be highlighted that several mouthrinses are sold on the general market. A prescription may or may not be necessary to buy these products, depending on the laws of different countries. Additionally, as many of these products are designed for continuous usage, a pleasant flavor is a pivotal factor for the ongoing use of the mouthrinse and adherence to the recommended treatment.

A VAS was used in order to measure participants' gustatory perceptions. This is a valid method for quantifying the taste[19,20]. In the present study, participants performed the evaluation immediately after rinsing, without having access to their previous evaluations, which did not allow comparisons. It was found that the most pleasant gustatory perception was reported for the EO not containing alcohol. Therefore, these results must be taken into consideration when prescribing an EO mouthrinse, as individuals with alcohol restrictions and those with higher sensitivity to alcohol should not receive an EO mouthrinse with alcohol.

The present study used commercially available solutions with essential oils. Additionally, a saline solution with mint essence was used. This was a double-blind and cross-over study, which reduced the chance of any interference from knowledge of which substance was tested. Furthermore, the washout period of at least seven days allowed a decrease in any potential residual effects during the study. The order of the tested solutions was determined randomly, avoiding any adaptation among the individuals throughout the study. In the present study, twenty individuals were included. Although this number may seem small, previous studies regarding the chemical control of biofilm have used similarly sized and valid samples[21,22]. Regarding the study participants, it is important to highlight that no dropouts occurred and no adverse events were reported.

Some limitations to the present study must be acknowledged, such as the fact that each rinsing was performed only once. Additionally, the included individuals were young and some of them were students of the School of Dentistry of the Federal University of Rio Grande do Sul. In this sense, these characteristics may have interfered with the study results, decreasing the external validity of the present study[23]. Therefore, further clinical trials involving only non-health professionals are warranted.

In addition, the present study included a higher number of female participants, and a lower internal validity might be expected for male individuals.

In conclusion, no significant difference was observed in the antimicrobial potential of EO with and without alcohol. However, it was concluded that the mouthrinse containing EO without alcohol was widely evaluated as having a more pleasant taste when compared to the flavor of an EO mouthrinse with alcohol.

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References

1. Van der Weijden FA, Slot DE. Efficacy of homecare regimens for mechanical plaque removal in managing gingivitis a meta review. J Clin Periodontol. 2015 Apr;42 Suppl 16:S77-91. doi: 10.1111/jcpe.12359.
2. Haas AN, Pannuti CM, Andrade AK, Escobar EC, Almeida ER, Costa FO, et al. Mouthwashes for the control of supragingival biofilm and gingivitis in orthodontic patients: evidence-based recommendations for clinicians. Braz Oral Res. 2014 Jul 11;28(spe):1-8. doi: 10.1590/1807-3107bor-2014.vol28.0021.
3. de Andrade Meyer AC, de Mello Tera T, da Rocha JC, Jardini MA. Clinical and microbiological evaluation of the use of toothpaste containing 1% chlorhexidine and the influence of motivation on oral hygiene in patients with motor deficiency. Spec Care Dentist. 2010;30(4):140-5. doi: 10.1111/j.1754-4505.2010.00140.x.
4. Van Leeuwen MP, Slot DE, Van der Weijden GA. Essential oils compared to chlorhexidine with respect to plaque and parameters of gingival inflammation: a systematic review. J Periodontol. 2011 Feb;82(2):174-94. doi: 10.1902/jop.2010.100266.
5. Haas AN, Wagner TP, Muniz FWMG, Fiorini T, Cavagni J, Celeste RK. Essential oils-containing mouthwashes for gingivitis and plaque: Meta-analyses and meta-regression. J Dent. 2016 Dec;55:7-15. doi: 10.1016/j.jdent.2016.09.001.
6. Van Leeuwen MP, Slot DE, Van der Weijden GA. The effect of an essential-oils mouthrinse as compared to a vehicle solution on plaque and gingival inflammation: a systematic review and meta-analysis. Int J Dent Hyg. 2014 Aug;12(3):160-7. doi: 10.1111/idh.12069.
7. Cantarelli R, Ribas ME, Daudt FARL, Rosing CK. [Use profile of mouthwash used by patients at the UFRGS School of Dentistry]. Perionews. 2011;5:361-7. Portuguese.
8. Lynch MC, Cortelli SC, McGuire JA, Zhang J, Ricci-Nittel D, Mordas CJ, et al. The effects of essential oil mouthrinses with or without alcohol on plaque and gingivitis: a randomized controlled clinical study. BMC Oral Health. 2018 Jan 10;18(1):6. doi: 10.1186/s12903-017-0454-6.
9. Cantarelli R, Negrini TC, Muniz FW, Oballe HJ, Arthur RA, Rösing CK. Antimicrobial potential and gustatory perception of chlorhexidine gluconate mouthwashes with or without alcohol after a single rinse - a randomized controlled crossover clinical trial. Int J Dent Hyg. 2017 Nov;15(4):280-6. doi: 10.1111/idh.12255.
10. Cortelli JR, Cogo K, Aquino DR, Cortelli SC, Ricci-Nittel D, Zhang P, et al. Validation of the anti-bacteremic efficacy of an essential oil rinse in a Brazilian population: a cross-over study. Braz Oral Res. 2012 Sep-Oct;26(5):478-84. doi: 10.1590/s1806-83242012005000021.

11. Erriu M, Pili FM, Tuveri E, Pigliacampo D, Scano A, Montaldo C, et al. Oil Essential mouthwashes antibacterial activity against aggregatibacter actinomycetemcomitans: a comparison between antibiofilm and antiplanktonic effects. Int J Dent. 2013;2013:164267. doi: 10.1155/2013/16426.

12. Ajay Rao HT, Bhat SS, Hegde S, Jhamb V. Efficacy of garlic extract and chlorhexidine mouthwash in reduction of oral salivary microorganisms, an in vitro study. Anc Sci Life. 2014;34(2):85-8. doi: 10.4103/0257-7941.153465.

13. Ros-Llor I, Lopez-Jornet P. Cytogenetic analysis of oral mucosa cells, induced by chlorhexidine, essential oils in ethanolic solution and triclosan mouthwashes. Environ Res. 2014 Jul;132:140-5. doi: 10.1016/j.envres.2014.03.032.

14. Gunsolley JC. A meta-analysis of six-month studies of antiplaque and antigingivitis agents. J Am Dent Assoc. 2006 Dec;137(12):1649-57. doi: 10.14219/jada.archive.2006.0110.

15. Stoeken JE, Paraskevas S, van der Weijden GA. The long-term effect of a mouthrinse containing essential oils on dental plaque and gingivitis: a systematic review. J Periodontol. 2007 Jul;78(7):1218-28. doi: 10.1902/jop.2007.060269.

16. Sissons CH, Wong L, Cutress TW. Inhibition by ethanol of the growth of biofilm and dispersed microcosm dental plaques. Arch Oral Biol. 1996 Jan;41(1):27-34. doi: 10.1016/0003-9969(95)00103-4.

17. Marchetti E, Tecco S, Caterini E, Casalena F, Quinzi V, Mattei A , et al. Alcohol-free essential oils containing mouthrinse efficacy on three-day supragingival plaque regrowth: a randomized crossover clinical trial. Trials. 2017 Mar 31;18(1):154. doi: 10.1186/s13063-017-1901-z.

18. Simón-Soro A, Tomás I, Cabrera-Rubio R, Catalan MD, Nyvad B, Mira A. Microbial geography of the oral cavity. J Dent Res. 2013 Jul;92(7):616-21. doi: 10.1177/0022034513488119.

19. Miller MD, Ferris DG. Measurement of subjective phenomena in primary care research: the Visual Analogue Scale. Fam Pract Res J. 1993;13(1):15-24.

20. Kimberlin CL, Winterstein AG. Validity and reliability of measurement instruments used in research. Am J Health Syst Pharm. 2008 Dec;65(23):2276-84. doi: 10.2146/ajhp070364.

21. Sennhenn-Kirchner S, Wolff N, Klaue S, Mergeryan H, Borg-von Zepelin M. Decontamination efficacy of antiseptic agents on in vivo grown biofilms on rough titanium surfaces. Quintessence Int. 2009;40(10):e80-8.

22. Marchetti E, Casalena F, Capestro A, et al. Efficacy of two mouthwashes on 3-day supragingival plaque regrowth: a randomized crossover clinical trial. Int J Dent Hyg. 2017 Feb;15(1):73-80. doi: 10.1111/idh.12185.

23. Kim KJ, Komabayashi T, Moon SE, Goo KM, Okada M, Kawamura M. Oral health attitudes/behavior and gingival self-care level of Korean dental hygiene students. J Oral Sci. 2001 Mar;43(1):49-53. doi: 10.2334/josnusd.43.49.