Effect of antimicrobial activity of *Teucrium polium* on oral *Streptococcus mutans*: a randomized cross-over clinical trial study

Somayeh Khoramian Tusi
Alborz University of Medical Sciences
Ahmad Jafari (ajafari@tums.ac.ir)
Tehran University of Medical Sciences Dental School https://orcid.org/0000-0001-9164-8264
Seyed Mahmoud Amin Marashi
Qazvin University of Medical Sciences
Salomeh Faramarzi Niknam
Alborz University of Medical Sciences
Malihe Farid
Alborz University of Medical Sciences
Mehdi Ansari
Kerman University of Medical Sciences

Research article

Keywords: mouthwash, medicinal plants, salvia, herbal extract, dental caries, oral hygiene

Posted Date: February 5th, 2020

DOI: https://doi.org/10.21203/rs.2.22624/v1

Version of Record: A version of this preprint was published at BMC Oral Health on May 1st, 2020. See the published version at https://doi.org/10.1186/s12903-020-01116-4.

Abstract

Background : The purpose of this study was to determine the effect of a mouthwash containing *Teucrium polium* herb on Streptococcus mutans in mouth. Methods : This study was a randomized, crossover, double-blind clinical trial, where twenty-two volunteer dental students were randomly divided into two groups. The study had two phases. In each phase, one group was chosen as the intervention group, while the other one was the control group. Both the intervention and control groups were given the mouthwash with and without *Teucrium polium* , respectively. *S. mutans* of saliva were measured before and after each phase to compare the effects of the mouthwashes. A three-week washout period was considered between the two phases. An independent two-sample t-test was used to compare the mean number of *S. mutans* colonies. Additionally, a standard AB/BA crossover model was used to find the results of the treatment and the carryover effect on the residual biological effects. The significance level was considered 0.05 in this experiment. Results : There was no significant difference between the two groups in the number of *S. mutans* before using the mouthwashes. Using the mouthwash containing Teucrium polium extract significantly decreased the number of *S. mutans* colonies in both phases (P = 0.002). Conclusion : The results of this study showed that the mouthwash containing aqueous extract of *Teucrium polium* can significantly reduce the colonization of *S. mutans* in human saliva.

Background

A healthy mouth is the gate of a healthy body. The effect of oral health on many systemic diseases and cancers has already been proved [1–3]. Amongst a wide variety of microorganisms, Streptococcus pathogens such as Streptococcus mutans (S. mutans) not only cause dental caries, but they also decrease the general health and oral hygiene. Ultimately, these bacteria can impose medical expenses or teeth loss. Therefore, finding substances that have the capability to remove or minimize these microbial species is of high importance in every respect. In recent years, there has been great attention towards using herbal medicines (based on the knowledge of traditional medicine) for preventing and treating illnesses. Dental science keeps up with other medical specialties to use herbal and traditional medicine in oral health. Some studies have been carried out to investigate the effect of medicinal plants as antibacterial agents in dentistry [4, 5]. Additionally, herbal extracts have been used in oral hygiene products. Polei-gamander, with the scientific name of Teucrium polium (T. polium), is a kind of peppermint that is a component of aromatic plants. It contains some organic materials with antibacterial effects [6]. T. polium or Polei-gamander is one of the medicinal herbs in traditional medicine that is used to treat cancer [6], gastrointestinal disorders [7], kidney and kidney stones [8], wound healing [9], and diabetes [10].

In dentistry, T. polium has been used to produce probiotic compounds containing herbal extracts [11]. Antimicrobial mouthwashes work in a variety of ways, including the connection to the bacterial cell wall and the destruction of the wall. Extensive studies, however, are needed to fully understand the mechanism of the effect of antibacterial activity of T. polium on S. mutans. The aim of this study was to investigate the effect of a mouthwash containing T. polium herb on the number of salivary Streptococcus mutans.

Methods

An AB/BA randomized, crossover, double-blind clinical trial study was designed (Fig. 1). Ethical issues were approved by the Ethics Committee of the Rafsanjan University of Medical Sciences with the approval number of 937/9/31 and Iranian Registry of Clinical Trials (IRCT) code No. IRCT2013121815842N1.

Inclusion criteria:

Inclusion criteria included 1) not using antibiotics in the last month, 2) not having active caries, 3) plaque index equal to or less than 20%, 4) aging from eighteen to twenty-five years old, and 5) signing the consent form before participating in the study.

Samples:

Samples were selected from dental students of Rafsanjan School of Dentistry. Sample size was calculated based on a previous study [12]. The following formula was used to estimate the sample size:

$$n_1 = n_2 = \frac{\sigma_d^2\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2}{2\Delta^2} \quad (\alpha = 0.05 \rightarrow Z_{1-\frac{\alpha}{2}} = 1.96;\ \beta = 0.10 \rightarrow Z_{1-\beta} = 1.29;\ \sigma_d = 5.61;\ \Delta = 4)$$

Therefore: $n_1 = n_2 \approx 11$. So, each group needed eleven samples.

Twenty-two volunteers of dental students were randomly divided into the intervention and control groups, each including 11 individuals of both genders.

The numbers one and two representing intervention and control groups respectively were written on twenty-two pieces of paper (11 each), and were put in a bowl. Students were asked to pull a piece of paper one by one, so that other students would not be aware of each other's grouping. Their groups were assigned based on the random selection of the papers. Each student was given a two-digit code (01 to 22), and these codes were used during the trial instead of their names.

The study had two phases. In each phase, one group was chosen as intervention, while the other group was the control group. Intervention and control groups were given the mouthwash with and without T. polium, respectively. The codes of the students were written on mouthwash bottles and were given to the students, so that neither the students nor the laboratory personnel were aware of the type of mouthwash (double-blinded).

The volunteers were asked not to change their usual health care practices, but keep 15 mL of the mouthwash in their mouth for 30 seconds twice a day for two weeks. They, however, were requested to neither have food or beverages nor wash their mouth for almost 30 minutes [13]. After these two weeks, they entered the 3-week washout phase (not using mouthwash) in order for their saliva's S. mutans value to return to initial levels [14]. In this phase, opposite to the first phase, placebo and T. polium mouthwashes were given to the first and second groups. Then, they were again asked not to change their usual health care habits, but keep 15 mL of the mouthwash in their mouth for 30 seconds twice a day for two weeks.

Mouthwash preparation:

The extract of T. polium was prepared at the Faculty of Pharmacy, Kerman University of Medical Sciences. T. polium was collected from the mountain area around Kerman, the capital city of Kerman province in the South East of Iran, in June. Plant flowers were cleaned, then washed with cold water and deionized water (Zolal Teb Shimi, Tehran, Iran), and were dried in a place away from direct sunlight. The dried samples were grounded with an electric mill (Moulinex 1043, Paris, France), and passed through a sieve with a mesh size of 32. Next, 250 g of the powder was soaked in 2L of water for 48 hours and was filtered with a vacuum pump (Eyela A-35, Tokyo, Japan), a Buchner funnel (Isolab, Hannover, Germany), and a filter paper (Munktell, Bavaria, Germany). Obtained extracts were concentrated by vacuum distillation method at 52 °C using a rotary machine (Lab Tech EV 311-V, Rome, Italy). Eventually, concentrated extracts were dried by oven (Memmert UF-55, Frankfurt, Germany) at 42 °C for 3 days. Extraction yield was 10%. Dried extract was kept in a freezer at -22 °C for further experiment.

All components of the mouthwash except for T. polium extract were utilized for the preparation of the placebo. Distilled water (Zolal Teb Shimi, Tehran, Iran) was used instead of T. polium extracts to prepare the placebo.

Prepared mouthwashes were packed in matte plastic containers, and then were tagged with specific codes that were only known to the researchers, and were given to the volunteers. Each compound was prepared at the earliest time to its consumption to ensure the highest drug stability and the highest amount of active ingredients. After extract preparation, 25% concentration of mouthwash was chosen for further experiment due to its adequate taste. The mouthwash ingredients were as below: 1L deionized water, 2 g T. polium extracts, 1 g artificial sweetener powder of 0.1% aspartame (Merck, Darmstadt, Germany), and 5 g powder of 0.5% coffee flavor (Nestle, Netherland, Holland).

Saliva Sampling

To sampling the volunteers' saliva, they were asked not take food or beverage for almost one hour prior to sampling. Sampling, however, was carried out every day at 10 a.m. in which 2 mL of their unprovoked saliva was collected in a sterile container [15].Unprovoked saliva sample of all individuals was taken at the beginning of the study, before using the mouthwash. The second test was arranged at last day of the first phase of experiment using a spitting method by which the volunteers were asked to take a minute's saliva in their mouth and then pour in a sterile falcon tub. Third and fourth tests were conducted before and after the second phase. During the experiment, the volunteers were asked not change their daily health care practices and brush their teeth using Crest toothpaste and Oral B toothbrush.

Colony Count

To determine the number of Streptococcus mutans colonies, samples were sent to the Laboratory of Microbiology, University of Rafsanjan, Iran. One day before saliva sampling the culture medium was prepared. The TYCSB (Tryptone-Yeast-Cysteine-Sucrose-Bacitracin) medium of S. mutans was prepared as mentioned in the standard protocol [16]. Normal saline sterile (Zolal Teb Shimi, Tehran, Iran) was used to make a 1:1000 dilution of the samples, and then they were transferred to the plates containing 20 mL Agar TYCSB using a 0.01 ml standard loop (Lab Tron, Tehran, Iran) [17].The plates were placed in a CO_2 incubator (Gallenkamp, Munich, Germany) at37 °Cfor 48 h.

Thereafter, Gram's method was used to distinguish between gram negative and positive bacteria. Gram-positive colonies (cocci) were selected to perform the catalase test. Next, catalase-negative cocci were incubated under biochemical tests for 48 hours at 37 ° C. Based on previous recommendations [18, 19], colonies of positive mannitol, positive vogues-proskauer (VP), negative arginine, positive dextran, negative urease, and positive bile esculin were the target colonies. Eventually, Streptococcus mutans' colonies were calculated and were multiplied by dilution ratio to determine the number of colonies per one mL of each participant's saliva (CFU/mL).

Statistical Analysis

Data was analyzed by SPSS software (Version 18.0). Quantitative data were reported as mean value and standard deviation. Qualitative data was reported as number and percentage. Paired t-test was used to determine the effect of each mouthwash on the Streptococcus mutans's colonies. Moreover, an independent two-sample t-test was used to compare the mean number of Streptococcus mutans's colonies after mouthwash consumption in each phase of the experiment. Besides, crossover analysis was used to compare the mean of Streptococcus mutans's colonies after using T. polium and placebo mouthwashes during the study. Finally, a standard AB/BA crossover model was used to find the results of treatment and the carryover effect on the residual biological effects after using the mouthwash until the final phase [20]. The significant level, however, was considered to be 0.05 in this experiment.

Ethical Consideration

Ethical issues approved by the Ethics Committee of the Rafsanjan University of Medical Sciences with the approval number of 937/9/31 and IRCT code No. IRCT2013121815842N1. An Informed consent was taken from participants and there was no compulsion to participation in the study. Attending or not attending had no effect on student education.

Consolidated standards of reporting trials (CONSORT) flow diagram

CONSORT flow diagram is seen in Fig. 2.

Results

Twenty-two dental students (11 male and 11 female students) participated in this study. The average age of participants was 23 (21 to 26 years old. The age difference between the two groups was not significant. The first group were the students who used *T. polium* mouthwash in the first phase, and then used the placebo mouthwash during the second phase. The second group of students, however, used the *T. polium* and placebo mouthwash conversely. The first phase lasted for two weeks, and was followed by a three-week washout period. The second phase commenced after the washout period and lasted for two weeks. The results showed that there was no statistical difference between the numbers of *Streptococcus mutans*'s colonies per one mL of saliva in the groups before using the mouthwashes (Table1).

In the first phase, *T. polium* mouthwash significantly pulled down the number of *S. mutans*' colonies (P< 0.001). However, placebo mouthwash did not significantly decrease the number of *S. mutans*' colonies (P= 0.340). Also, the mean change (decrease) in *S. mutans'* colonies was significantly higher in *T. polium* mouthwash than placebo mouthwash (P< 0.001) (Fig 3, Table 2).

At the second phase, after three weeks of washout period (without using the mouthwashes), the first group used the placebo mouthwash, while the second group used the *T. polium* mouthwash. Placebo mouthwash, again, did not significantly decrease the number of *S. mutans'* colonies (P = 0.279). The use of *T. polium* mouthwash resulted in a significant reduction in the number of *S. mutans'* colonies (P <0.001). Moreover, the mean change (decrease) in the number of *S. mutans'* colonies in the *T. polium* group was significantly higher than the placebo group (P= 0.001) (Table 2).

Finally, standard AB/BA crossover model analysis was conducted to find the carryover effect. The difference between the change in *S. mutans* colonies before and after using mouthwashes in first phase (Y1) and second phase (Y2) were calculated. This difference was significant (P<0.001). It showed that an effective treatment or carryover effect existed between the treatments. To find whether the effect was due to the treatment or carry effect, the second part of this analysis was done. In this part the sum of Y1 and Y2 was analyzed, and showed no significant differences, which approves that there is no carryover effect. Comparison of *T. polium* and placebo mouthwashes using crossover analysis showed that *T. polium* mouthwash significantly reduced the number of *S. mutans*' colonies (P<0.001) (Table 3).

Discussion

Tooth decay is an epidemic problem in the world. While chemical mouthwashes have their own side effects such as discolorations of tongue and tooth surfaces, altered taste perception, soft tissue irritations and burning sensation [21], herbal mouthwashes can be an appropriate substitute for them [22, 23, 24]. Results of this study proved that a mouthwash containing *T. polium* can effectively decrease *S. mutans* population in the mouth, and this effect can last for at least three weeks after using the mouthwash.

During the first phase of the study students became aware of their treatments due to a tangible difference in the taste of the mouthwash containing *T. polium*. However, this limitation was predicted before the study and several steps were designed to reduce its impact on the outcomes. First, students voluntarily participated in the study and the importance of following the study protocol was discussed with them. Additionally, samples were selected among dental students (not from the society) and were more likely to cooperate with the research team. Second, the study was designed as an AB/BA cross-over study, and a standard AB/BA crossover statistical model was utilized to analyze the outcomes and test the possible carryover effects.

S. mutans, is considered to be the most important and pathogenic decay microorganism and plays a major role in the onset of caries [25]. In the present study, the effect of *T. polium* mouthwash on saliva's *S. mutans* has been investigated. The design of the study in cross-over model has been considered as its strongpoint since its two-step design (two phases) increases its accuracy. The positive results of the two phases, however, emphasize the efficacy of the antibacterial properties of the polei-gamander plant on *S. mutans*. In this cross-over study, two positive effects of *T. polium* mouthwash were observed. These effects include a reduction in the number of *S. mutans* colonies during the time when *T. polium* mouthwash was used and the continuous antimicrobial effect of the mouthwash in the washout period. In the intervention group, there was a significant reduction in *S. mutans* in both phases, while in the control group only a slight decrease in the microbial colonies was obtained due to the regular use of mouthwash. The counting of microbial colonies of saliva before and after each phase shows the capability of this study to be effective.

In studies that investigate the antimicrobial effects of mouthwashes, the duration of the washout period is usually two to three weeks [24, 26]. In this study, however, the washout period was three weeks after which the *S. mutans* value did not return to the initial level. This indicates the potent antibacterial effect of the *T. polium* mouthwash. Consequently, at the end of the second phase, when both groups had used *T. polium* mouthwash, similar results of a reduction in the microbial colony were observed. Using standard AB/BA crossover model analysis [20] showed there were no carryover effects in the study.

Studies on antibacterial activity of herbal plants have had different results. The findings of this study showed that *T. polium* mouthwash, compared to the placebo mouthwash, significantly reduced the rate of salivary *S. mutans*. Mehta *et al* (2013) showed that a commercial herbal mouthwash had similar effect on reducing the salivary *S. mutans* as chlorhexidine (P = 0.639) [24]. Shah *et al*. (2018) had a randomized controlled pilot study on comparing an herbal mouthwash and a mouthwash containing 0.2% chlorhexidine on reducing salivary *S. mutans*. Their herbal mouthwash containing twelve herbal plants was significantly more effective than the 0.2% chlorhexidine mouthwash in reducing oral *S. mutans* population (P < 0.001). They also showed that their herbal mouthwash had no side effects on oral or dental tissues [23]. Additionally, a systematic review in 2018 showed that herbal products exerted almost comparable antibacterial effect against *S. mutans* comparing with chlorhexidine [27]. Haffajee *et al*. [28] found that herbal mouthwashes can be effective in preventing the growth of oral microbes and can be useful in controlling dental plaques and inflammation of the gum. The range of effectiveness of antibacterial effect for polei-gamander plant has been widely reported in different studies. Mosadegh *et al*. [29] reported a weak antibacterial effect of ethanolic extract of polei-gamander plant on *Staphylococcus*, *Micrococcus luteus*, and *Escherichia coli* while the antibacterial effect in our study was strong. The differences in such reports might be due to different types of bacteria, and different extraction methods. Our study showed a positive result, however, the effects of *T. polium* mouthwash has not been compared with that of chlorhexidine as a gold standard. Nevertheless, it is suggested to investigate and compare the effect of polei-gamander plant's active ingredients on different bacterial strains with that of chlorhexidine in future studies.

Conclusion

The results of the present research indicate that the aqueous extract of *T. polium* significantly reduces colonization of *Streptococcus mutans* in human saliva. This decline was visible even after three weeks of washout period. Overall, it can be concluded that periodically, the use of *T. polium* mouthwash reduces the risk of tooth decays. It can also be used in other oral hygiene materials like toothpastes or chewing gums.

Abbreviations

S. mutans
Streptococcus mutans
T. polium
Teucrium polium
IRCT
Iranian Registry of Clinical Trials
TYCSB
Tryptone-Yeast-Cysteine-Sucrose-Bacitracin
VP
vogues-proskauer
CFU
colony forming unit
CONSORT
Consolidated Standards of Reporting Trials

Declarations

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Rafsanjan University of Medical Sciences with the approval number of 937/9/31 and Iranian Registry of Clinical Trials (IRCT) code No. IRCT2013121815842N1.

Consent for publication

Not Applicable

Availability of data and materials

SPSS File for Teucrium Polium Mouth Wash Effect Study is attached as a supplemental file named S 1 SPSS file.

Competing interests

The authors declare that there are not any competing interests.

Funding

No funding was obtained for this study

Authors’ contributions

SKT made contributions to the conceptual design of the work, and also drafted the work and revised early drafts.

AJ made contributions to the conceptual design of the work, and also drafted the work and revised early drafts and submitted the manuscript.

SMAM made contributions to supervise the microbial tests.

SFN wrote the manuscript and revised early drafts.

MF helped in the acquisition, analysis, and interpretation of data.

MA made contributions to prepare the extract of Teucrium polium and mouthwashes.

All authors read and approved the final manuscript.

Acknowledgments

The authors wish to thank Rafsanjan University of Medical Sciences for their all supports and to be grateful for students' participating in the study.

References

1. Farrell, J. J., Zhang, L., Zhou, H., Chia, D., Elashoff, D., Akin, D., ... & Wong, D. T. Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer. Gut. 2012;61(4):582-588.
2. Weidlich, P., Cimões, R., Pannuti, C. M., & Oppermann, R. V. Association between periodontal diseases and systemic diseases. Brazilian oral research. 2008;22:32-43.
3. Meyer, K., Geurtsen, W., & Günay, H. An early oral health care program starting during pregnancy. Clinical oral investigations. 2010;14(3):257-264.
4. Palombo, E. A. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evidence-Based Complementary and Alternative Medicine. 2011;2011.
5. Vlachojannis, C., Winsauer, H., & Chrubasik, S. Effectiveness and safety of a mouthwash containing essential oil ingredients. Phytotherapy Research. 2013;27(5):685-691.
6. Rajabalian, S. Methanolic extract of Teucrium polium L potentiates the cytotoxic and apoptotic effects of anticancer drugs of vincristine, vinblastine and doxorubicin against a panel of cancerous cell lines. Experimental Oncology.2008
7. Ali-Shtayeh, M. S., Yaniv, Z., & Mahajna, J. Ethnobotanical survey in the Palestinian area: a classification of the healing potential of medicinal plants. Journal of Ethnopharmacology. 2000;73(1-2):221-232.
8. Al-Khalil, S. A survey of plants used in Jordanian traditional medicine. International Journal of Pharmacognosy. 1995;33(4):317-323.
9. Alizadeh, A. M., Sohanaki, H., Khaniki, M., Mohaghgheghi, M. A., Ghmami, G., & Mosavi, M. The effect of Teucrium Polium Hney on the wound healing and tensile strength in rat. Iranian journal of basic medical sciences. 2011;14(6):499.
10. Alzweiri, M., Al Sarhan, A., Mansi, K., Hudaib, M., & Aburjai, T. Ethnopharmacological survey of medicinal herbs in Jordan, the Northern Badia region. Journal of Ethnopharmacology. 2011;137(1):27-35.
11. Mahmoudi R, Kazeminia M, Ghajarbeygi P,Pakbin B. An introductory review on increasing the survival of probiotic bacteria in dairy products using essential oil. Journal of Dental and Oral Health.069:(4)3;2017

12. Khosravanifard B, Ghasemi M, Rastegarian H, Sajadi H, Emami H, Amani M. Effect of three different mouthrinses on *mutans Streptococci* accumulation around orthodontic brackets. J Res Dent Sci. 2008; 5(1):38-46. [Abstract in English]
13. Karami M, Mazaheri R, Mesripour M. (). Comparing the effectiveness of two fluoride mouth rinses on Streptococcus mutans. J Mash Dent Sch. 2011;35(2):115-22. [Abstract in English]
14. Vieira, A. R., Deeley, K. B., Callahan, N. F., Noel, J. B., Anjomshoaa, I., Carricato, W. M., ... & Brandon, C. A. Detection of Streptococcus mutans genomic DNA in human DNA samples extracted from saliva and blood. ISRN dentistry. 2011;2011:1- 6.
15. Emamieh S, Khaterizadeh Y, Goudarzi H, Ghasemi A, BaghbanAA, Torabzadeh H. The effect of two types chewing gum containing casein phosphopeptide-amorphous calcium phosphate and xylitol on salivary Streptococcus mutans. J Conserv Dent. 2015;18(3):192–5.
16. Luce, E. Koneman's color atlas and textbook of diagnostic microbiology. Plastic and Reconstructive Surgery. 2010;125(1):414-415.
17. Srinagesh, J., Krishnappa, P., & Somanna, S. N. Antibacterial efficacy of triphala against oral streptococci: An in vivo study. Indian Journal of Dental Research. 2012;23(5):696.
18. Hamada, S., & Slade, H. D. Biology, immunology, and cariogenicity of Streptococcus mutans. Microbiological reviews. 1980;44(2):331.
19. Whiley, R.A. and Hardie, J.M., Streptococcus vestibularis sp. nov. from the human oral cavity. International Journal of Systematic and Evolutionary Microbiology. 1988;38(4):335-339.
20. Reed James F. Analysis of two-treatment, two-period crossover trials in emergency medicine. Annals of emergency medicine. 2004;43(1):54-58.
21. Gürgan, C. A., Zaim, E., Bakirsoy, I., & Soykan, E. Short-term side effects of 0.2% alcohol-free chlorhexidine mouthrinse used as an adjunct to non-surgical periodontal treatment: a double-blind clinical study. Journal of periodontology. 2006;77(3):370-384.
22. Jassoma E, Baeesa L, Sabbagh H. The antiplaque/anticariogenic efficacy of Salvadora persica (Miswak) mouthrinse in comparison to that of chlorhexidine: a systematic review and meta-analysis. BMC Oral Health. 2019;19(1):64.
23. Shah, S., Bargale, S., Dave, B. H., Deshpande, A., Kariya, P. B., & Karri, A. Comparison of antimicrobial efficacy of (between) 0.2% chlorhexidine and herbal mouthwash on salivary Streptococcus mutans: A randomized controlled pilot study. Contemporary clinical dentistry. 2018;9(3):440-445.
24. Mehta S, Pesapathy S, Joseph M, Tiwari PK, Chawla S. Comparative evaluation of a herbal mouthwash (Freshol) with chlorhexidine on plaque accumulation, gingival inflammation, and salivary Streptococcus mutans growth. J Int Soc Prev Community Dent. 2013;3(1):25-8.
25. Johansson, I., Witkowska, E., Kaveh, B., Lif Holgerson, P., & Tanner, A. C. R. The microbiome in populations with a low and high prevalence of caries. Journal of dental research. 2016;95(1):80-86.
26. Moeiny P, Jamei N, Mohamadi M, SHafizadeh N, Valaei N, Rahbar M, KHosravi K. Effect of a probiotic yogurt produced in Iran on the salivary counts of Streptococcus mutans. Res Dent Sci. 2013;10(2):73-82.
27. Jacob, B., & Nivedhitha, M. S. Comparative Assessment of the Antibacterial Efficacy of Natural Products and Chlorhexidine Mouthwash against Streptococcus Mutans: A Systematic Review. Journal of Clinical & Diagnostic Research. 2018;12(12):1-7
28. Haffajee, A. D., Yaskell, T., & Socransky, S. S. (). Antimicrobial effectiveness of an herbal mouthrinse compared with an essential oil and a chlorhexidine mouthrinse. The Journal of the American Dental Association. 2008;139(5):606-611.

29. Mosadegh, M., DEHMOUBED, S. A., NASIRI, P., Esmaeili, S., & Naghibi, F. The study of phytochemical, antifungal and antibacterial effects of Teucrium polium and Cichourium intybus.1-6:(1)7;2002

Tables

Table 1. Variables in both A and B groups before starting the study

Variables			groups	
			A	B
Gender	Male	Count (percent)	5 (45.5%)	6 (54.5%)
	Female	Count (percent)	6 (54.5%)	5 (45.5%)
Age	Mean year		23.09(±1.64)	23.18(±1.78)
_Salivas' *S. mutans*_population before starting the trial	Mean(*10^6 CFU/mL)		7.24 (±1.69)	7.86(±1.28)

Table 2. Colony count of *Streptococcus mutans* before and after of each phase, and significances of effectiveness of using mouthwashes intergroups and intragroups

		Group 1	Group 2	p-value *
Treatment in the first phase		Treatment A	Treatment B	
1st period_S.mutans_before	Mean	7.24(±1.69)	7.86(±1.28)	
1st period_S.mutans_after	Mean	5.39(±1.24)	7.68(±1.40)	
Difference of S.mutans before and after the use of mouthwash in first phase	Mean	1.85(±0.87)	0.18(±0.60)	<0.001
p-value in treatment		<0.001	=0.340	
Treatment in the second phase		Treatment B	Treatment A	
2nd period_S.mutans_before	Mean	6.93(±1.41)	8.34(±1.42)	
2nd period_S.mutans_after	Mean	6.67(±1.17)	6.62(±0.98)	
Difference of S.mutans before and after the use of mouthwash in second phase	Mean	0.25(±0.74)	1.72(±1.09)	=0.001
p-value in treatment		=0.279	<0.001	

* p-value in decreasing the colonies between two groups at the end of each period

Table 3. Standard AB/BA crossover model analysis

groups		N	Mean($*10^6$ CFU/mL)	Std. Deviation	Std. Error Mean
y1- y2	AB	11	-1.5909	1.25495	0.37838
	BA	11	1.5364	1.22904	0.37057

		F*	Sig.	t-test	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
y1-y2	Equal variances assumed	0.146	0.707	-5.905	20	0.000	-3.12727	0.52962	-4.23204	-2.02251
	Equal variances not assumed			-5.905	19.991	0.000	-3.12727	0.52962	-4.23207	-2.02248

* Levene's Test for Equality of Variances

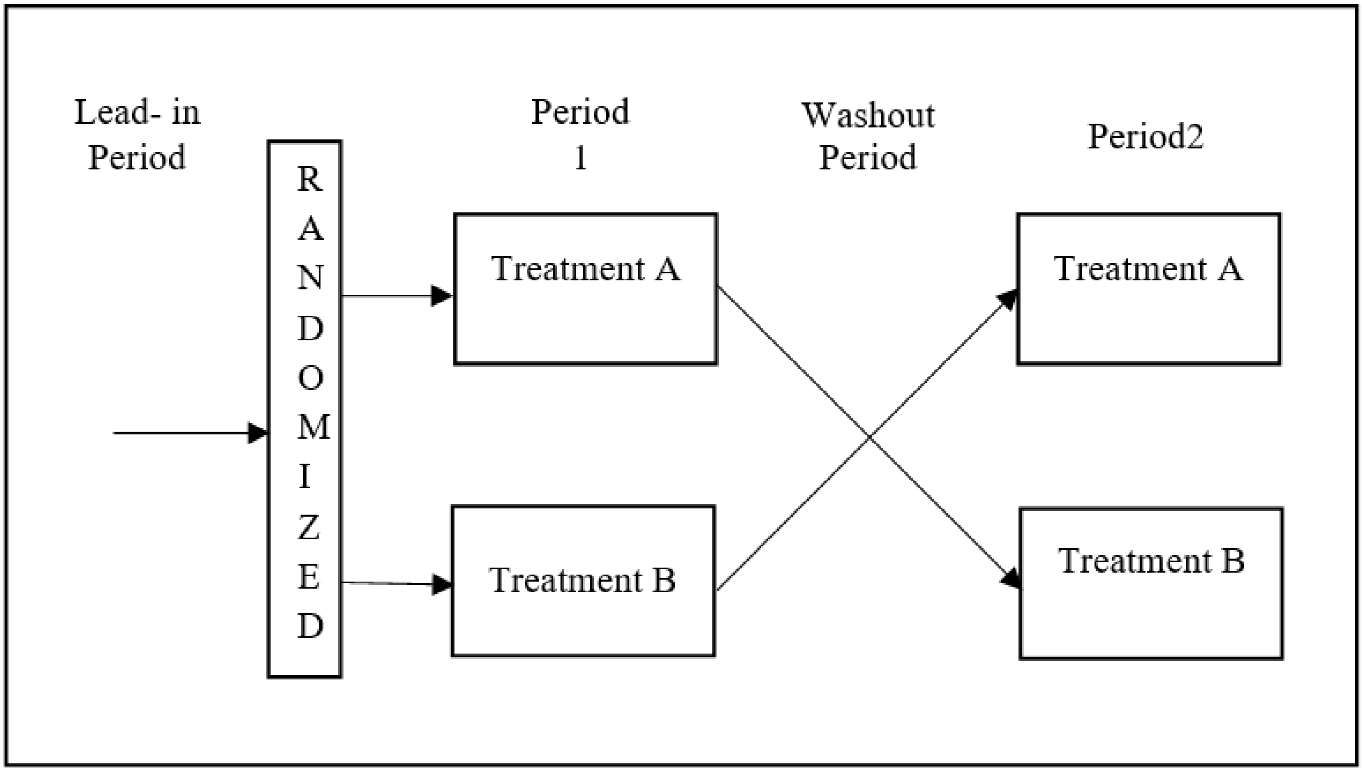
groups		N	Mean	Std. Deviation	Std. Error Mean
y1+ y2	AB	11	2.1000	1.01587	0.30630
	BA	11	1.9000	1.25379	0.37803

		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
y1+ y2	Equal variances assumed	0.433	0.518	0.411	20	0.685	0.20000	0.48655	-0.81492	1.21492
	Equal variances not assumed			0.411	19.175	0.686	0.20000	0.48655	-0.81772	1.21772

* Y1: Difference of *S. mutans* before and after the use of mouthwash in first phase

** Y2: Difference of *S. mutans* before and after the use of mouthwash in second phase

Figures



Treatment A: Using *T. polium* mouthwash
Treatment B: Using the placebo mouthwash

Figure 1

AB/BA randomized, crossover study design

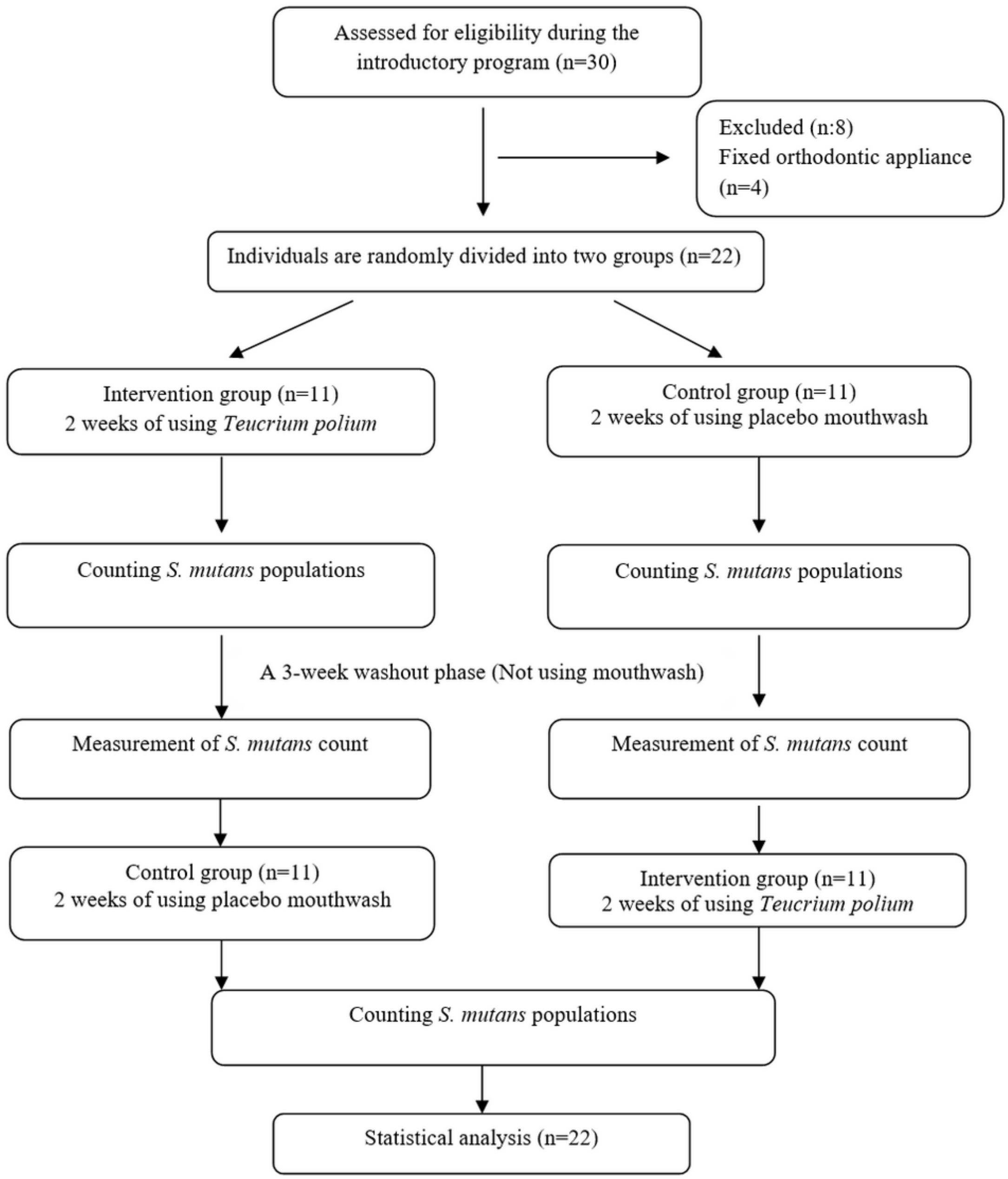


Figure 2

CONSORT flow diagram

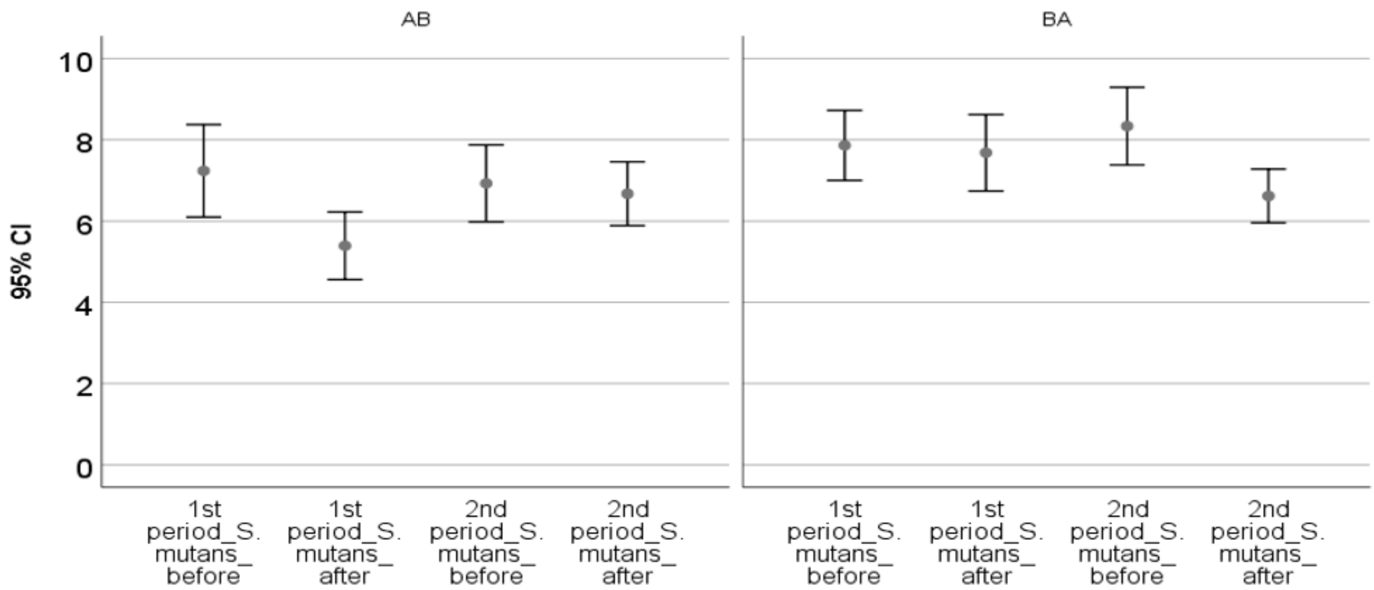


Figure 3

Colony count of Streptococcus mutans before and after each phase

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- DataandvariablesFileforT.Polium.xls