Evaluation of new Seawater-based Mouth Rinse Versus Chlorhexidine 0.2% Reducing Plaque and Gingivitis Indexes. A Randomized Controlled Pilot Study

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Featured Application: The use of this seawater mouth rinse reduce the effect of aggressive oral bacteria, taste, and numbness problems.

Abstract: For a long time, Chlorhexidine digluconate (CHX) has been considered the most used mouth-rinse for reducing plaque and gingivitis. Sea4® Encias is a new seawater-based mouth rinse with a similar action to CHX. Its prolonged use produces fewer side effects. This study compared the effects of two oral rinses: Chlorhexidine 0.20% (Group A); Sea4® Encias (Group B); and a placebo saline solution (Group C) mouth-rinses for reducing plaque and gingivitis indices. Plaque and gingival inflammation (Löe and Silness test) were evaluated at baseline and after each study stage. Group A reduced plaque growth and gingivitis significantly compared with Groups B and C; Group B was more effective than Group C. The mean P.I. decreased similarly in groups A and B. However, Group A showed the statistically significant value compared with other groups. Group A and group B, showed greater reduction in Gingival Indexes compared with group C. The Sea4 mouthwash showed better inhibitory activity on plaque (1.32 ± 0.22) compared with CHX (1.97 ± 0.34) and saline (2.78 ± 0.11). Sea4 Encias and Chlorhexidine 0.20% mouth-rinses significantly reduce plaque growth and G.I. compared with saline mouth-rinse; Sea4 Encías mouth-rinse is more effective against plaque regrowth than Chlorhexidine in this pilot study.

Keywords: chlorhexidine; gingivitis; oral mouth rinse; plaque; seawater mouth rinse
1. Introduction

Many bacteria present in the mouth cannot be eliminated properly with daily brushing, and can be present in the preservation of healthy dental and periodontal tissues [1]. Gingivitis begins with plaque build-up, which will end in periodontitis [2]. In this context, much research has investigated the efficacy of several types of mouthwash, which are designed to improve plaque and gingivitis levels. V-Sol containing alcohol, as well as essential oils (E.O.), have been shown to offer beneficial effects on plaque and gingivitis index, where it seems to significantly improve oral health six months after use [3,4]. Different mouthwashes containing natural compounds (NCCM) versus E.O. mouthwash (Listerine®) demonstrated their effectiveness for plaque control [4,5]. Another study evaluated the effects of E.O. that reduces plaque and gingivitis compared to cetylpyridinium chloride (CPC) and a placebo. Essential oils showed a better effect than mechanical plaque control (MPC) and CPC, reducing plaque and gingival inflammation in patients who may suffer gingivitis. Therefore, mouthwashes containing E.O. should be the first option for mechanical plaque control [6–8]. The therapeutic application of natural compounds showed that these compounds have some efficacy in the fight against gingivitis and periodontitis [9–13]. However, evidence that the efficiency and effectiveness of NCCM, as routine methods that help mechanical oral hygiene practices for plaque control and gingivitis has remained low, and it seems that some NCCMs may offer various oral health benefits by reducing the plaque and gingivitis [14]. The main side effects of alcohol-containing mouthwashes (ACM) were oral pain, burning sensation, and taste disturbances [14–17]. When the clinical efficacy of two 0.2% chlorhexidine mouthwash (CHX) formulations are compared, the alcohol-free chlorhexidine rinse demonstrated action levels similar to the use of frequently used alcohol-formed rinses [18]. Some of the most frequent side effects from the use of CHX are staining of the tongue and teeth, as well as taste alteration, as CHX is a rinse that is very effective in reducing plaque and gingivitis. Some authors describe moderate evidence when oxygenating agent (OA) and CHX work together to reduce teeth staining, without interfering with the inhibition of plaque growth [19,20]. Mouthwashes with seawater could offer a promising alternative for CHX to overcome its possible adverse effects. Balneotherapy with spa water or the Dead Sea water can be used to treat inflammatory skin diseases (psoriasis and atopic dermatitis). This effect of seawater has been attributed to the preservation of the skin barrier induced by NaCl and KCl [21]. Only one study has investigated the use of a mouthwash solution with sea salt in street children in Manila, demonstrating its effectiveness in reducing mild to severe forms of periodontal disease. The authors proposed sea salt mouthwash for its antimicrobial action, which is highly effective and well-tolerated by the user and could be implemented worldwide using low-cost resources [22]. This is considered to be the first pilot study which compares the clinical efficacy of 0.2% chlorhexidine with a new mouthwash formulated with seawater (analyzed for the first time) and a saline solution, in reducing gingiva inflammation and plaque formation.

2. Material and Methods

Undergraduate students, hygienists and doctors of the Universidad Católica de Murcia (UCAM, Catholic University of Murcia), School of Medicine and Dentistry, volunteered to take part in the study. The sample included 39 males and 54 females aged between 19 and 42 years, as a pilot study. This double-blind crossover study recruited 93 volunteer dental students (eighty one), hygienists (four) and doctors (eight). The UCAM Research and Ethics Committee at the Faculty of Medicine and Dentistry approved the study protocol, and subjects gave their informed consent to participate. The study was conducted in accordance with the CONSORT statement.

The inclusion criteria were as follows:

- Healthy patients with at least 26–32 permanent teeth;
Non-smokers, with good oral health, presenting probing depths \( \leq 3 \text{ mm} \), no active dental caries, no fixed or removable prostheses, and orthodontic devices.

Exclusion criteria
Exclusion criteria were as follows:

- Subjects with systemic diseases, caries, or active periodontal disease;
- Subjects with crowns, or an orthodontic treatment;
- Subjects taking antibiotics or anti-inflammatory drugs, or any other medication;
- Subjects with an allergy to any of the ingredients of the mouth rinse.

The presence of plaque was evaluated using the Plaque Index (PI Silness and Löe). These index measurements were performed at three points of both, the lingual and buccal surfaces of all teeth, classifying the data as a 0–3 score: 0 = absence of plaque; 1 = no plaque; 2 = moderate accumulation of plaque; 3 = abundant accumulation of plaque on the dental surfaces. Gingival Inflammation was evaluated using the Gingival Index (GI Löe and Silness) on the same surfaces as the P.I., classifying the results with the same 0-3 score: 0 = no inflammation; 1 = light swelling but lack of bleeding during probing; 2 = redness, swelling, and bleeding during probing; 3 = intense redness, swelling, and spontaneous bleeding.

2.1. Experimental Protocol

The twenty-one volunteers completed a pre-treatment phase that included early prophylaxis (thorough mechanical cleaning) and, after receiving instruction in oral hygiene practices, performed the recommended regime for one week (without mouth rinse). Plaque and gingival indexes were registered at baseline and the end of the pre-treatment phase (Figure 1). The randomization process was carried out, and in the flowchart, explain the method of our study.

2.2. Calibration Trial

One single doctor (J.L.C-G) recorded probing pocket depth (PPD) and soft tissue recession (G.R.) in all 93 subjects. These measurements were repeated 1 week later. The researcher checked all the measurements; ± 2 mm tolerance value is accepted as appropriate. All repeated measurements of the Gingival index (G.I.) and plaque index (P.I.) were performed at the same time, every week for four weeks of the study. During the treatment phase, the subjects used the three mouth rinses as part of

![Figure 1. Material and methods following in this study.](image-url)
their oral hygiene regime as follows: First mouth rinse used for 10 days followed by a resting period of 10 days; the second mouth rinse for another 10 days followed by a resting period of 10 days; the third mouth rinse during the last 10 days, followed by one week of plaque removal through prophylaxis according to oral hygiene instruction. The mouth rinse groups under investigation were as follows: Group A (n = 7) solution with seawater Sea4® Encias (Blue Sea Laboratories, Alicante, Spain); Group B (n = 7) Chlorhexidine Digluconate 0.20 % (Laboratorios KIN, S.A., Barcelona, Spain) and Group C (n = 7) placebo/control saline solution (Betafar, Madrid, Spain). The mouth rinses were given to blinded the study subjects at each respective period of using, in the same packaging with only the following indications: Bottle A, Bottle B and Bottle C (Figure 2). The 93 volunteers rinsed three times a day for 1 min after meals, with 10 ml of solution provided randomly in Bottle A, Bottle B or Bottle C. After the end of each mouth rinse period of ten days, initial and final clinical measurements were obtained (Day 0 – Day 10: P.I., G.I.). Afterwards, and before the beginning of the next ten day period, a resting phase of 10 days was scheduled when subjects exercised prophylaxis (according to instruction in oral hygiene) for plaque removal. This allowed some time to eliminate the effects of the previous mouth rinse. Rinsing was unsupervised throughout the study period.

![Figure 2. The mouth rinses used in the study: (a) Bottle A (Sea4), (b) Bottle B (PerioKin CHX 0.20%), and (c) Bottle C (saline).](image)

2.3. Patient Variables

Patient data was assessed by J.L.C-G, to evaluate the residual liquid (ml) in the patient’s returned bottles from the subjects. Patients’ perception of the product and possible adverse effects were registered in a questionnaire the subjects were asked to complete on the last visit by placing a mark on a 10 cm visual analog scale (VAS) (Table 1). All study subjects were evaluated in a dental office by a principal investigator (J.L.C-G). G.I. and P.I. Indexes were registered at days 0 and 7 of the pre-treatment phase), day 17 (end of the first rinse period), day 27 (end of the first resting phase), day 37 (end of the second rinse period), day 47 (end second resting phase), and day 57 (end of the third rinse period) and day 67 (end of the fourth rinse period). After the experiment was concluded, the participants received the last week of plaque removal through prophylaxis (according to instructions in oral hygiene).
Table 1. Patients’ perception of the product—VAS. n.s., no statistically significant difference between each treatment, \( p > 0.0125 \). * Using Friedman test, \( p < 0.05 \). * Using Wilcoxon signed-rank test with Bonferroni adjustment for multiple comparison, \( p < 0.0125 \).

| Questionaire                                                                 | Positive Control | CHX          | Seal ENCIAS | Intergroup Statistics |
|------------------------------------------------------------------------------|------------------|--------------|-------------|-----------------------|
| Do you have stains in your teeth and tongue after rinsing?                   | 0.66             | 0.89         | 0.29        | 0.0131 *              |
| Not, yes, very much                                                          | 0.11             | 0.28         | 0.22        |                       |
| Mouthwash flavour: Quality                                                   | 6.12             | 6.29         | 7.45        | 0.0351 *              |
| How was the taste of the Product?                                            | 0.72             | 0.41         | 0.51        |                       |
| (very good—very bad)                                                         |                  |              |             |                       |
| Mouthwash flavour: Duration                                                  | 5.45             | 6.81         | 7.78        | 0.451                 |
| How long did the taste remain in the mouth? (short period of time—long period of time) | 0.23             | 0.15         | 0.43        |                       |
| How was your taste of food and drink after mouth rinse use?                  | 5.42             | 8.56         | 5.54        | 0.0248 *              |
| Was the use of the mouth rinse convenient?                                   | 0.37             | 1.94         | 0.76        |                       |
| Do you feel oral numbness or a burning sensation on your mucosa due to the mouth rinse used? | 7.56             | 7.91         | 8.34        | 0.3729                |
| absolutely not—yes, very much                                                | 0.76             | 0.92         | 0.74        |                       |
| What was your perception of the plaque reduction?                            | 1.92             | 5.51         | 3.81        | 0.0231 *              |
| Do you feel oral numbness or a burning sensation on your mucosa due to the mouth rinse used? | 0.23             | 0.66         | 0.56        |                       |
| absolutely not—yes, very much                                                | 0.66             | 0.66         | 0.12        | 0.1689                |
| What is your overall opinion of the Mouth rinse used in this study?          | 1.87             | 7.89         | 8.51        | 0.0141 *              |
| very bad—very good                                                           | 0.61             | 0.66         | 0.12        |                       |
| What is your overall opinion of the Mouth rinse used in this study?          | 2.1              | 7.65         | 9.76        |                       |
| very bad—very good                                                           | 0.7              | 0.32         | 0.23        |                       |
2.4. Statistical Analysis

As this was a pilot study, the sample size of 31 patients per group was selected for convenience. Quantitative clinical variables were calculated per patient and per visit, and then by group. Means, standard deviations and 95% confidence intervals (CI) were calculated in clinical variables, such as per patient, per visit, and then by group. The Kolmogorov–Smirnov test was used to evaluate the normality and significant distribution of the samples, and ANOVA Test was used to identify significant differences between groups at the initial visit, and significant changes before, and after, treatment within each group. Wilcoxon signed-rank test was used to the non-normal distribution of the frequency of the indexes, with a significance level of \( p < 0.05 \). Statistical analyses were performed separately for the gingivitis and plaque evaluations. Comparisons, between the baseline scores for G.I. and P.I. of subjects in the three different groups, were performed separately using an independent t-test. Intra-group comparisons regarding baseline G.I. and P.I. Scores versus follow-up G.I. and P.I. Scores were performed using a paired t-test. Inter-group comparisons regarding baseline-adjusted G.I. and P.I. Scores at the follow-up examinations were performed using ANOVA. All statistical tests of hypotheses were two-sided and employed a level of significance of \( \alpha = 0.05 \). All analyses were performed using the IBM software package SPSS, v.20 (IBM Corp., Armonk, NY, USA).

3. Results

**Patients’ Perception of Mouth Rinses**

The differences in patients’ perception of the products tested between small groups and the reduction of plaque scores in SEA 4 and Chlorhexidine 0.20% when compared to saline after first week onwards; however, reduction of plaque is higher in group SEA 4 than Chlorhexidine in second, third and fourth week.

No clinical signs of mucosal changes were found after the use of the three mouth rinses at any point during the study. Most of the volunteers reported a mouthwash taste sensation in the mouth, which remained for 45 minutes after rinsing.

The periodontal status of all subjects was evaluated at the time of recruitment. Probing depth was controlled ranged between 1.3 and 3.1 mm, while plaque indexes ranged between 0.1 and 1.2 mm. All subjects (N = 21; SEA 4 Group N = 7; Chlorhexidine Group N = 7; Saline water Group N = 7) complied with the experimental protocol, and the data obtained was complete. The amounts of the products returned in the patient’s bottles when the study was finished suggested the right level of subject uses with the study protocol. No side effects were reported during the study.

There were significant differences in the Plaque index between the three groups. The Sea4 Group showed more significant inhibitory effect on plaque regrowth compared with the CHX group in the first week; in fact, the mean overall plaque index was 0.86 in the SEA 4 Group, compared with 1.26 in the Chlorhexidine Group, and 1.56 in the saline water Group. The follow-up analysis found no stained teeth or taste alteration in the SEA 4 Group or the Saline Water Group, but some patients showed some taste alteration reporting that food lacked flavor, and some dental staining was observed in the Chlorhexidine Group.

After using SEA 4 Encías, some patients reported a taste alteration from salty to sweet menthol. Significant differences were found between the mean P.I. measured at the beginning of the study period (habitual oral hygiene) and mean P.I. obtained with SEA 4 Encías and Chlorhexidine (\( p < 0.01 \)). After the first and second-week, plaque and gingival scores decreased in Group A and Group B, and Gingival scores showed a greater reduction in Group A and Group B, than in Group C in the second, third and fourth weeks, but the reduction was more significant in Group A than Groups B and C (Figure 3). SEA 4 Encías and Chlorhexidine presented similar levels of efficacy in delaying plaque growth in the fourth week, which was higher with Chlorhexidine than with saline water. SEA 4 Encías produced a more significant reduction in plaque at 4 weeks than the other two mouth rinses (Figure 4). Gingival scores decreased significantly more in Groups A and B than Group C during the entire study period.
Figure 3. Plaque index frequency. Sea4 Encias produced a more significant reduction in plaque at four weeks than the other two mouth rinses.

Figure 4. Gingival Index frequency. Seawater was effective in gingival index reduction compared with Chlorhexidine 0.20% and saline.

4. Discussion

For the first time, this pilot study investigated a new SEA 4 Encías® seawater-based mouthwash, compared to the most commonly used mouthwash, such as Chlorhexidine. As this is the first clinical trial of SEA 4, it is impossible to compare the results with any other published research. However, the results provided clear evidence that mouthwash with seawater reduced gingivitis and plaque formation more effectively than 0.2% chlorhexidine digluconate and a placebo saline solution throughout the study period.

Van der Weijden et al. found that the most effective mouthwashes in plaque reduction are those containing Chlorhexidine (CHX) and essential oils (E.O.) [6,8,23–25].
Arunachalam et al. compared the intraoral distribution of 0.2% chlorhexidine without alcohol (CHX-Alc) and 0.1% chlorhexidine with alcohol (CHX + Alc) at different rinse times (10 s, 20 s, 30 s) and described that the shortest rinse-time will assist in monitoring and continuity of the patient’s compliance [26]. Another short-term study by Shiloah and Patters found no statistically significant differences between two commercially available CHX types of mouthwash (0.2% and 0.12%) in plaque inhibition with a 30-second rinse time, reporting side effects of spots and taste disturbances, limiting their potential for long-term use [25].

Some oral antiseptics, containing octenidine or polyhexamethylene biguanide, will be considered potential alternatives to chlorhexidine-based preparations to control oral microorganisms (Fusobacterium nucleatum, Streptococcus mutans, Streptococcus sanguinis and Candida albicans) [27–29].

It has been clinically proven that three common therapeutic agents found in mouthwashes produce essential benefits for the control of gingivitis and plaque, such as Chlorhexidine, CPC, and essential oils. The SEA 4 Encías®mouthwash includes CPC in its formulation, demonstrating that in vitro and clinical studies are useful both, in biofilms and in the reduction of the common gingival inflammation and plaque index.

The use of seawater mouthwashes will be the best alternative to the use of Chlorhexidine, with the same essential anti-bacterial activity, reducing the side effects of dental staining and taste disturbances.

5. Conclusions

This clinical study tested a newly formulated seawater mouthwash SEA 4 Encías, containing CPC and hyaluronic acid, and compared it with 0.20% chlorhexidine and a placebo mouth-rinse. Our research with larger sample sizes confirmed the results, and provided clear evidence that seawater mouthwash was more effective against plaque and gingivitis than Chlorhexidine 0.20% rinse in 4 weeks study. It was also more effective against plaque regrowth than the chlorhexidine 0.20% rinse and placebo. These findings provide clinical evidence that this mouthwash may be recommended to patients as a useful adjunct to their oral hygiene practices, avoiding staining and taste disturbances.

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References

1. Lang, N.P.; Cumming, B.R.; Le, H. Toothbrushing frequency as it relates to plaque development and gingival health. J. Periodontol. 1973, 44, 396–405. [CrossRef] [PubMed]
2. Lindhe, J.; Okamoto, H.; Yoneyama, T.; Haïfajee, A.; Socransky, S.S. Longitudinal changes in periodontal disease in untreated subjects. J. Clin. Periodontol. 1989, 16, 662–670. [CrossRef] [PubMed]
3. Van Leeuwen, M.P.C.; Slot, D.E.; Van der Weijden, G.A. 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, 12, 160–167. [CrossRef] [PubMed]
4. Stoeken, J.E.; Paraskevas, S.; Van DerWeijden, G.A. The long-term effect of a mouthrinse containing essential oils on dental plaque and gingivitis: A systematic review. J. Periodontol. 2007, 78, 1218–1228. [CrossRef] [PubMed]
5. Stoeken, J.E.; Versteeg, P.A.; Rosema, N.A.; Timmerman, M.F.; van der Velden, U.; van der Weijden, G.A. Inhibition of "de novo" plaque formation with 0.12% chlorhexidine spray compared to 0.2% spray and 0.2% chlorhexidine mouthwash. *J. Periodontol.* 2007, 78, 899–904. [CrossRef]

6. Haas, A.N.; Wagner, T.P.; Muniz, F.W.; Fiorini, T.; Cavagni, J. Celeste RK Essential oils-containing mouthwashes for gingivitis and plaque: Meta-analyses and meta-regression. *J. Dent.* 2016, 55, 7–15. [CrossRef]

7. Haas, A.N.; Pannuti, C.M.; Andrade, A.K.; Escobar, E.C.; Almeida, E.R.; Costa, E.O.; Cortelli, J.R.; Cortelli, S.C.; Rode, S.D.; Pedrazzi, V.; et al. Mouthwashes for the control of supragingival biofilm and gingivitis in orthodontic patients: Evidence-based recommendations for clinicians. *Braz. Oral Res.* 2014, 28, 1–8. [CrossRef]

8. Haas, A.N.; Silva-Boghossian, C.M.; Colombo, A.P.; Albandar, J.; Oppermann, R.V.; Rösing, C.K.; Susin, C. Predictors of clinical outcomes after periodontal treatment of aggressive periodontitis: A 12-month randomized trial. *Braz. Oral Res.* 2016, 20, 30–31. [CrossRef]

9. Haas, A.N.; Pannuti, C.M.; Andrade, A.K.; Escobar, E.C.; Almeida, E.R.; Costa, E.O.; Cortelli, J.R.; Cortelli, S.C.; Rode, S.D.; Pedrazzi, V.; et al. Mouthwashes for the control of supragingival biofilm and gingivitis in orthodontic patients: Evidence-based recommendations for clinicians. *Braz. Oral Res.* 2014, 28, 1–8. [CrossRef]

10. Surathu, N.; Kurumathur, A.V. Traditional therapies in the management of periodontal disease in India and China. *Periodontol.* 2000 2011, 56, 14–24. [CrossRef]

11. Chen, Y.; Wong, R.W.; Seneviratne, C.J.; Hagg, U.; McGrath, C.; Samaranayake, L.P. The effects of a natural compound containing mouth rinses on patients with fixed orthodontic appliance treatment: Clinical and microbiological outcomes. *Int. J. Pediatr. Dent.* 2013, 23, 452–459.

12. Laing, E.; Ashley, P.; Gill, D.; Naini, F. An update on oral hygiene products and techniques. *Dent. Update* 2008, 35, 270–279. [CrossRef] [PubMed]

13. Zimmer, S.; Korte, P.; Verde, P.; Ohmann, C.; Naumova, E.; Jordan, R.A. Randomized controlled trial on the efficacy of new alcohol-free chlorhexidine mouthrinses after 8 weeks. *Int. J. Dent. Hyg.* 2015, 13, 110–116. [CrossRef] [PubMed]

14. Chen, Y.; Wong, R.W.; McGrath, C.; Hagg, U.; Seneviratne, C.J. Natural compounds containing mouthrinses in the management of dental plaque and gingivitis: A systematic review. *Clin. Oral Investig.* 2014, 18, 1–16. [CrossRef] [PubMed]

15. Garcia-Gargallo, M.; Zurlohe, M.; Montero, E.; Alonso, B.; Serrano, J.; Sanz, M.; Herrera, D. Evaluation of new Chlorhexidine- and cetylpyridinium chloride-based mouth rinse formulations adjunctive to scaling and root planing: A pilot study. *Int. J. Dent. Hyg.* 2017, 15, 269–279. [CrossRef] [PubMed]

16. Poggi, P.; Rodriguez y Baena, R.; Rizzo, S.; Rota, M.T. Mouthrinses with alcohol: Cytotoxic effects on human gingival fibroblasts in vitro. *J. Periodontol.* 2003, 74, 623–629. [CrossRef]

17. Fine, D.H.; Markowitz, K.; Furgang, D.; Goldsmith, D.; Charles, C.H.; Lisante, T.A.; Lynch, M.C. Effect of an essential oil-containing antimicrobial mouthrinse on specific plaque bacteria in vivo. *J. Clin. Periodontol.* 2007, 34, 652–657. [CrossRef]

18. Papaoannou, W.; Vassilopoulos, S.; Vrotsos, I.; Margaritis, V.; Panis, V. A comparison of a new alcohol-free 0.2% chlorhexidine oral rinse to an established 0.2% chlorhexidine rinse with alcohol for the control of dental plaque accumulation. *Int. J. Dent. Hyg.* 2016, 14, 272–277. [CrossRef] [PubMed]

19. Mor-Reinoso, C.; Pascual, A.; Nart, J.; Quirynen, M. Inhibition of de novo plaque growth by a new 0.03% chlorhexidine mouth rinse formulation applying a non-brushing model: A randomized, double-blind clinical trial. *Clin. Oral Investig.* 2016, 20, 1459–1467. [CrossRef]

20. Van Maanen-Schakel, N.W.; Slot, D.E.; Bakker, E.W.; Van der Weijden, G.A. The effect of an oxygenating agent on chlorhexidine-induced extrinsic tooth staining: A systematic review. *Int. J. Dent. Hyg.* 2012, 10, 198–208. [CrossRef]

21. Yoshizawa, Y.; Tanojo, H.; Kim, S.J.; Maibach, H.I. Seawater or its components alter experimental irritant dermatitis in man. *Skin Res. Technol.* 2001, 7, 36–39. [CrossRef] [PubMed]

22. Michel, J.; Michel, M.G.; Nandan, J.; Nowzari, H. The street children of Manila are affected by early-in-life periodontal infection: Description of a treatment modality: Sea salt. *Refuat Hapeh Vehashinayim* 2013, 30, 6–13, 67.

23. Van der Weijden, F.A.; Van der Sluijs, E.; Ciancio, S.G.; Slot, D.E. Can Chemical Mouthwash Agents Achieve Plaque/Gingivitis Control? *Dent. Clin. N. Am.* 2015, 59, 799–829. [CrossRef] [PubMed]
24. Cortelli, S.C.; Cortelli, J.R.; Wu, M.M.; Simmons, K.; Charles, C.A. Comparative antiplaque and antigingivitis efficacy of a multipurpose essential oil-containing mouth rinse and a cetylpyridinium chloride-containing mouth rinse: A 6-month randomized clinical trial. *Quintessence Int.* 2012, 43, e82–e94. [PubMed]

25. Shiloah, J.; Patters, M.R. DNA probe analyses of the survival of selected periodontal pathogens following scaling, root planning, and intra-pocket irrigation. *J. Periodontol.* 1994, 65, 568–575. [CrossRef] [PubMed]

26. Arunachalam, L.T.; Merugu, S.; Sudhakar, U. Comparison of intraoral distribution of two commercially available chlorhexidine mouthrinses with and without alcohol at three different rinsing periods. *J. Int. Soc. Prev. Commun. Dent.* 2012, 2, 20–24. [CrossRef]

27. Todkar, R.; Sheikh, S.; Byakod, G.; Muglikar, S. Efficacy of chlorhexidine mouthrinses with and without alcohol—A clinical study. *Oral Health Prev. Dent.* 2012, 10, 291–296.

28. Jose, A.; Butler, A.; Payne, D.; Maclure, R.; Rimmer, P.; Bosma, M.L. A randomized clinical study to evaluate the efficacy of alcohol-free or alcohol-containing mouth rinses with Chlorhexidine on gingival bleeding. *Br. Dent. J.* 2015, 219, 125–130. [CrossRef]

29. Rohrer, N.; Widmer, A.F.; Waltimo, T.; Kulik, E.M.; Weiger, R.; Filipuzzi-Jenny, E.; Walter, C. Antimicrobial efficacy of 3 oral antiseptics containing octenidine, polyhexamethylene biguanide, or Citroxx: Can Chlorhexidine be replaced? *Infect. Control Hosp. Epidemiol.* 2010, 31, 733–739. [CrossRef]