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Evaluation of Safety, Patient Perception and Efficacy of a New Cymenol-Based Mouth Rinse Formulation: A Randomized Clinical Trial

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Featured Application: The use of a cymenol-based mouth rinse is safe and well-tolerated by patients, with no reports of side effects or adverse events.

Abstract: The aim of this study was to evaluate a newly formulated mouth rinse containing cymenol in patients undergoing supportive periodontal care in terms of safety (primary outcome) and the impact on dental biofilm and gingival inflammation (secondary outcomes). This research was designed as a pilot, controlled, randomized, parallel, triple-blinded, single-center, clinical trial of a 12-week duration. Adverse events and product perception were assessed by a questionnaire. Clinical, patient-reported outcomes (PROs), compliance, tooth staining, dentin hypersensitivity and microbiological variables were also evaluated. Student T, Mann–Whitney-U and Chi-square tests were applied. Thirty participants (15 per group) were included, randomized and followed for 12 weeks. No adverse events were reported. The questionnaire showed an overall rating of 7.2 (out of 10) in the experimental group and of 8.2 in the control group ($p = 0.165$) at 12 weeks. No statistically significant differences were observed in terms of gingival health, tooth staining, dentin hypersensitivity or microbiological outcomes between groups at baseline, 6- and 12-week visits. The adjuvant use of the new mouth rinse formulation proved to be as safe as the control product and no significant differences were observed in terms of clinical efficacy.

Keywords: cymenol; mouth rinse; tolerability; dental biofilm; gingival inflammation

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1. Introduction

Mechanical biofilm control, including tooth brushing or interdental cleaning, are the cornerstone in supragingival dental biofilm control [1], although a lack of compliance, poor motivation, inadequate skills and hard-to-reach areas can hinder effective mechanical biofilm control, whereas the adjunctive use of chemotherapeutic agents delivered as mouth rinses or dentifrices can significantly enhance dental biofilm management. These agents provide substantial benefits in maintaining oral and periodontal health [2].

The advent of new dentifrice/mouth rinse formulations requires appropriate testing to evaluate their actual antiplaque/antigingivitis effect, since the inclusion of a well-known active agent in a formulation does not guarantee its clinical efficacy [3]. The final clinical recommendations should be based on 6-month, home-use, randomized clinical trials (RCTs), following accepted regulations [4], while recently, the American Dental Association (ADA) Council on Scientific Affairs also endorsed 3-month studies [5].

A new mouth rinse formulation (*Lacer Oros Acción Integral*, Lacer SA, Barcelona, Spain) incorporating O-Cymen-5-ol, potassium nitrate, zinc chloride, dipotassium glycyrrhizate, sodium fluoride, panthenol and xylitol in its formulation has been recently proposed, which makes an evaluation of its safety and efficacy relevant.

It was, therefore, the main objective of this RCT to evaluate the safety of this mouth rinse formulation, by assessing its tolerability and the advent of adverse events. As secondary objectives, outcomes related to its efficacy were also evaluated: (1) the impact on plaque and gingival inflammation, (2) the impact on dentin hypersensitivity, (3) the patient's evaluation of the product, by means of patient-reported outcomes (PROs), and (4) the microbiological impact.

2. Materials and Methods

The study protocol received approval from the local ethics committee (CEIm Hospital Clínico San Carlos, Madrid, Spain) on 2 December 2020 (code 20/750-EC_X). It was a priori registered in the URI+i system of the Faculty of Odontology, Complutense University of Madrid (code 36-161220, 16 December 2020) and in ClinicalTrials.gov (NCT04881357).

2.1. Study Population

Consecutive subjects enrolled in a supportive periodontal care program (SPC) underwent screening at the Post-Graduate Periodontal Clinic at the University Complutense of Madrid to determine eligibility based on the following criteria:

2.1.1. Inclusion Criteria

- Age of 35–64 years.
- Periodontally treated patients undergoing SPC for at least 6 months, with the last SPC visit being within the previous 6 months.
- Patients should be generally healthy, according to the criteria of the American Society of Anesthesiologists (ASA), and categorized as ASA type I or II (refer to the exclusion criteria as well).
- There must be at least three assessable teeth in each quadrant.
- Moderate gingival inflammation: $\geq$40% bleeding on marginal probing (BOMP) [6], and $\geq$30% Gingival Bleeding Index (GBI) [7].
- Inadequate plaque control (Turesky plaque index $\geq$ 1.5) [8].
- Patients should not have any orthodontic bands or removable prostheses.
- Subjects willing to participate and adhere to the study requirements.
- Subjects must report dentin hypersensitivity in at least one assessable tooth. Confirmation of dentin hypersensitivity was determined using an evaporative sensitivity test [9], with a minimum score of 1 [10]. To be eligible, the selected tooth must not currently undergo desensitizing therapy, should not have been restored within the last 3 months or have a crown or significant restoration. Only incisors, canines and premolars were considered [11].

2.1.2. Exclusion Criteria

- Presence of untreated or uncontrolled periodontitis.
- Regular use of mouth rinses containing antiseptics or anti-hypersensitivity agents.
- Antibiotic use within the past month.
- Excessive acid exposure due to conditions like eating disorders or chronic regurgitation.
- Chronic use of pain relievers or anti-inflammatory medications.
- Pregnancy.
- Any significant medical history (such as diabetes, osteoporosis or immunosuppression) or long-term medication use (including chemotherapy, immunosuppressive therapy and medications linked to gingival overgrowth, such as phenytoin, phenobarbital, lamotrigine, vigabatrin, ethosuximide, topiramate, primidone, nifedipine, amlodipine, verapamil and cyclosporine) that affects gingival health.
- Conditions which require antibiotic prophylaxis (infectious endocarditis, cardiac valve prosthesis. . .).

2.2. Study Design

A 12-week clinical trial was conducted as a pilot study with a parallel design, employing triple-blinding, randomization and placebo control.

2.2.1. Screening Visit

After completing a questionnaire that covered the subject's medical health status, use of medications and smoking habits (with a smoker defined as someone currently smoking 9 or more cigarettes per day [12]), a thorough oral health assessment was conducted, including a full-mouth BOMP/GBI evaluation. Subjects who met the inclusion and exclusion criteria were then informed by the investigator about the study's objectives and details and were invited to participate voluntarily. Upon acceptance (by signing the Institutional Review Board (IRB)-approved informed consent form), they were enrolled in the study.

2.2.2. Baseline Visit

At this visit, the oral health of each participant, identified by a unique trial number, was examined. This trial number was linked to the randomization list and was consecutively assigned, as patients were enrolled in the study, by a single examiner (A.A.) who was blinded to this allocation. Subjects were randomly assigned by the study promotor using true random numbers from a computer-generated list to either the experimental or placebo groups:

(1) The experimental group used a supplied manual toothbrush with a sodium fluoride toothpaste three times a day, followed by the test mouth rinse (*Lacer Oros Acción Integral*—new formula, LACER SA, Barcelona, Spain), with O-Cymen-5-ol, potassium nitrate, zinc chloride, dipotassium glycyrrhizate, sodium fluoride, panthenol and xylitol.
(2) The control group used the same provided manual toothbrush with a sodium fluoride dentifrice with the same frequency, but followed by a control mouth rinse (*Lacer Oros Acción Integral*—new formula, without active ingredients, LACER SA, Barcelona, Spain).

All subjects were blinded to their group allocation and received a kit containing their assigned products, which included a manual toothbrush (GingiLacer toothbrush, LACER SA, Barcelona, Spain), a toothpaste (fluoridated toothpaste with 1450 ppm fluoride, LACER SA, Barcelona, Spain) and the adequate amount of the assigned mouth rinse (coded A or B) with dose caps. Participating subjects were instructed to maintain their usual oral hygiene habits and were specifically directed to brush with the toothpaste for 2 min and rinse with 10 mL of the mouth rinse for 1 min, three times daily. No additional specific instructions were provided. Each subject received standardized verbal and written instructions on the proper usage of the assigned products. They were also provided with compliance forms to record daily usage of their allocated products throughout the study period. At this baseline visit, clinical outcome measurements were recorded around all teeth, except the third molars, and subgingival microbiological samples were collected. After this examination, no other treatment was provided due to the pilot nature of the study design.

2.2.3. Six-Week Visit

After 6 weeks of product use, a comprehensive oral examination was performed, including the assessment of the clinical variables and the collection of subgingival microbiological samples.

All participants were asked to complete a questionnaire on product usage and perceptions (PROs) and report any adverse effects experienced. The study coordinator gathered and reviewed the compliance forms as well as the empty and unused bottles. Afterward, participants received a new kit comprising the designated products and corresponding compliance forms.

2.2.4. Twelve-Week Visit

After 12 weeks of product use, a thorough oral examination was conducted, which included assessing clinical variables and obtaining new subgingival microbiological samples. Subjects were then asked to complete a new questionnaire regarding product usage and perceptions (PROs) and to report any adverse effects. The study coordinator collected the compliance forms as well as the empty and unused bottles. All subjects were provided with professional mechanical plaque removal (PMPR) at the end of the study.

2.3. Outcome Variables

Examinations were performed by one calibrated examiner (A.A.). The examiner was blinded to the treatment allocation and to data from previous visits. Evaluations were performed in the following sequence:

- Overall assessment of oral condition.
- Photographs of buccal area of lower and upper anterior teeth, from canine to canine.
- Dentin hypersensitivity.
- Plaque index (PlI).
- BOMP/GBI, evaluated during periodontal probing.
- Periodontal probing, including evaluation of BOMP/GBI, starting with the teeth evaluated in the first place for BOMP.
- Microbiological sampling.

2.3.1. Adverse Events' Evaluation

Comprehensive oral examinations were performed at follow-up visits and subjects were asked to report on the occurrence of any adverse effects.

2.3.2. Patient-Reported Outcomes (PROs)

A predefined questionnaire on product usage and perceptions, including nine questions, was filled by all the subjects.

2.3.3. Assessment of Compliance

At each study visit, the coordinator collected the compliance forms completed by the patients, along with the empty and unused bottles of mouth rinse.

2.3.4. Periodontal Clinical Parameters

Dental plaque was assessed using a disclosing solution (PlacControl®, Dentaid, Barcelona, Spain) with the Turesky et al. [8] modification of the Quigley and Hein index [13], scored at six sites per tooth.

The gingival condition was assessed using:

- The BOMP index by recording the presence or absence of bleeding within 30 s of probing on a scale 0–2 [6,14].
- GBI [7], by dichotomously assessing bleeding after gentle probing.

Staining of teeth was scored using the Gründemann modification of the stain index (GMSI) [15], recorded at four areas per tooth [Mesial (A), Distal (A), Gingival (G) and Incisal (I)] [16]. Stain was graded using the intensity stain index of Lobene [17]. Staining presence was evaluated in the upper and lower anterior buccal sites using standardized clinical photographs reviewed by a single calibrated examiner (A.A.). Additional information on the clinical indices used is presented as an Appendix in Supplementary files.

Probing depth (PD), gingival recession (REC) and clinical attachment level (CAL) were measured at six sites per tooth with a periodontal probe (North Carolina) in millimeters.

2.3.5. Dentin Hypersensitivity

Dentin hypersensitivity was explored using an evaporative stimulus and evaluated objectively using the Schiff scale [9] and subjectively by each participating subject using a

visual analogue scale (VAS). Dentin hypersensitivity was scored in just one tooth, identified as part of the inclusion criteria, at baseline and follow-up visits. If a patient identified multiple teeth with dentin hypersensitivity at baseline, the tooth experiencing the highest level of pain (according to the patient's assessment) was selected, although the clinical investigator took the final decision concerning the selected tooth.

2.4. Microbiological Evaluation

2.4.1. Microbiological Sampling

Four sites were chosen, one from each quadrant, based on bleeding observed during the screening visit. The same sites were sampled during follow-up visits. These sites were isolated using cotton rolls and gently dried with compressed air. The sites were isolated with cotton rolls and dried gently using air spray. Two consecutive sterile paper points (medium size, Maillefer, Ballaigues, Switzerland) were inserted as deeply as possible into the sulcus and left in place for 10 s. The paper points were then transferred to a vial containing 1.5 mL of reduced transport fluid [18] and combined with the other paper points collected.

2.4.2. Microbiological Processing

The vials were sent to the laboratory and processed within 24 h. At the laboratory, the samples were vortexed for 30 s and then analyzed using both culture methods and quantitative polymerase chain reaction (qPCR).

Culture

Samples were serially diluted and plated on various media: blood agar (Oxoid No. 2; Oxoid Ltd., Basingstoke, UK), enriched with 5% horse blood, hemin (5 mg/L) and menadione (1 mg/L), as well as on a selective medium for *Aggregatibacter actinomycetemcomitans* [19]. The blood agar plates were examined after 7 and 14 days of anaerobic incubation (80% N_2, 10% H_2, 10% CO_2 at 37 °C), while the selective plates were checked after 3 to 5 days of incubation at 37 °C in air with 5% CO_2. The plates were carefully inspected for the identification of *A. actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia/nigrescens*, *Tannerella forsythia*, *Parvimonas micra*, *Capnocytophaga* spp., *Eikenella corrodens* and *Fusobacterium* spp., based on colony morphology and various standard biochemical tests to confirm the initial identification (RapID ^{TM} ANA II System, Remel, Lenexa, KS, USA). Other significant colonies (those that constituted a notable portion of the microbiota) were also isolated for additional characterization. The total number of colony-forming units (CFU) per milliliter, along with the count of each bacterial species, was determined on a representative plate containing between 30 and 300 colonies. Counts of *A. actinomycetemcomitans* were performed on the selective plates based on its typical colony morphology, a catalase reaction and a set of specific enzymes.

Quantitative Polymerase Chain Reaction (qPCR)

Samples were processed with a commercial kit for DNA extraction (MoIYsis Complete5, Molzym Gmbh and Co. KG., Bremen, Germany) following manufacturer's instructions. Subgingival DNA samples were analyzed using multiplex qPCR to quantify the bacterial DNA of three putative periodontal pathogens (*A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythia*). The qPCR amplification was performed on Taqman Probes using specific primers targeted against 16S rRNA gene. For multiplex qPCR, the oligonucleotide probes of each bacterium were labeled with different fluorescent dyes, which have been selected on the bases of different fluoresce wavelengths [20].

PCR amplification was performed in a total reaction mixture volume of 10 µL, which included 5 µL of 2 × TaqMan master mixture (LC 480 Probes Master, Roche Diagnostic GmbH, Mannheim, Germany), optimal concentrations of primers and hydrolysis probe (300, 300 and 100 nM for *A. actinomycetemcomitans*; 300, 300 and 300 nM for *P. gingivalis* and 300, 300 and 200 nM for *T. forsythia*) and 2.5 µL of DNA from the samples. Each plate

featured a standard curve using DNA from the target bacteria for internal calibration, as well as a no-template control (NTC). The samples underwent an initial amplification step at 95 °C for 10 min, followed by 40 cycles consisting of 15 s at 95 °C and 1 min at 60 °C, using the LightCycler® 480 II thermocycler (Roche Diagnostic GmbH, Mannheim, Germany). The assays were designed to have a linear quantitative detection range with a slope between 3.3 and 3.6 cycles per log decade, an r^2 value greater than 0.997 and efficiency ranging from 1.9 to 2.0.

2.5. Data Analysis

2.5.1. Sample Size Calculation

Since this study was designed as a pilot study, to initially evaluate safety of a new formulation, as well as exploring parameters of efficacy, a convenience sample of 30 patients (15 patients per group) was selected.

2.5.2. Data Analysis

For all variables, the patient was the statistical unit for the analysis. PROs were determined as the average values for each of the nine questionnaire items. Additionally, calculations were made for total anaerobic counts, counts of individual pathogens, proportions of microbiota (pathogen counts relative to total counts) and the presence or absence of each pathogen. The primary outcome variable was safety (adverse events and PROs). Secondary outcomes were the mean values and changes in clinical (PlI, BOMP, GBI, PD, REC and CAL) and microbiological outcomes, staining (GMSI), dentin hypersensitivity and compliance.

Categorical outcome data were compared using the Chi-square test or Fisher's exact test. The Shapiro–Wilk goodness-of-fit test was employed to assess the normal distribution of quantitative variables. Differences between groups at baseline, 6 weeks and 12 weeks, as well as changes from baseline to 6 weeks, baseline to 12 weeks and 6 weeks to 12 weeks were analyzed using the Student's *t*-test or Mann–Whitney U test for quantitative outcomes. Statistical significance was set at $p < 0.05$ (IBM® SPSS® Statistics 27.0, IBM Corporation, Armonk, NY, USA).

2.5.3. Calibration

Intra-examiner calibration for periodontal variables was carried out on two patients and three evaluations per patient were completed with a difference of, at least, one hour. Calibration for PlI (unweighted agreement: 85.5%; kappa = 0.80; 95% confidence interval—95% CI [0.74; 0.85]; $p < 0.001$), BOMP (unweighted agreement: 89.1%; kappa = 0.58; 95% CI [0.46; 0.71]; $p < 0.001$), PD (intraclass correlation coefficient—ICC = 0.80; 95% CI [0.74; 0.84]; $p < 0.001$) and REC (ICC = 0.82; 95% CI [0.77; 0.85]; $p < 0.001$).

3. Results

Recruitment for this study started on September 2021 and the last follow-up visit was carried out in June 2022. During the screening phase, 58 subjects were selected, but 30 participants were finally included in this study, 15 in each group. The reasons for excluding 28 subjects are listed in the study flow chart Figure 1.

All included participants (n = 30) attended the three visits (baseline, 6 weeks and 12 weeks). No patient was lost to follow-up. The main characteristics of this sample are reported in Table 1, showing no statistically significant differences between groups in terms of age, sex, smoking habit, systemic diseases, chronic medication intake, psychological stress or allergies.

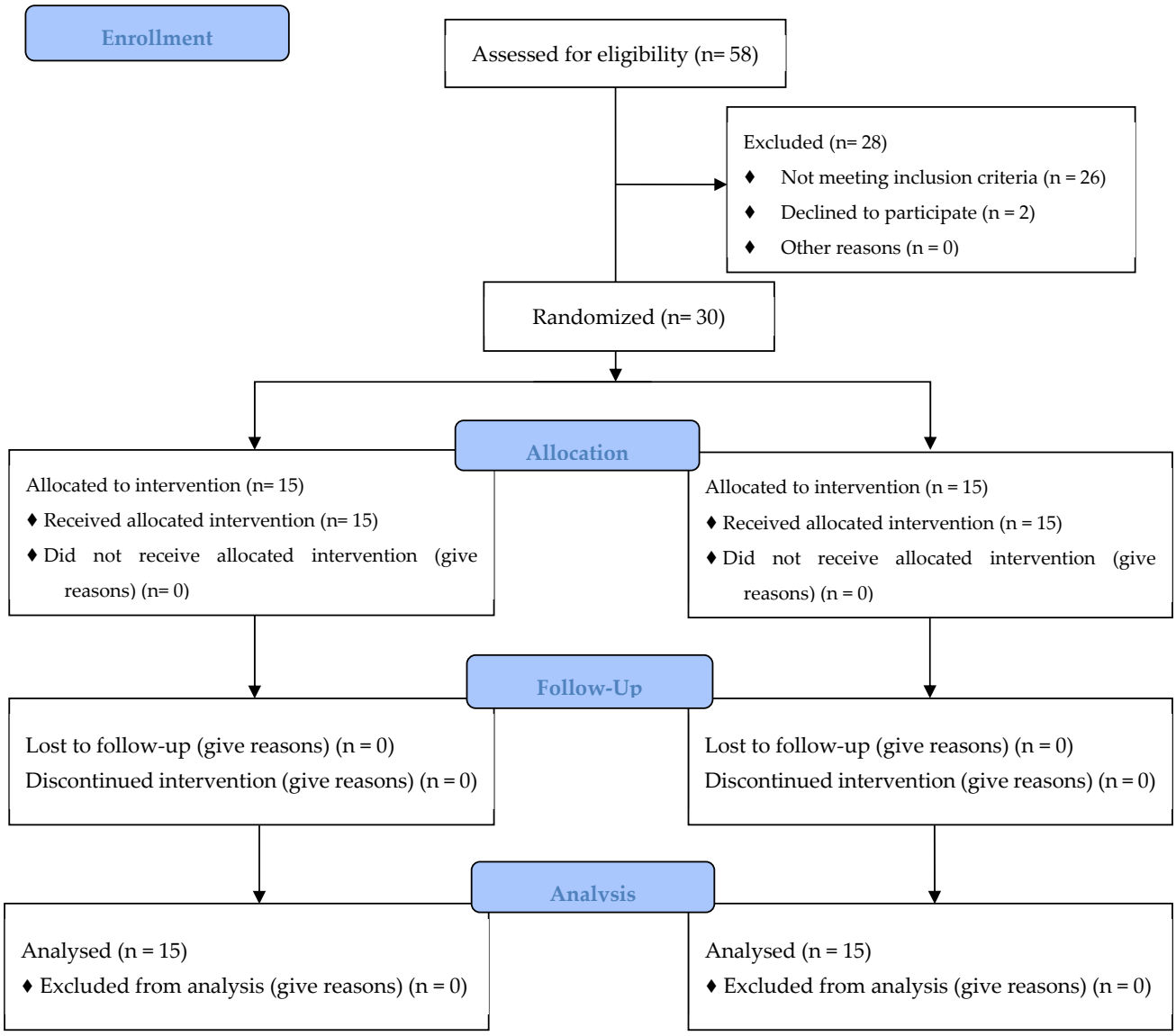

Figure 1. Flow chart of the study.

Table 1. Baseline demographic information.

	Test		Control		*p*-Value
	n	%	n	%	
Sex					
Male	6	40	4	26.7	0.439
Female	9	60	11	73.3	
Tobacco					
Non-smoker	14	93.3	14	93.3	1.000
Smoker	1	6.7	1	6.7	
Systemic Diseases					
No	10	66.7	12	80	0.409
Yes	5	33.3	3	20	
Medication					
No Medication	7	46.7	7	46.7	1.000
Medication	8	53.3	8	53.3	
Stress					
No Stress	13	86.7	12	80	0.624
Stress	2	13.3	3	20	
Allergies					
No Allergies	13	86.7	13	86.7	1.000
Allergies	2	13.3	2	13.3	

3.1. Safety and Tolerability

No adverse effects were reported or observed during the duration of this study in any of the study groups.

3.2. Patient-Reported Outcomes (PROs) and Compliance

Table 2 reports the results from the PROs and compliance.

Table 2. Compliance and patient-reported outcomes (PROs) at 6 and 12 weeks.

		6 Weeks							12 Weeks						
Variable	**Group**	**N**	**Mean**	**SD**	**Mean Diff.**	**95% CI Lower**	**95% CI Upper**	***p* Value**	**N**	**Mean**	**SD**	**Mean Diff.**	**95% CI Lower**	**95% CI Upper**	***p* Value**
Liquid left	Control	15	877.2	2540.0	−20.2	−1916.8	1876.5	0.603	15	778.8	2557.4	567.5	−789.6	1924.5	0.256
	Test	15	897.4	2531.4					15	211.3	207.1				
n miss	Control	15	10.7	13.8	−0.9	−9.7	7.8	0.454	15	11.9	13.2	−7.0	−20.1	6.1	0.327
	Test	15	11.6	9.0					15	18.9	21.1				
n right	Control	15	106.9	32.5	−7.5	−25.3	10.4	0.884	15	89.8	46.0	−17.3	−44.6	10.0	0.442
	Test	15	114.4	9.0					15	107.1	21.1				
n not registered	Control	15	8.4	32.5	8.4	−9.6	26.4	0.317	15	24.2	50.2	24.2	−3.6	52.0	0.073
	Test	15	0.0	0.0					15	0.0	0.0				
Q1	Control	15	8.1	1.5	0.8	−0.4	2.0	0.194	15	8.0	0.8	0.7	−0.3	1.7	0.490
	Test	15	7.3	1.8					15	7.3	1.7				
Q2	Control	15	5.5	2.3	0.3	−1.1	1.7	0.720	15	5.5	2.0	0.3	−1.2	1.9	0.556
	Test	15	5.3	1.3					15	5.1	2.1				
Q3	Control	15	5.4	1.3	0.5	−0.4	1.4	0.190	15	5.3	0.8	0.2	−0.6	1.0	0.758
	Test	15	4.9	1.1					15	5.1	1.3				
Q4	Control	15	1.5	2.1	−0.2	−1.6	1.2	0.343	15	2.0	1.5	0.1	−1.2	1.5	0.357
	Test	15	1.7	1.8					15	1.9	2.0				
Q5	Control	15	1.3	1.0	−1.8	−3.3	−0.3	0.016	15	1.9	1.8	−1.3	−3.1	0.5	0.122
	Test	15	3.1	2.6					15	3.3	2.9				
Q6	Control	15	1.0	0.0	−0.7	−1.7	0.3	0.073	15	1.2	0.6	−0.5	−1.6	0.7	0.916
	Test	15	1.7	1.8					15	1.7	2.1				
Q7	Control	15	1.6	2.3	−0.2	−1.6	1.2	0.053	15	1.5	1.5	−1.8	−3.7	0.1	0.046
	Test	15	1.8	1.1					15	3.3	3.1				
Q8	Control	15	8.3	1.5	1.8	0.4	3.2	0.022	15	8.2	1.2	1.0	−0.3	2.3	0.165
	Test	15	6.5	2.3					15	7.2	2.1				
Q9	Control	15	6.1	2.5	0.5	−1.2	2.3	0.322	15	6.3	1.8	0.4	−1.3	2.1	0.831
	Test	15	5.6	2.1					15	5.9	2.8				

SD, standard deviation; CI, confidence interval; Diff., difference; Q, question. Q1: Mouth rinse flavor (1: very bad; 10: very good). Q2: How much time does the mouth rinse flavor last in your mouth (1: very low; 10: too much). Q3: Which is your perception of the food and drinks flavor when using the mouth rinse (1: much worse, 10: better). Q4: Do you notice the teeth and the mucosa more sensitive after using the mouth rinse (1: no, absolutely; 10: yes, much more). Q5: Do you notice a drier mouth after using the mouth rinse (1: no, absolutely; 10: yes, much more). Q6: Do you notice burning feeling after using the mouth rinse (1: no, absolutely; 10: yes, much more). Q7: Do you notice some staining on the teeth or tongue due to the use of the mouth rinse (1: no, absolutely; 10: yes, much more). Q8: Which is your general opinion after using the mouth rinse in this study (1: very bad; 10: very good). Q9: Do you think that the mouth rinse use has improved your mouth health (1: no, absolutely; 10: yes, much more).

For PROs, statistically significant differences were found in questions 5 and 8 of the questionnaire. For question 5 ("Do you notice a drier mouth after using the mouth rinse?"), the mean value in the test group was 3.1 (standard deviation, SD = 2.6), while the control group had a mean value of 1.3 (SD = 1.0), with statistically significant differences at 6 weeks

(p = 0.016) that disappeared at 12 weeks (p = 0.122). For question 8 ("Which is your general opinion after using the mouth rinse in this study?"), after 6 weeks, the mean value in the test group was 6.5 (SD = 2.3), while the control group had a mean value of 8.3 (SD = 1.5), with statistically significant differences (p = 0.022); after 12 weeks, the mean score in the test group increased up to 7.2 (SD = 2.1) and it was maintained in the control group at 8.2 (SD = 1.2), with no statistically significant differences between the groups (p = 0.165).

Regarding compliance, no statistically significant differences were observed between the groups at 6 and 12 weeks and a similar amount of liquid was left in the bottles in both groups.

3.3. Periodontal Clinical Outcomes

Table 3 presents the periodontal parameters in both treatment groups at each study visit, while the mean changes between visits are presented in Table S1.

For PlI, statistically significant differences were observed in the mean changes in PlI between the groups from the baseline to the 12-week visit: an increase in PlI of 0.2 (SD = 0.3) in the test group and a decrease of 0.1 (SD = 0.3) in the control group, with a mean difference of 0.3 (95% CI [−0.5; −0.1]; p = 0.012).

For BOMP and GBI, no statistically significant differences between groups were detected at follow-up visits or in the changes between visits. Both groups experienced a decrease in BOMP over the course of the study: at the baseline, it was 0.4 (SD = 0.2) for the test and 0.4 (SD = 0.2) for the control group and at 12 weeks, it was 0.2 (SD = 0.1) and 0.2 (SD = 0.1), respectively. Similarly, reductions were also observed for GBI: at the baseline, the mean percentages were 39.9% and 41.9%, respectively, and after 12 weeks, decreased to 26.7% and 26.4%, respectively.

For PD, REC and CAL, no statistically significant differences between groups at any of the three visits of the study or in the changes from the baseline–week 6 or baseline–week 12 were observed.

3.4. Dentin Hypersensitivity

Table S2 depicts the dentin hypersensitivity values over time. Differences between groups were not statistically significant at any of the study visits or in the changes between visits. Both groups presented reductions in dentin hypersensitivity values over time.

3.5. Tooth Staining

Table S3 presents tooth staining at 6 and 12 weeks, with similar values in both study groups.

3.6. Microbiological Outcomes—Culture

Tables 4 and 5 present the microbiological outcomes expressed as the frequency of detection, counts and proportions of each target bacterial species at the three evaluation visits and Table S4 depicts the changes between visits.

Table 3. Periodontal clinical outcomes at each study visit.

			Baseline							6 Weeks							12 Weeks						
Variable	**Sites**	**Group**	**N**	**Mean**	**SD**	**Mean Diff.**	**95% CI Lower**	**95% CI Upper**	***p* Value**	**N**	**Mean**	**SD**	**Mean Diff.**	**95% CI Lower**	**95% CI Upper**	***p* Value**	**N**	**Mean**	**SD**	**Mean Diff.**	**95% CI Lower**	**95% CI Upper**	***p* Value**
n teeth		Control	15	23.6	4.5	1.0	−1.7	3.6	0.181	15	23.6	4.5	1.1	−1.6	3.8	0.162	15	23.6	4.5	1.1	−1.6	3.8	0.156
		Test	15	22.6	2.4					15	22.5	2.5					15	22.5	2.4				
PII (0–5)	**All**	Control	15	1.7	0.5	0.1	−0.2	0.5	0.384	15	1.7	0.6	0.0	−0.4	0.4	0.983	15	1.7	0.5	−0.2	−0.6	0.2	0.290
		Test	15	1.6	0.4					15	1.7	0.3					15	1.8	0.6				
	Buccal	Control	15	1.5	0.5	0.1	−0.2	0.4	0.548	15	1.5	0.6	0.0	−0.4	0.4	0.983	15	1.5	0.5	−0.3	−0.8	0.2	0.272
		Test	15	1.4	0.4					15	1.5	0.4					15	1.8	0.7				
	Lingual	Control	15	2.0	0.7	0.1	−0.3	0.6	0.548	15	1.9	0.7	0.0	−0.5	0.4	0.663	15	1.8	0.6	−0.1	−0.4	0.3	0.934
		Test	15	1.8	0.6					15	1.9	0.5					15	1.9	0.5				
	Prox	Control	15	2.0	0.5	0.2	−0.2	0.5	0.419	15	1.9	0.6	0.0	−0.4	0.4	0.917	15	1.9	0.5	−0.1	−0.5	0.2	0.373
		Test	15	1.8	0.4					15	1.9	0.4					15	2.0	0.5				
GBI (%)	**All**	Control	15	41.9%	12.8%	2.0%	−6.1%	10.2%	0.756	15	31.5%	10.3%	1.7%	−6.1%	9.6%	0.619	15	26.4%	10.4%	−0.3%	−7.2%	6.5%	0.694
		Test	15	39.9%	8.6%					15	29.7%	10.6%					15	26.7%	7.7%				
	Buccal	Control	15	33.7%	14.4%	1.9%	−7.3%	11.1%	0.868	15	23.4%	11.6%	0.9%	−7.0%	8.8%	0.575	15	20.4%	9.2%	1.0%	−5.8%	7.8%	0.803
		Test	15	31.8%	10.0%					15	22.5%	9.5%					15	19.4%	9.0%				
	Lingual	Control	15	50.2%	14.3%	2.1%	−8.3%	12.6%	0.740	15	39.5%	14.4%	2.6%	−8.5%	13.7%	0.724	15	32.4%	14.6%	−1.7%	−10.9%	7.4%	0.351
		Test	15	48.1%	13.7%					15	36.9%	15.2%					15	34.1%	9.2%				
	Prox	Control	15	46.1%	12.2%	5.6%	−2.6%	13.7%	0.237	15	31.6%	9.9%	0%	−8%	8%	0.967	15	28.2%	10.1%	1%	−7%	8%	0.803
		Test	15	40.5%	9.4%					15	31.5%	11.0%					15	27.4%	9.8%				
BOMP (0–2)	**All**	Control	15	0.4	0.2	0.0	−0.1	0.2	0.507	15	0.3	0.2	0.1	0.0	0.2	0.078	15	0.2	0.1	0.0	−0.1	0.1	0.967
		Test	15	0.4	0.2					15	0.2	0.1					15	0.2	0.1				
	Buccal	Control	15	0.3	0.2	0.0	−0.1	0.1	0.481	15	0.2	0.1	0.0	−0.1	0.1	0.481	15	0.2	0.1	0.0	−0.1	0.1	0.934
		Test	15	0.3	0.1					15	0.2	0.1					15	0.2	0.1				
	Lingual	Control	15	0.6	0.2	0.1	−0.1	0.3	0.330	15	0.4	0.3	0.2	0.0	0.3	0.093	15	0.3	0.2	0.0	−0.1	0.2	0.663
		Test	15	0.5	0.2					15	0.3	0.2					15	0.3	0.1				
	Prox	Control	15	0.5	0.2	0.1	0.0	0.2	0.237	15	0.3	0.2	0.1	0.0	0.2	0.147	15	0.2	0.1	0.0	−0.1	0.1	0.724
		Test	15	0.4	0.2					15	0.2	0.1					15	0.2	0.1				

Table 3. *Cont.*

			Baseline							6 Weeks							12 Weeks						
Variable	**Sites**	**Group**	**N**	**Mean**	**SD**	**Mean Diff.**	**95% CI Lower**	**95% CI Upper**	***p* Value**	**N**	**Mean**	**SD**	**Mean Diff.**	**95% CI Lower**	**95% CI Upper**	***p* Value**	**N**	**Mean**	**SD**	**Mean Diff.**	**95% CI Lower**	**95% CI Upper**	***p* Value**
PD (mm)	**All**	Control	15	2.4	0.4	0.1	−0.1	0.4	0.254	15	2.5	0.4	0.1	−0.1	0.4	0.221	15	2.4	0.4	0.1	−0.2	0.3	0.395
		Test	15	2.3	0.2					15	2.3	0.3					15	2.3	0.2				
	Buccal	Control	15	2.4	0.3	0.2	−0.1	0.4	0.141	15	2.4	0.3	0.1	−0.1	0.4	0.237	15	2.3	0.3	0.1	−0.1	0.3	0.494
		Test	15	2.2	0.2					15	2.3	0.2					15	2.2	0.2				
	Lingual	Control	15	2.5	0.5	0.1	−0.2	0.4	0.330	15	2.5	0.4	0.1	−0.1	0.4	0.245	15	2.5	0.4	0.0	−0.2	0.3	0.534
		Test	15	2.4	0.3					15	2.4	0.4					15	2.4	0.3				
	Prox	Control	15	2.7	0.4	0.1	−0.1	0.4	0.191	15	2.7	0.4	0.1	−0.2	0.3	0.494	15	2.6	0.4	0.0	−0.2	0.2	0.820
		Test	15	2.6	0.3					15	2.6	0.3					15	2.6	0.2				
%PD < 4 mm		Control	15	87%	8%	−5%	−11%	0%	0.065	15	89%	8%	−3%	−8%	2%	0.309	15	89%	8%	−2%	−7%	3%	0.443
		Test	15	93%	5%					15	92%	6%					15	91%	5%				
%PD 4–5 mm		Control	15	12%	8%	5%	0%	10%	0.120	15	10%	8%	3%	−2%	8%	0.351	15	11%	7%	3%	−2%	7%	0.372
		Test	15	7%	4%					15	7%	5%					15	8%	5%				
%PD > 5 mm		Control	15	1%	2%	0%	−1%	1%	0.714	15	1%	1%	0%	−1%	1%	0.963	15	0%	1%	0%	−1%	0%	0.449
		Test	15	1%	1%					15	1%	1%					15	1%	1%				
REC (mm)	**All**	Control	15	0.7	0.8	−0.3	−0.9	0.4	0.361	15	0.7	0.8	−0.2	−0.9	0.5	0.419	15	0.7	0.8	−0.2	−0.9	0.4	0.330
		Test	15	1.0	1.0					15	0.9	1.1					15	0.9	1.1				
	Buccal	Control	15	0.8	0.9	−0.2	−0.9	0.5	0.290	15	0.8	0.9	−0.2	−0.9	0.5	0.330	15	0.8	0.9	−0.2	−0.9	0.5	0.372
		Test	15	1.0	1.0					15	1.0	1.0					15	1.0	1.0				
	Lingual	Control	15	0.6	0.7	−0.3	−1.0	0.4	0.419	15	0.6	0.7	−0.2	−0.9	0.5	0.678	15	0.6	0.7	−0.3	−0.9	0.4	0.604
		Test	15	0.9	1.1					15	0.8	1.1					15	0.8	1.1				
	Prox	Control	15	0.6	0.7	−0.3	−1.0	0.4	0.395	15	0.6	0.7	−0.2	−0.9	0.5	0.648	15	0.6	0.7	−0.3	−0.9	0.4	0.520
		Test	15	0.8	1.1					15	0.8	1.1					15	0.8	1.1				

Table 3. *Cont.*

Variable	Sites	Group	Baseline							6 Weeks							12 Weeks						
			N	Mean	SD	Mean Diff.	95% CI Lower	95% CI Upper	*p* Value	N	Mean	SD	Mean Diff.	95% CI Lower	95% CI Upper	*p* Value	N	Mean	SD	Mean Diff.	95% CI Lower	95% CI Upper	*p* Value
CAL (mm)	**All**	Control	15	1.7	0.9	0.4	−0.3	1.1	0.110	15	1.8	0.8	0.4	−0.3	1.1	0.178	15	1.7	0.9	0.3	−0.4	1.0	0.178
		Test	15	1.3	1.0					15	1.4	1.1					15	1.4	1.1				
	Buccal	Control	15	1.6	1.0	0.4	−0.3	1.1	0.059	15	1.6	1.0	0.4	−0.4	1.1	0.106	15	1.5	1.0	0.3	−0.4	1.1	0.237
		Test	15	1.2	0.9					15	1.3	1.0					15	1.2	1.0				
	Lingual	Control	15	1.9	0.8	0.4	−0.3	1.1	0.178	15	1.9	0.7	0.4	−0.4	1.1	0.254	15	1.9	0.8	0.3	−0.4	1.0	0.340
		Test	15	1.5	1.0					15	1.6	1.2					15	1.6	1.2				
	Prox	Control	15	2.2	0.8	0.4	−0.2	1.1	0.085	15	2.1	0.8	0.3	−0.4	1.0	0.237	15	2.1	0.8	0.3	−0.5	1.0	0.419
		Test	15	1.8	1.0					15	1.8	1.1					15	1.8	1.1				
n open pock.	**All**	Control	15	3.5	3.4	1.0	−1.7	3.7	0.134	15	2.7	2.8	0.7	−1.4	2.8	0.328	15	1.5	1.1	0.1	−0.9	1.1	0.588
		Test	15	2.5	3.7					15	1.9	2.8					15	1.3	1.6				

SD. standard deviation; CI. confidence interval; Diff. difference. PlI, plaque index; Prox, proximal; GBI, gingival bleeding index; BOMP, bleeding on marginal probing; PD, probing depth; REC, gingival recession; CAL, clinical attachment loss; pock., pocket.

Table 4. Prevalence of target bacterial species at each study visit.

Prevalence	Bacteria	Group	Baseline				6 Weeks				12 Weeks			
			N	n	%	*p* Value	N	n	%	*p* Value	N	n	%	*p* Value
Culture	Aa	Control	15	0	0.0		15	0	0.0		14	0	0.0	
		Test	15	0	0.0		14	0	0.0		14	0	0.0	
	Pg	Control	15	7	46.7	0.713	15	6	40.0	0.876	14	5	35.7	0.256
		Test	15	6	40.0		14	6	42.9		14	8	57.1	
	Pi	Control	15	4	26.7	0.361	15	1	6.7	0.054	14	7	50.0	0.445
		Test	15	2	13.3		14	5	35.7		14	9	64.3	
	Tf	Control	15	1	6.7	0.309	15	0	0.0	0.292	14	4	28.6	0.663
		Test	15	0	0.0		14	1	7.1		14	3	21.4	
	Pm	Control	15	0	0.0	0.032	15	3	20.0	0.316	14	0	0.0	0.309
		Test	15	4	26.7		14	1	7.1		14	1	7.1	
	Fn	Control	15	13	86.7	1.000	15	8	53.3	0.550	14	13	92.9	0.541
		Test	15	13	86.7		14	9	64.3		14	12	85.7	
	Cr	Control	15	3	20.0	0.624	15	0	0.0		14	3	21.4	0.067
		Test	15	2	13.3		14	0	0.0		14	0	0.0	
	Ec	Control	15	3	20.0	0.232	15	2	13.3	0.033	14	7	50.0	0.705
		Test	15	6	40.0		14	7	50.0		14	6	42.9	
qPCR	Aa	Control	15	1	6.7	1.000	15	1	6.7	1.000	15	1	6.7	1.000
		Test	15	0	0.0		15	0	0.0		15	0	0.0	
	Pg	Control	15	6	40.0	0.705	15	12	80.0	0.427	15	10	66.7	0.705
		Test	15	5	33.3		15	9	60.0		15	9	60.0	
	Tf	Control	15	13	86.7	0.390	15	13	86.7	1.000	15	12	80.0	0.427
		Test	15	10	66.7		15	12	80.0		15	9	60.0	

qPCR: quantitative polymerase chain reaction; Aa: *Aggregatibacter actinomycetemcomitans*; Pg: *Porphyromonas gingivalis*; Pi: *Prevotella intermedia*; Tf: *Tannerella forsythia*; Pm: *Parvimonas micra*; Fn: *Fusobacterium nucleatum*; Cr: *Campylobacter rectus*; Ec: *Eikenella corrodens*.

No statistically significant differences between groups were observed at any study visit for the frequency of the detection of the target species. *A. actinomycetemcomitans* was not detected. *P. gingivalis* was detected in 40.0–46.7% of samples at the baseline and 35.7–57.1% at the final visit after 12 weeks. *T. forsythia* remained low from the baseline to 6 weeks for both study groups (0.0–6.7% at the baseline, 0.0–7.1% at 6 weeks) and slightly increased at 12 weeks (21.4–28.6%).

For the bacterial counts and proportions of the total counts of target species, both test and control groups showed similar trends, with no statistically significant differences between groups. For the proportions of the total microbiota, *P. gingivalis* showed the baseline's highest mean proportions of 9.06–9.35%, with some reductions with time, of a higher magnitude in the control group. For the mean bacterial counts, *P. gingivalis* also presented the highest values, similar at the baseline in both groups: 1.37×10^5 CFU/mL (SD = 2.87×10^5) for the test and 1.32×10^5 CFU/mL (SD = 2.57×10^5) for the control.

Table 5. Counts and proportions of target bacterial species at each study visit.

Method	Variable	Group	Baseline							6 Weeks							12 Weeks						
			N	Mean	SD	Mean Diff.	95% CI Lower	95% CI Upper	*p* Value	N	Mean	SD	Mean Diff.	95% CI Lower	95% CI Upper	*p* Value	N	Mean	SD	Mean Diff.	95% CI Lower	95% CI Upper	*p* Value
Culture	Counts (CFU/mL)																						
	All	Control	15	1.3×10^6	1.8×10^6	3.9×10^5	-7.1×10^5	1.5×10^6	0.787	15	6.5×10^5	1.2×10^6	-1.7×10^6	-4.4×10^6	1.1×10^6	0.116	14	2.7×10^6	5.3×10^6	1.8×10^6	-1.3×10^6	4.9×10^6	0.383
		Test	15	9.3×10^5	1.1×10^6					14	2.3×10^6	5.1×10^6					14	8.8×10^5	1.3×10^6				
	Aa	Control	15	0.0	0.0					15	0.0						14	0.0					
		Test	15	0.0	0.0					14	0.0						14	0.0					
	Pg	Control	15	2.5×10^5	5.9×10^5	1.4×10^5	-1.9×10^5	4.6×10^5	0.854	15	6.6×10^3	1.0×10^4	-4.2×10^5	-1.1×10^6	2.5×10^5	0.608	14	3.4×10^5	1.1×10^6	2.2×10^5	-3.9×10^5	8.4×10^5	0.424
		Test	15	1.1×10^5	1.9×10^5					14	4.3×10^5	1.2×10^6					14	1.1×10^5	2.5×10^5				
	Pi	Control	15	1.2×10^5	4.0×10^5	1.2×10^5	-1.0×10^5	3.4×10^5	0.299	15	6.7×10^1	2.6×10^2	-4.2×10^3	-1.0×10^4	2.0×10^3	0.049	14	1.1×10^5	3.7×10^5	9.5×10^4	-1.1×10^5	3.0×10^5	0.720
		Test	15	6.7×10^2	2.6×10^3					14	4.2×10^3	1.1×10^4					14	1.2×10^4	2.1×10^4				
	Tf	Control	15	3.3×10^3	1.3×10^4	3.3×10^3	-3.8×10^3	1.0×10^4	0.317	15	0.0	0.0	-1.8×10^3	-5.6×10^3	2.1×10^3	0.301	14	5.1×10^3	1.3×10^4	-2.9×10^3	-1.5×10^4	9.0×10^3	0.856
		Test	15	0.0	0.0					14	1.8×10^3	6.7×10^3					14	8.0×10^3	1.7×10^4				
	Pm	Control	15	0.0	0.0	-3.7×10^3	-9.4×10^3	2.1×10^3	0.035	15	6.1×10^4	2.3×10^5	5.6×10^4	-7.2×10^4	1.8×10^5	0.344	14	0.0	0.0	-2.9×10^3	-9.0×10^3	3.3×10^3	0.317
		Test	15	3.7×10^3	1.0×10^4					14	5.0×10^3	1.9×10^4					14	2.9×10^3	1.1×10^4				
	Fn	Control	15	5.3×10^4	1.2×10^5	3.7×10^4	-2.8×10^4	1.0×10^5	0.394	15	5.6×10^3	8.7×10^3	-2.2×10^4	-4.2×10^4	-1.3×10^3	0.108	14	2.5×10^4	3.7×10^4	-7.1×10^1	-2.8×10^4	2.8×10^4	0.854
		Test	15	1.6×10^4	2.1×10^4					14	2.7×10^4	3.5×10^4					14	2.5×10^4	3.6×10^4				
	Cr	Control	15	2.1×10^3	5.6×10^3	6.7×10^2	-3.4×10^3	4.7×10^3	0.632	15	0.0					1.000	14	3.6×10^4	1.3×10^5	3.6×10^4	-4.1×10^4	1.1×10^5	0.072
		Test	15	1.4×10^3	5.2×10^3					14	0.0						14	0.0	0.0				
	Ec	Control	15	2.2×10^3	5.6×10^3	-1.3×10^3	-5.6×10^3	3.1×10^3	0.293	15	1.3×10^3	4.4×10^3	-6.5×10^3	-1.7×10^4	3.5×10^3	0.043	14	6.6×10^2	1.1×10^3	-1.1×10^3	-3.2×10^3	1.0×10^3	1.000
		Test	15	3.5×10^3	6.1×10^3					14	7.8×10^3	1.7×10^4					14	1.7×10^3	3.5×10^3				
	Proportions (%)																						
	Aa	Control	15	0.0						15	0.0						14	0.0					
		Test	15	0.0						14	0.0						14	0.0					
	Pg	Control	15	9.4	15.1	0.3	−11.7	12.3	0.731	15	2.6	7.1	−6.1	−18.0	5.8	0.696	14	5.0	11.3	−2.2	−10.3	5.9	0.272
		Test	15	9.1	16.8					14	8.7	19.8					14	7.1	9.4				
	Pi	Control	15	2.4	7.7	2.3	−1.9	6.6	0.285	15	0.0	0.0	−0.6	−1.5	0.3	0.049	14	2.3	4.1	1.3	−1.1	3.8	0.720
		Test	15	0.1	0.3					14	0.6	1.6					14	1.0	1.3				
	Tf	Control	15	0.1	0.2	0.1	−0.1	0.2	0.317	15	0.0	0.0	−1.4	−4.6	1.7	0.301	14	0.6	2.0	0.1	−1.1	1.3	0.809
		Test	15	0.0	0.0					14	1.4	5.4					14	0.5	1.0				
	Pm	Control	15	0.0	0.0	−0.9	−1.9	0.1	0.035	15	3.9	13.9	2.9	−5.0	10.7	0.344	14	0.0	0.0	−0.2	−0.5	0.2	0.317
		Test	15	0.9	1.8					14	1.0	3.8					14	0.2	0.6				
	Fn	Control	15	3.8	3.7	1.0	−1.5	3.4	0.383	15	1.9	3.1	−6.5	−17.8	4.8	0.353	14	2.4	3.0	−5.5	−14.3	3.2	0.250
		Test	15	2.8	2.9					14	8.3	19.4					14	7.9	15.0				
	Cr	Control	15	0.1	0.3	−0.1	−0.4	0.2	0.725	15	0.0						14	0.8	2.1	0.8	−0.4	2.1	0.072
		Test	15	0.2	0.5					14	0.0						14	0.0	0.0				
	Ec	Control	15	0.3	0.8	−0.2	−0.8	0.4	0.250	15	0.8	2.8	0.1	−1.6	1.7	0.040	14	0.2	0.3	−0.3	−0.9	0.2	0.653
		Test	15	0.5	0.8					14	0.7	1.0					14	0.6	1.0				

Table 5. *Cont.*

Method	Variable		Group	Baseline							6 Weeks							12 Weeks						
				N	Mean	SD	Mean Diff.	95% CI Lower	95% CI Upper	*p* Value	N	Mean	SD	Mean Diff.	95% CI Lower	95% CI Upper	*p* Value	N	Mean	SD	Mean Diff.	95% CI Lower	95% CI Upper	*p* Value
qPCR	Counts (CFU/mL)	Aa	Control	15	6.5×10^3	2.5×10^4	6.5×10^3	-7.5×10^3	2.0×10^4	0.317	15	3.7×10^2	1.4×10^3	3.7×10^2	-4.2×10^2	1.2×10^3	0.317	15	6.1×10^4	2.4×10^5	6.1×10^4	-7.0×10^4	1.9×10^5	0.317
			Test	15	0.0	0.0					15	0.0	0.0					15	0.0	0.0				
		Pg	Control	15	1.3×10^5	2.6×10^5	-5.3×10^3	-2.1×10^5	2.0×10^5	0.792	15	8.2×10^4	2.5×10^5	-1.1×10^5	-4.1×10^5	1.9×10^5	0.690	15	5.5×10^4	1.5×10^5	-3.1×10^4	-1.8×10^5	1.2×10^5	0.750
			Test	15	1.4×10^5	2.9×10^5					15	1.9×10^5	5.1×10^5					15	8.5×10^4	2.5×10^5				
		Tf	Control	15	1.7×10^6	3.4×10^6	8.7×10^5	-1.1×10^6	2.9×10^6	0.327	15	5.9×10^5	1.2×10^6	-6.3×10^5	-2.2×10^6	9.4×10^5	0.633	15	7.5×10^5	1.6×10^6	-3.3×10^5	-1.6×10^6	9.7×10^5	0.344
			Test	15	7.9×10^5	1.6×10^6					15	1.2×10^6	2.6×10^6					15	1.1×10^6	1.8×10^6				

SD, standard deviation; CI, confidence interval; Diff, difference; CFU, colony-forming units; qPCR: quantitative polymerase chain reaction. Aa: *Aggregatibacter actinomycetemcomitans*; Pg: *Porphyromonas gingivalis*; Pi: *Prevotella intermedia*; Tf: *Tannerella forsythia*; Pm: *Parvimonas micra*; Fn: *Fusobacterium nucleatum*; Cr: *Campylobacter rectus*; Ec: *Eikenella corrodens*.

3.7. Microbiological Outcomes—qPCR

Tables 4, 5 and S4 present the microbiological outcomes, expressed as counts, and the frequency of detection of each target bacterial species at each time point and in changes between visits. No statistically significant differences between groups were detected at any study visit.

A. actinomycetemcomitans was detected in only one participant from the control group in all visits. *P. gingivalis* showed an increase in prevalence over time in both groups, 33.3–40.0% at the baseline to 60–80% at 6 weeks, reaching 60.0–66.7% at 12 weeks. *T. forsythia* had a consistently high prevalence throughout the study for both groups, 66.7–86.7% at the baseline, 80.0–86.7% at 6 weeks and 60–80% at 12 weeks.

For *T. forsythia's* bacterial counts, both groups experienced an increase at 6 weeks and a slight decrease at 12 weeks.

4. Discussion

The primary objective of the present pilot clinical trial was to evaluate the safety of a newly formulated mouthwash containing cymenol, potassium nitrate and zinc chloride. This objective was confirmed in terms of the lack of adverse events and a good user's tolerability. As secondary objectives this study also evaluated the clinical and microbiological effect of this newly formulated mouth rinse, when compared to a standard control mouth rinse, demonstrating a similar effect (no statistically significant differences were demonstrated).

In terms of safety, no adverse events were reported during the study. The subject's perceptions on the assigned mouth rinse used, assessed through self-reported questionnaires, showed statistically significant differences between groups at the 6-week visit in questions 5 and 8, favoring the control group, since users of the experimental mouthwash reported a greater sensation of dry mouth ($p = 0.016$) and reported a lower score on their overall opinion of the trial mouthwash ($p = 0.022$), although this opinion improved at 12 weeks ($p > 0.05$). In 2006, a panel of experts assessed the safety of different ingredients used as cosmetic biocides/preservatives and/or fragrances, including cymenol. They concluded that o-Cymen-5-ol was safe at concentrations up to 0.5% [21]. These data are in line with a previous study (2011) where 17 adverse events were reported, but only one occurred in the experimental group after the use of a toothpaste containing cymenol and zinc chloride and it was considered as not related to the treatment itself [22]. Conversely, in another study, a mild case of lip tingling, associated with the use of a toothpaste containing 0.1% cymenol, 0.6% zinc chloride and sodium fluoride, was reported [23].

In terms of the effect of the tested mouth rinse on gingival health, statistically significant differences between the groups were only observed when evaluating the changes in PlI between the baseline and the 12-week visit, with small intragroup changes, but with better results in the control group. These results differ from those observed in previous studies, where the mean PlI score in the cymenol and zinc chloride group was 2.05 at 12 weeks, while in the fluoride control toothpaste, it was 2.71 ($p < 0.0001$) [22]. In another study, evaluating in situ plaque formation with a specific software, differences after 4 days and after 8 days suggested a short-term anti-plaque effect of a 0.10% cymenol mouthwash [24].

The effect on dentin hypersensitivity (Schiff and VAS) showed a trend towards reduction in both groups, but no statistically significant differences between the groups were found. These results, using a formulation with potassium nitrate, are in line with those from a systematic review, reporting the lesser efficacy of potassium, as compared with arginine in a treatment for dentin hypersensitivity [25]. Similarly, a clinical study reported that a control mouth rinse containing 0.2% sodium fluoride reduced hypersensitivity after 2 and 6 weeks of use was equally efficient as the experimental mouthwash containing 3% potassium nitrate and 0.2% sodium fluoride at 2 weeks, although at 6 weeks, significant differences were reported in favor of the experimental group in terms of general sensitivity and response to cold air tests [26].

The impact on tooth staining, a significant side effect of antiseptics, was also evaluated, revealing no statistically significant differences between the groups. While in the experimental group there was a minor increase of 0.4 (SD = 0.3) at 6 weeks and 0.4 (SD = 0.4) at 12 weeks, in the control group, there was a small decrease.

In recent years, there has been a growing interest in using plant-based antimicrobial substances, such as cymenol, as alternatives to traditional antiseptics like chlorhexidine and triclosan. This is primarily due to the possibility that these natural compounds may cause fewer side effects. Cymenol, a natural phenolic compound derived from isopropyl cresol, works by altering the permeability of the cell wall and cell membrane [24]. Zinc salts are combined with cymenol for their antimicrobial properties and their capacity to inhibit microbial enzymatic activities in the oral cavity. An in vitro study showed that the cymenol/zinc combination directly inhibited oral pathogens such as *Streptococcus mutans, Actinomyces viscosus, P. gingivalis, F. nucleatum* and *Candida albicans*. Additionally, there was a synergistic effect between cymenol and zinc against anaerobic bacteria like *F. nucleatum* and *P. gingivalis* [27], which led authors to suggest that this synergistic effect could delay bacterial growth in the supragingival biofilm after toothbrushing, hence reducing the microbial challenge of dental plaque [27]. Furthermore, the presence of zinc salts should have an impact on the levels of volatile sulfur compounds (VSCs), which has been demonstrated with the use of a toothpaste containing 0.1% cymenol and 0.6% zinc chloride, 12 h after brushing [23].

The present study is not free of limitations, with the most important being the small sample size, justified due to the study design (pilot study) and the primary objective (assessing safety and tolerability). In addition, the selected sample belongs to a population undergoing periodontal maintenance, with a higher level of engagement with mechanical biofilm control, which could make it difficult to find the additional impact of mouthwashes with anti-plaque and/or anti-gingivitis effects. Another limitation is the possible influence of the Hawthorne effect in both groups because they felt observed since they were going to be periodically assessed, which could have improved their oral hygiene performance, as reported in previous studies assessing oral hygiene products [28]. A future approach could consider selected patients with less optimal supragingival biofilm control, for whom chemical control would have higher chances of improving clinical outcomes, and recruiting a larger sample.

5. Conclusions

Within the limitations of the present study, it can be concluded that the use of a mouth rinse containing cymenol, and other active agents, was found to be safe and well-tolerated by the patients, with no reports of side effects or adverse events. No significant differences were observed in terms of clinical or microbiological efficacy.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14156595/s1, Table S1: Changes in clinical outcomes between visits: baseline–6 weeks, baseline–12 weeks and 6–12 weeks; Table S2: Dentin hypersensitivity outcomes at each study visit; Table S3: Tooth staining at 6 and 12 weeks; Table S4: Changes in counts and proportions of target bacterial species between visits: baseline–6 weeks, baseline–12 weeks and 6–12 weeks. Appendix Clinical Indices. References [6,8,13–17,29,30] are cited in the Supplementary Materials.

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Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki (Edinburgh revision of October 2000) and the protocol was approved by the

local ethics committee (CEIm Hospital Clínico San Carlos), Madrid, Spain, on 2 December 2020 with the following registration number: 20/750-EC_X.

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: All data are available on request due to restrictions. The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy, data protection and ethical issues.

Conflicts of Interest: A.A., E.F., J.S., S.R., B.A., M.S. and D.H. declare no potential direct conflicts of interest related to the present work.

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