

# Effect of Herbal, Essential Oil, and Chlorhexidine Mouthrinses on the Composition of the Subgingival Microbiota and Clinical Periodontal Parameters

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## Abstract

- **Objective:** The purpose of the present investigation was to determine if antimicrobial mouthrinses with different formulations could affect the composition of the subgingival microbiota and clinical parameters of adjacent tissues in periodontal maintenance subjects.
- **Methods:** One-hundred and sixteen subjects, who had been treated for chronic periodontitis and were in a maintenance program, were randomly assigned to one of four mouthrinses, to be used twice daily for three months. The mouthrinses were herbal 1, herbal 2, essential oil, and chlorhexidine. Clinical measurements and subgingival plaque samples were taken at baseline and at three months. Plaque samples were individually evaluated for 18 test species/taxa using checkerboard DNA-DNA hybridization. Significance of differences between baseline and three months for both microbiological and clinical parameters were determined using the Wilcoxon Signed Ranks test. Significance of difference among groups for change in clinical and microbiological parameters was determined using analysis of covariance (ANCOVA), adjusting for baseline values.
- **Results:** Shifts in species proportions differed significantly for 9/18 test species/taxa among the four mouthrinse groups. *Streptococcus* and *Capnocytophaga* species were reduced most in the herbal rinse groups, while *Veillonella parvula* was reduced most in the essential oil and chlorhexidine groups. *Actinomyces* were also markedly reduced in the chlorhexidine group. Mean Plaque (PI) and Gingival Indices (GI) were reduced between baseline and three months in each group. Results emphasize that chlorhexidine ( $p < 0.001$ ) and herbal ( $p < 0.05$ ) rinses significantly reduced PI. Some subjects in each group responded better than others.
- **Conclusion:** All four mouthrinses tested produced shifts in the composition of subgingival microbiota, although the results differed among the groups. The observed microbial changes were accompanied by improvements in clinical parameters in the periodontal maintenance subjects.

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## Introduction

It has been known for a considerable period of time that the accumulation of supragingival plaque at the gingival margin can lead to gingivitis.<sup>1</sup> Further, bacterial species present in supragingival biofilms can move subgingivally and initiate periodontitis. Regular removal of supragingival plaque can diminish gingivitis and alter the composition of the subgingival biofilm.<sup>2,3</sup> While the common method of achieving this goal is self-performed tooth brushing and/or flossing, the removal of plaque through mechanical means may not be properly performed by the majority of the population. Therefore, the use of antimicrobial mouthrinses may be an effective adjunct for specifically controlling supragingival plaque that, in turn, can impact the subgingival environment and the clinical manifestations of gingivitis and periodontitis. Thus, the question asked in the present study was whether regularly performed antimicrobial mouth rinsing could affect the composition of the subgingival microbiota and the clinical status of the adjacent periodontal tissues.

To examine this question, mouthrinses with different active ingredients and mechanisms of action were chosen for study. Because only active ingredients in the mouthrinses were compared (*i.e.*, positive control), a placebo was not included. The effectiveness of antimicrobial mouthrinses in inhibiting the development of plaque and gingivitis is well documented.<sup>4</sup> In

particular, the long-term plaque- and gingivitis-reducing characteristics of two specific antimicrobial mouthrinses, one based on an essential oil (Listerine® Cool Mint®, Johnson & Johnson, New Brunswick, NJ, USA) and the other on chlorhexidine (Peridex™, Zila Pharmaceuticals, Phoenix, AZ, USA) have been demonstrated.<sup>5-7</sup> The essential oil mouthrinse contains menthol (a local anesthetic), thymol (an antiseptic), and methyl salicylate (a cleansing agent), as well as eucalyptol and between 22 and 27% ethanol, depending on the flavor. Studies of the essential oil mouthrinse have indicated that it is effective in reducing plaque and gingivitis,<sup>8-10</sup> as well as mean bacterial counts in supra- and subgingival plaque samples and biofilm samples from the tongue dorsum.<sup>11,12</sup>

Chlorhexidine (CHX) has been widely used to prevent plaque development on the teeth since the classic studies of Løe, *et al.* that examined the effects of CHX on plaque levels and gingivitis.<sup>13-15</sup> Further, once-daily rinsing with CHX for two years decreased the number of bacteria in saliva by 30 to 50%.<sup>16</sup> Oral use of 0.2% CHX also affected the supragingival plaque bacteria by increasing levels of *Streptococcus sanguis* (*Streptococcus sanguinis*) and decreasing levels of *Streptococcus milleri* (*Streptococcus anginosus*, *Streptococcus constellatus*, *Streptococcus intermedius*).<sup>17</sup> Later studies have demonstrated that rinsing with CHX reduces plaque bacteria,<sup>18-20</sup> including species in the genera

*Streptococcus*, *Actinomyces*, *Fusobacterium*, *Veillonella* and *Capnocytophaga*.<sup>20-23</sup>

An herbal mouthrinse (Natural Dentist Healthy Gums Oral Rinse, Natural Dentist, Inc., Medford, MA, USA), containing several naturally occurring anti-inflammatory agents, such as aloe vera and calendula, and antimicrobial agents such as golden-seal and grapefruit seed,<sup>24-31</sup> has also been shown to reduce gingival bleeding and gingivitis<sup>32</sup> and inhibit the growth of aerobic, microaerophilic, and anaerobic bacteria.<sup>33</sup> It was also shown to have *in vitro* efficacy against three specific species of oral bacteria (*Streptococcus mutans*, *S. sanguinis*, and *Actinomyces viscosus*).<sup>34</sup> Further, data from recent *in vitro* testing suggest that the herbal mouthrinse may provide oral health benefits by inhibiting the growth of such periodontal or cariogenic pathogens as *Eubacterium nodatum*, *Tannerella forsythia*, *Prevotella* species, and *S. mutans*.<sup>35</sup>

The herbal, essential oil, and chlorhexidine mouthrinses have been shown to be effective in reducing dental plaque and gingivitis potentially by different mechanisms of action given the different active ingredients in these products. Therefore, the purpose of this study was to examine and compare the effect of these four rinses on the microbiota and, further, to examine the clinical effect on periodontal tissues.

## Materials and Methods

### Subject Population

One-hundred and twenty-two subjects, who had been treated for chronic periodontitis and were in a maintenance program, were recruited. The subjects were older than 20 years of age, with at least 20 natural teeth, and had at least four teeth with pocket depths (PD) > 4 mm and attachment level (AL) > 3 mm prior to therapy. Subjects were randomly assigned to one of the four mouthrinse groups using a random number table. Subjects had no known systemic disorders that could affect periodontal disease status or clinical monitoring and sample taking. The subjects had received maintenance scaling and root planing (SRP) three months prior to the start of the study. Subjects were excluded if they were pregnant, nursing, or had any systemic condition that might influence the course of periodontal disease or treatment (e.g., diabetes, AIDS), any systemic condition that required antibiotic coverage for routine periodontal procedures (e.g., heart conditions, joint replacements, etc.), any known allergy to mouthrinse ingredients, or were current smokers. The Forsyth Institutional Review Board approved the study protocol that conformed to the guidelines of the Declaration of Helsinki. All subjects provided signed consent prior to the start of the study.

### Microbiological Sample Taking and Enumeration

Subgingival plaque samples were taken at baseline and after three months of self-performed mouthrinse administration. Prior to clinical monitoring, subgingival plaque samples were collected from 14 teeth (one randomly assigned maxillary and mandibular quadrant) in each subject by the clinician assigned to the subject. After removal of supragingival plaque, samples of subgingival plaque were taken using separate sterile Gracey curettes. Each sample was placed in a separate tube containing 0.15 ml TE (10 mM Tris-HCL, 0.1 mM EDTA, pH 7.6), after

which 0.10 ml of 0.5 M NaOH was immediately added. Each sample was analyzed for its content of 18 bacterial species/groups using checkerboard DNA-DNA hybridization.<sup>36,37</sup>

The samples were lysed and the DNA placed in lanes on a nylon membrane using a 60-lane checkerboard (Immunitics, Cambridge, MA, USA) that accommodated 56 plaque samples run against 18 DNA probes. After fixation of the DNA to the membrane, the membrane was placed in a Miniblotter<sup>®</sup> 45 (Immunitics), with the lanes of DNA at 90° to the lanes of the device. Digoxigenin-labeled whole genomic DNA probes to 18 bacterial taxa were hybridized in individual lanes of the Miniblotter. After hybridization, the membranes were washed at high stringency, and the bound DNA probes detected using antibody to digoxigenin conjugated with alkaline phosphatase and chemi-fluorescence detection. Signals were detected using AttoPhos<sup>®</sup> Substrate (Amersham Life Sciences, Arlington Heights, IL, USA) and read using a Storm<sup>™</sup> FluorImager (Molecular Dynamics, Sunnyvale, CA, USA), a computer-linked instrument that reads the intensity of the fluorescence signals resulting from the probe-target hybridization.

Two lanes in each run contained standards at the concentration of 10<sup>5</sup> and 10<sup>6</sup> cells of each species. The sensitivity of the assay was adjusted to permit the detection of 10<sup>4</sup> cells of a given species by adjusting the concentration of each DNA probe. Signals were evaluated using the Storm FluorImager and converted to absolute counts by comparison with standards on the same membrane. Failure to detect a signal was recorded as zero. A total of 2,867 subgingival samples were evaluated (12.4 samples per subject per visit).

### Clinical Measures

Clinical monitoring was performed by the same clinician at baseline and three months for a given subject. Each subject was clinically monitored at a baseline visit at six sites per tooth, excluding third molars, for a total of up to 168 sites for Gingival Index<sup>39</sup>(GI), Plaque Index<sup>38</sup> (PI), Bleeding on Probing (BOP; 0,1), Pocket Depth (PD; mm), and Attachment Level (AL; mm). PD and AL were measured twice, and the mean of the pair of measurements was used in the analyses. Clinical measurements were repeated at three months.

### Mouthrinse Assignment and Utilization

After baseline monitoring and randomization to mouthrinse groups, subjects were provided with a three-month supply of the assigned test agent. The four test mouthrinses were Listerine Cool Mint, Peridex, The Natural Dentist Healthy Gums Oral Rinse, and The Natural Dentist Healthy Gums Oral Rinse minus bloodroot. Subjects were instructed to rinse for one minute, twice a day (*i.e.*, after morning and evening tooth brushing) for three months. The mouthrinses were provided in indistinguishable bottles marked only with the specific code so neither the examining clinician nor the subject was aware of the identity of the prescribed product. At the end of the study, after the three-month monitoring, subjects received a full-mouth prophylaxis. Natural Dentist supplied the herbal mouthrinses, and the formulations were described in Haffajee, *et al.*<sup>35</sup> The formulations for the two herbal mouthrinses employed in this study were identical, except that herbal

rinse 1 contained bloodroot. The Forsyth Institute purchased the essential oil and chlorhexidine mouthrinses.

### Sample Size

Since no published data were found that examined the effect of mouthrinses on the subgingival microbiota, surrogate markers were sought that could indicate a change was taking place as a result of the use of a mouthrinse. Thus, the number of subjects necessary to demonstrate a significant effect of mouthrinses on PI was determined by study power calculations that were based on paired comparisons testing the significance of differences between the baseline mean values and the mean values at three months. Data were available from two previous investigations of plaque reduction using home care procedures. The mean standard deviation (SD) of differences was 0.49 for one study and 0.54 for the second study. The mean reduction in PI considered to be significant was 0.33, typically a reduction > 25% from baseline mean PI values of 1.30 (based on earlier studies). Thus, using an alpha of 0.05 and a power of 0.80, it was found that 22 subjects would be required for each test group.

### Data Analysis

Clinical and microbiological data were available from 116 subjects who completed both the baseline and three-month monitoring visits. The percentage that each species made up of the total DNA probe count at each of the 14 sample sites in each subject was determined. The percentages for each species were averaged within a subject, and then across subjects in each treatment group and time point separately. Significance of differences between baseline and three months was determined using the Wilcoxon Signed Ranks test. Significance of differences among mouthrinse groups in the mean change in proportions of each species/group from baseline to three months was determined using analysis of covariance (ANCOVA), adjusting for baseline values. All microbial analyses were run without adjusting for and with adjustments for 18 comparisons.<sup>40</sup>

Each clinical parameter was measured at up to 168 sites per subject. The data for each parameter were averaged within a subject and then averaged across subjects at each time point in each mouthrinse group separately. Significance of differences between baseline and three months was determined using the Wilcoxon Signed Ranks test. Mean differences in clinical parameters from baseline to three months were computed, and the significance of differences of each parameter among mouthrinse groups was determined using (ANCOVA), adjusting for baseline values.

## Results

The mean baseline clinical parameters of the 116 subjects in the four mouthrinse groups who completed both the baseline and three months monitoring appointments are presented in Table I. There were no statistically significant differences among treatment groups at baseline.

### Microbiological Findings

It was hypothesized that a reduction in the supragingival plaque by oral mouthrinses would affect the amount and com-

**Table I**  
Means and Standard Deviations of Clinical Parameters at Baseline for Subjects in the Four Mouthrinse Groups

	Herbal 1	Herbal 2	Essential Oil	CHX
N	29	28	28	31
Age (years)	54 ± 12	53 ± 13	47 ± 14	51 ± 14
% males	41	39	39	48
Plaque Index	1.27 ± 0.71	1.14 ± 0.82	0.91 ± 0.61	1.09 ± 0.71
Gingival Index	0.81 ± 0.41	0.76 ± 0.43	0.78 ± 0.36	0.81 ± 0.39
% sites with Bleeding				
on Probing	22.5 ± 13.1	22.0 ± 18.9	5.4 ± 9.2	20.2 ± 14.5
Mean Pocket				
Depth (mm)	2.46 ± 0.32	2.53 ± 0.49	2.41 ± 0.22	2.45 ± 0.23
Mean Attachment				
Level (mm)	2.40 ± 0.62	2.67 ± 1.11	2.30 ± 0.62	2.43 ± 0.74
N missing teeth	1.79 ± 2.30	3.04 ± 2.77	2.14 ± 2.03	2.32 ± 2.45

None of the parameters differed significantly using the Kruskal Wallis test.

position of subgingival biofilms. This hypothesis appeared to be correct in that regression analysis demonstrated that subjects in whom the PI was reduced exhibited a significant reduction in total counts of bacterial species ( $r = 0.34$ ,  $p < 0.001$ ). There also were alterations in the composition of the subgingival microbiota, which differed among groups, as can be seen in Table II and Figure 1. The mean proportions ( $\pm$  SD) of the 18 test species/groups at baseline and three months in each mouthrinse group are presented in Table II. After adjusting for 18 comparisons, only the *Actinomyces* species showed significant reductions between baseline and three months in subjects in the CHX group.

When the change in mean proportions of each of the test species from baseline to three months in each group was examined, the different mouthrinses appeared to have different effects on the subgingival microbiota (Figure 1). For example, the species reduced most by CHX were primarily *Actinomyces*, *Actinomyces naeslundii* genospecies 2, *Actinomyces israelii*, and *Actinomyces gerencseriae*. In addition, proportions of *Veillonella parvula* and *Selenomonas noxia* were also reduced more by this mouthrinse. The species that were affected most by the essential oil rinse included *V. parvula* and *Prevotella*. The herbal mouthrinses affected species in the genus *Streptococcus*, including *Streptococcus mitis*, *Streptococcus oralis*, *S. sanguinis*, *Streptococcus gordonii*, the "milleri streptococci," *S. intermedius*, *S. anginosus*, and *S. constellatus*, as well as *S. mutans*. It is also interesting that, although not statistically significant, the herbal mouthrinses appeared to have a greater numerical effect on species of the pathogenic red complex, *T. forsythia*, *Porphyromonas gingivalis*, and *Treponema denticola*, as well as other suspected periodontal pathogens, such as those in the genus *Fusobacterium* and *Parvimonas micra*.

### Clinical Findings

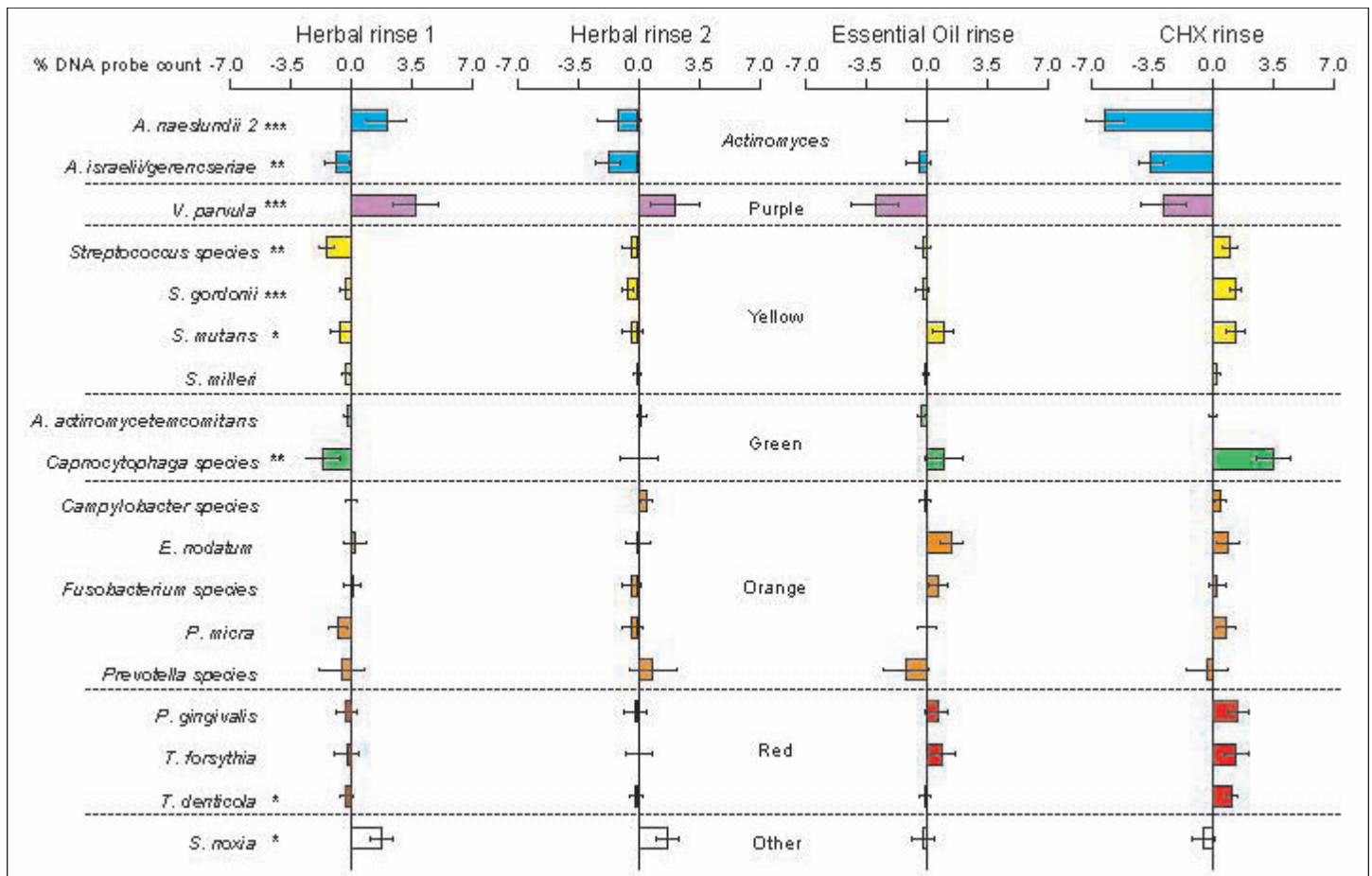
Mean ( $\pm$  SD) clinical parameters at baseline and three months for each mouthrinse group are presented in Table III. In general, mean PI, mean GI, and mean percent of sites with BOP decreased in all treatment groups with the exception of percent of sites with BOP in the herbal rinse 2 and essential oil groups, where there was a small non-significant increase. The decrease in PI was statistically significant in the herbal rinse 1 and 2 and CHX groups, while GI showed a statistically significant

**Table II**  
Mean Percent DNA Probe Counts and Standard Deviations for the 18 Test Taxa at Baseline and Three Months for Subjects in the Four Mouthrinse Groups

	Herbal Rinse 1		Herbal Rinse 2		Essential Oil Rinse		Chlorhexidine Rinse	
	Baseline	3 Months	Baseline	3 Months	Baseline	3 Months	Baseline	3 Months
N	29	29	26	26	28	28	31	31
<i>A. naeslundii</i> 2	11.27 ± 9.18	13.35 ± 8.53	10.21 ± 8.04	9.75 ± 5.89	13.95 ± 9.12	12.43 ± 8.13	<b>9.97 ± 6.21</b>	<b>4.52 ± 4.82</b>
<i>A. israelii/gerencseriae</i>	10.30 ± 6.63	9.08 ± 4.54	8.66 ± 5.55	7.39 ± 4.00	11.29 ± 7.54	9.92 ± 6.03	<b>8.20 ± 3.65</b>	<b>5.37 ± 3.65</b>
<i>V. parvula</i>	<b>9.94 ± 7.12</b>	<b>13.88 ± 9.26</b>	10.05 ± 4.86	12.28 ± 5.26	<b>9.56 ± 8.42</b>	<b>7.01 ± 5.34</b>	<b>11.48 ± 6.72</b>	<b>7.94 ± 8.92</b>
<i>S. mitis/oralis/sanguinis</i>	<b>3.60 ± 6.28</b>	<b>1.79 ± 1.34</b>	2.98 ± 2.58	2.58 ± 2.54	2.69 ± 2.19	2.70 ± 2.51	<b>2.88 ± 2.59</b>	<b>4.00 ± 3.16</b>
<i>S. gordonii</i>	1.64 ± 1.24	1.44 ± 0.99	1.96 ± 1.88	1.27 ± 0.77	1.21 ± 0.75	1.26 ± 0.98	<b>2.49 ± 2.81</b>	<b>3.46 ± 3.39</b>
<i>S. mutans</i>	4.07 ± 4.99	3.07 ± 2.54	3.56 ± 3.93	3.06 ± 2.59	3.34 ± 3.75	4.28 ± 4.24	<b>2.40 ± 2.24</b>	<b>4.19 ± 4.30</b>
<i>S. milleri</i>	<b>0.87 ± 0.66</b>	<b>0.66 ± 0.53</b>	0.76 ± 0.92	0.94 ± 1.11	1.22 ± 1.63	0.95 ± 1.05	1.15 ± 1.14	1.22 ± 1.37
<i>A. actinomycetemcomitans</i>	1.67 ± 1.16	1.53 ± 1.35	1.82 ± 1.56	2.00 ± 1.89	1.59 ± 1.49	1.33 ± 1.00	1.98 ± 2.17	1.91 ± 1.40
<i>Capnocytophaga sp.</i>	8.07 ± 7.16	6.46 ± 4.06	6.22 ± 4.82	6.69 ± 3.71	9.96 ± 11.49	10.56 ± 13.0	<b>8.64 ± 7.80</b>	<b>12.06 ± 8.85</b>
<i>Campylobacter sp.</i>	2.51 ± 1.48	2.54 ± 2.27	2.83 ± 1.98	3.24 ± 2.20	2.11 ± 1.53	2.17 ± 1.61	2.96 ± 1.66	3.24 ± 1.92
<i>E. nodatum</i>	1.98 ± 1.14	2.63 ± 2.13	2.48 ± 1.54	2.57 ± 1.35	2.75 ± 1.92	4.09 ± 6.22	3.39 ± 2.95	3.87 ± 2.13
<i>Fusobacterium sp.</i>	5.62 ± 3.63	5.92 ± 3.48	6.92 ± 5.35	6.30 ± 3.62	5.29 ± 4.57	6.33 ± 4.71	7.21 ± 6.10	7.11 ± 4.71
<i>P. micra</i>	4.38 ± 3.10	3.58 ± 2.39	5.36 ± 3.26	4.46 ± 3.38	3.52 ± 3.32	3.83 ± 3.49	3.90 ± 2.84	4.82 ± 3.68
<i>Prevotella sp.</i>	17.3 ± 10.14	16.52 ± 9.58	16.87 ± 8.29	17.55 ± 12.2	16.83 ± 10.3	15.46 ± 8.53	14.47 ± 7.88	14.63 ± 7.92
<i>P. gingivalis</i>	2.21 ± 3.02	1.95 ± 2.01	2.28 ± 2.33	2.15 ± 2.45	2.62 ± 2.99	3.18 ± 3.19	<b>2.77 ± 2.99</b>	<b>4.20 ± 6.68</b>
<i>T. forsythia</i>	5.19 ± 4.02	5.19 ± 2.78	6.32 ± 6.17	6.20 ± 5.74	5.09 ± 4.22	6.23 ± 4.55	6.76 ± 4.79	7.81 ± 5.93
<i>T. denticola</i>	2.11 ± 2.18	2.17 ± 1.67	2.37 ± 1.98	2.42 ± 2.10	2.17 ± 2.49	2.30 ± 1.70	4.05 ± 5.30	4.45 ± 3.49
<i>S. noxia</i>	<b>7.34 ± 3.36</b>	<b>8.86 ± 4.00</b>	8.69 ± 5.42	9.38 ± 4.19	5.93 ± 3.97	6.32 ± 3.25	6.27 ± 3.14	6.18 ± 3.67

**Bold** numbers represent species that differed significantly (Wilcoxon, p < 0.05) from baseline to three months without adjusting for 18 comparisons.

**Italicized** numbers represent species that differed significantly (Wilcoxon, p < 0.05) from baseline to three months after adjusting for 18 comparisons.



**Figure 1.** Mean change (±SD) in proportions of the test species from baseline to three months in each of the mouthrinse groups. The mean change in proportions between baseline and three months was computed for each species in each subject, and then averaged across subjects in the four mouthrinse groups separately. The species have been ordered according to microbial complexes.<sup>36</sup> Significance of differences of species proportions among mouthrinse groups was determined using ANCOVA, adjusting for baseline values. \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001 adjusting for 18 comparisons.<sup>40</sup>

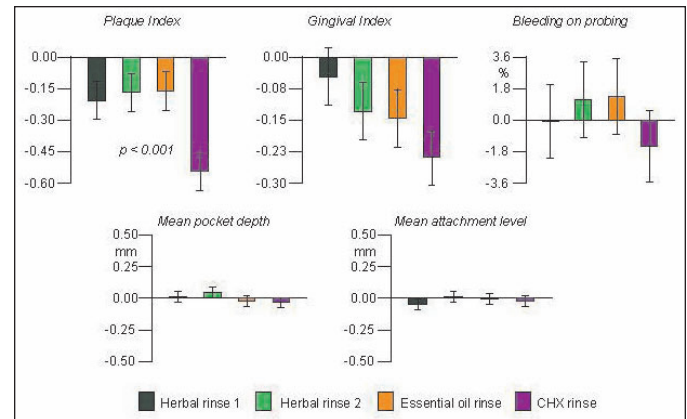
**Table III**  
Means and Standard Deviations of Clinical Parameters at Baseline and Three Months for Subjects in the Four Mouthrinse Groups

Herbal Rinse 1	Baseline	3 Months	Wilcoxon p
N	29	29	
Plaque Index	1.27 ± 0.71	0.99 ± 0.64	p < 0.01
Gingival Index	0.81 ± 0.41	0.75 ± 0.36	ns
% sites with Bleeding on Probing	22.54 ± 13.07	21.86 ± 12.00	ns
Mean PD (mm)	2.46 ± 0.32	2.47 ± 0.37	ns
Mean AL (mm)	2.40 ± 0.62	2.35 ± 0.68	ns
Herbal Rinse 2	Baseline	3 Months	Wilcoxon p
N	28	28	
Plaque Index	1.14 ± 0.82	0.96 ± 0.67	p < 0.05
Gingival Index	0.76 ± 0.43	0.64 ± 0.45	ns
% sites with Bleeding on Probing	22.03 ± 18.86	22.71 ± 21.11	ns
Mean PD (mm)	2.53 ± 0.49	2.58 ± 0.58	ns
Mean AL (mm)	2.67 ± 1.11	2.68 ± 1.20	ns
Essential Oil Rinse	Baseline	3 Months	Wilcoxon p
N	28	28	
Plaque Index	0.91 ± 0.61	0.84 ± 0.64	ns
Gingival Index	0.78 ± 0.36	0.65 ± 0.42	ns
% sites with Bleeding on Probing	15.37 ± 9.21	17.87 ± 11.82	ns
Mean PD (mm)	2.41 ± 0.22	2.38 ± 0.30	ns
Mean AL (mm)	2.30 ± 0.62	2.28 ± 0.67	ns
Chlorhexidine Rinse	Baseline	3 Months	Wilcoxon p
N	31	31	
Plaque Index	1.09 ± 0.71	0.55 ± 0.43	p < 0.001
Gingival Index	0.81 ± 0.39	0.56 ± 0.43	p < 0.001
% sites with Bleeding on Probing	20.16 ± 14.47	18.65 ± 15.05	ns
Mean PD (mm)	2.45 ± 0.23	2.41 ± 0.29	ns
Mean AL (mm)	2.43 ± 0.74	2.40 ± 0.78	ns

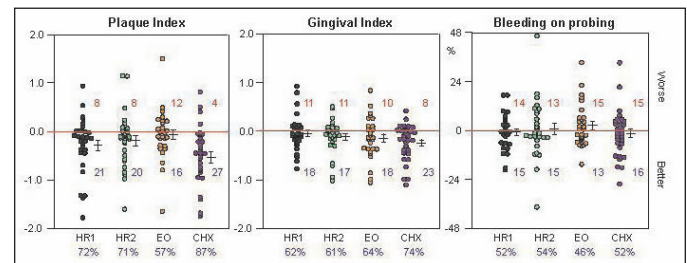
decrease in the CHX group only. As expected, mean PD and mean AL showed minimal change in all mouthrinse groups.

The changes in mean clinical parameters from baseline to three months in each of the four treatment groups are presented in Figure 2. The mean reductions in PI and GI in all mouthrinse groups were clearly observed, as well as the somewhat variable response in BOP and the minimal changes in mean PD and AL. The only statistically significant difference among mouthrinse groups in mean change at three months was found for PI ( $p < 0.001$ ).

While mean data can provide trends and significance testing can provide information regarding statistically significant differences among groups, the fate of individual subjects is obscured. Therefore, the data were analyzed for each subject separately in the four mouthrinse groups to demonstrate the individual mean changes in PI, GI, and percent of sites with BOP from baseline to three months (Figure 3). Eighty-seven percent of subjects showed an improvement in the PI in the CHX group (the best percentage) compared with 57% of subjects in the essential oil group (the lowest percentage). Seventy-two percent and 71% of subjects showed improvement in the PI at the end of the study in the two herbal rinse groups, respectively. The best response for GI was observed in the CHX group, with 74% of subjects showing improvement in this parameter from baseline to three months.



**Figure 2.** Mean change ( $\pm$  SD) in clinical parameters from baseline to three months in each mouthrinse group. The mean change between baseline and three months was computed for each parameter in each subject, and then averaged across subjects in the four mouthrinse groups separately. Significance of differences among mouthrinse groups was determined using ANCOVA, adjusting for baseline values. Only change in mean PI differed significantly among groups.



**Figure 3.** Plot of mean change in PI (left panel), GI (middle panel), and BOP (right panel) in each subject in each mouthrinse group. Each circle represents the mean change for an individual subject. The horizontal lines and whiskers to the right of each column represent the mean and SD for the group. The red horizontal line represents 0 (no change). Subjects below this line demonstrated an improvement in the parameter, while those above the line showed a worsening of the parameter. The red numbers above the red line indicate the number of subjects getting worse; the blue numbers below this line show the number of subjects improving. The percents below the mouthrinse group names represent the percent of subjects in each group that showed an improvement in the parameter. The differences among groups in % of subjects showing improvement did not differ significantly for any of the clinical parameters using chi square analysis. HR1 = Herbal rinse 1; HR2 = Herbal rinse 2; EO = Essential oil rinse; CHX = Chlorhexidine rinse.

The herbal rinse 1 and 2 and essential oil groups exhibited comparable percentages of subjects showing improvement; 62%, 61%, and 64%, respectively. There was little difference among the four mouthrinse groups for percent sites exhibiting BOP between baseline and three months, with about half of the subjects showing improvement in each group.

## Discussion

The purpose of the present investigation was to determine the influence of mouthrinses with different characteristics on the amount and composition of subgingival biofilms in previously treated subjects in a periodontal maintenance program. These subjects were chosen because they had completed their active treatment, but had a small number of residual pockets that were sufficiently deep to allow determination of altered subgingival biofilm composition. It was also of interest to determine the effectiveness of these mouthrinses on clinical parameters in

periodontal maintenance subjects. Since the question being asked regarded the effects of different mouthrinses on the subgingival biofilm composition, a pre-post design was essential. A control group was not utilized in this study since the objective was to specifically test and compare active controls/agents.

In the current investigation, the four test mouthrinses were shown to have effects on the microbial composition of subgingival plaque. Changes in proportions of the majority of test species in the subgingival plaque samples were modest in all mouthrinse groups. However, subjects in the chlorhexidine group did exhibit significant decreases in the proportions of the *Actinomyces* species, and subjects in the herbal mouthrinse groups (particularly group 1) showed significant decreases in the proportions of many of the *Streptococci*, although these reductions were not significant after adjusting for 18 comparisons.

Of interest was the finding that the four mouthrinses had different effects on species proportions in subgingival plaque. The chlorhexidine rinse affected primarily the *Actinomyces* species and *V. parvula* (major components of both supra- and subgingival plaque); the herbal mouthrinses affected the *Streptococci* and certain periodontal pathogens, and the essential oil rinse appeared to exert its major effect on *Prevotella* species, as well as *Aggregatibacter actinomycetemcomitans*. It should be stressed that most of these changes were not statistically significant after adjusting for 18 comparisons. However, these findings are interesting and may help to explain the modes of action and the effectiveness of the agents *in vivo*.

The differences in the effects of the different mouthrinses on the subgingival microbiota are likely due to the different active ingredients in each product. The herbal mouthrinses contain goldenseal, which has been shown to have antimicrobial properties against such oral pathogens as *S. mutans* and *Fusobacterium nucleatum*.<sup>25</sup> Grapefruit seed extract is also a proven antibacterial agent, having shown its effectiveness against a variety of bacteria, yeast, and viral strains, including a wide range of Gram-positive and Gram-negative organisms.<sup>24,26</sup> The essential oil mouthrinse has been shown to reduce *S. mutans* in plaque,<sup>46</sup> as well as significantly lower the level of subgingival organisms, including *P. gingivalis*, *F. nucleatum*, *Veillonella* species, and total anaerobes.<sup>47</sup> The mouthrinse containing the active ingredient 0.12% chlorhexidine has been shown to significantly reduce the number of total Gram-positive facultative cocci, streptococci, Gram-positive facultative rods, primarily *Actinomyces*, *Capnocytophaga*, and Gram-negative rods in subgingival and marginal plaque samples.<sup>48</sup> The active ingredient, 0.12% chlorhexidine, has demonstrated significant broad-spectrum antimicrobial results in reducing the number of both facultative and obligate anaerobes in plaque,<sup>22</sup> and also has been shown to significantly lower the levels of such species in the subgingival microbiota, including *Lactobacillus acidophilus*, *Eikenella corrodens*, *F. nucleatum* subspecies *nucleatum*, *T. denticola*, *Leptotrichia buccalis*, and *Eubacterium saburreum*.<sup>20</sup> The addition of blood-root to herbal rinse 1 appeared to have little impact on the clinical and microbiological findings, since both herbal rinses exhibited similar outcomes.

The changes in the subgingival microbiota, while modest and different among groups, were effective in reducing intraoral

plaque and improving the clinical parameters associated with gingivitis and periodontitis. Indeed, plaque levels were reduced at three months in more than 70% of subjects, except in the essential oil group, and gingivitis levels were improved in more than 60% of subjects in all groups. The change in the mean percentage of sites with BOP between baseline and three months was marginal in all mouthrinse groups, with approximately 50% of subjects showing an improvement in this parameter. As expected, changes in mean PD and AL in this maintenance group of subjects were minimal.

The clinical findings of the current investigation are in accord with the data from other studies that have indicated that regular rinsing with antibacterial mouthrinses can reduce the levels of plaque and gingival inflammation.<sup>4-7</sup> A number of six-month clinical investigations conducted during the past 25 years have demonstrated that the prescription, chlorhexidine-based mouthrinse<sup>6,7,41</sup> reduces plaque by between 21% and 60% and gingivitis by between 18% and 42%.<sup>42</sup> Similarly, clinical trials also have shown that an over-the-counter mouthrinse containing essential oil is also effective for the control of plaque and gingivitis,<sup>6,8,43-45</sup> reducing plaque between 13% and 56% and gingivitis by 14% and 40% during six-month investigations.<sup>42</sup> As previously mentioned, the herbal mouthrinse has been shown to reduce gingival bleeding and gingivitis.<sup>32,33</sup>

The results of this *in vivo* investigation suggest that the use of antibacterial mouthrinses not only reduce supragingival plaque levels, but affect the composition of the adjacent subgingival biofilm. All three categories of mouthrinse affected subgingival biofilm composition, but in different ways. The altered biofilm composition, in turn, was associated with positive changes in clinical status of the adjacent tissues as measured by gingival redness and BOP. Thus, the use of any of the three types of mouthrinses may provide benefits in terms of suppressing subgingival biofilm formation and minimizing clinical symptoms of periodontal disease in periodontal maintenance patients.

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