Reprint

The Ability of an Herbal Mouthrinse to Reduce Gingival Bleeding

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An In Vivo Comparison of the Antimicrobial Activities of Three Mouthrinses

J. Gultz, DDS  J.M. Kaim, DDS  J. DeLeo IV, BS  W. Scherer, DDS

An In Vitro Investigation of the Antimicrobial Activity of an Herbal Mouthrinse

J. M. Kaim, DDS  J. Gultz, DDS  L. Do, BS  W. Scherer, DDS

Clinical Efficacy of an Herbal Toothpaste

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Abstract

Forty healthy adult volunteers from the Junior Comprehensive Care Clinics at New York University College of Dentistry were accepted as subjects for this three-month, examiner-blinded, parallel-group clinical trial. To be eligible for a baseline clinical examination, subjects had to first indicate that during the previous six months they habitually brushed their teeth two or more times per day and had noticed "bleeding gums" or "bleeding the toothpaste" after brushing or after flossing their teeth. At the baseline examination subjects were enrolled in the study if they were found to have at least five Löe-Silness gingival bleeding sites and 20 natural teeth, including four molars. Subjects were assigned to one of the two following treatment groups: 1) Herbal Mouth and Gum Therapy, or 2) Control (distilled water and dye). Subjects were instructed to clean their teeth in their normal manner, not to use any other mouthrinses or oral irrigations products for the duration of the study. Subjects were to return for clinical examinations after three months of product use. At these examinations, gingivitis and gingival bleeding scores were recorded. An independent t-test before treatment indicated that there were no significant differences between the baseline evaluations of the two groups in the study. A one-way analysis of variance (ANOVA), comparing gingivitis and bleeding scores from baseline and three months with the baseline scores as a covariate, indicated that Herbal Mouth and Gum Therapy produced a statistically significant effect (p < 0.01) on both parameters relative to the control mouthrinse. The results of this study support the clinical efficacy of Herbal Mouth and Gum Therapy in reducing gingivitis and gingival bleeding. (J Clin Dent 9:97-100, 1998.)

Introduction

Over-the-counter and prescription products such as mouthrinses which often contain alcohol in high-volume percentages, have increased in sales and represent a substantial market share of at-home oral care products. In addition to alcohol, these products often contain other materials such as detergents, artificial sweeteners, emulsifiers, organic acids and dyes.1

Recently, the sales of "natural" products have grown as much as 20 percent per year.2 At the present time, as many as half of all Americans use supplements in one form or another whether it is a basic multivitamin, a homeopathic remedy or a botanical or herbal extract.3 As a result, the incorporation of natural ingredients into mouthrinses may provide a logical and practical approach toward providing the therapeutic benefits for oral hygiene procedures. One such product, Herbal Mouth and Gum Therapy, has recently been shown to demonstrate antimicrobial activity in vitro4 and in vivo.5

Herbal Mouth and Gum Therapy is composed of the following herbal extracts in a non-alcohol base: aloe vera gel, bloodroot, calendula, echinacea, goldenseal and grapefruit seed extract. According to the manufacturer, echinacea and goldenseal are the main active ingredients. The percentage of each extract in the formulation is proprietary information. The activities of all the ingredients may be found in Table I.6,7 It should be noted that although many of these herbal extracts have gained in popularity in recent years, many in the dental profession may be unaware of their possible benefits and modes of action. Therefore, the following description of some of the major herbal ingredients follows:

<table>
<thead>
<tr>
<th>Herbal Ingredient</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinacea</td>
<td>analgesic, anti-inflammatory, antibiotic</td>
</tr>
<tr>
<td>Goldenseal</td>
<td>anti-inflammatory</td>
</tr>
<tr>
<td>Calendula</td>
<td>anti-septic, antibiotic</td>
</tr>
<tr>
<td>Bloodroot</td>
<td>antibiotic, anti-inflammatory</td>
</tr>
<tr>
<td>Grapefruit Seed Extract</td>
<td>anti-fungal, antibiotic</td>
</tr>
<tr>
<td>Aloe Vera Gel</td>
<td>analgesic, anti-inflammatory, moisturizer</td>
</tr>
</tbody>
</table>

Echinacea

Echinacea, the purple coneflower, is a native American wildflower belonging to the sunflower family. It has now become the common name for several echinacea species, including E. angustifolia, E. purpurea and E. pallida. It was first used by American Indians for a variety of ailments, including the treatment of venous ulcers and external wounds. As a medicinal substance, it was very popular among medical professionals in the late nineteenth century. By the early part of the twentieth century, however, it had largely disappeared from United States medicine. More recently, it has regained popularity in the United States, and is now considered by some to be an immune system "booster," and antimicrobial and antiviral agent. Research on the immunostimulating action has shown that it promotes the production and action of antibodies, complement and B-cells.3,9 In regard to its antiviral properties, investigators have observed that echinacea stimulates T-cell lymphocytes which, in turn, produce interferon.10 Other investigators believe that echinacea actively competes with viruses for receptor sites on cell membrane surfaces.11 Antimicrobial studies have indicated that echinacea provides action against Streptococcus and Staphylococcal bacteria, and that the plant extract can possess a hyaluronidase inhibitory factor to inhibit bacteria.12,13

Goldenseal

Goldenseal is also a native American wildflower. Some of its chemical contents include berberine, biotin, candicid, choline and para-aminobenzoic acid.7 It has been used for its anti-inflammatory and antimicrobial properties,4,5 and immune-strengthening properties.16

Grapefruit Seed Extract

Grapefruit seeds contain a bioflavonoid complex known as procyanidolic oligomers (PCO).17 Investigators noted that PCO possessed anti-inflammatory activities, which are produced, in part, by the antioxidant effect of PCO, and by inhibiting the release and synthesis of certain compounds that influence inflammation, such as histamine, serine protease, prostaglandins and leukotrienes.18-20
Aloe Vera

The earliest recordings of the medicinal values of aloe vera are found in Egyptian papyri around 1500 B.C. Over the years, aloe vera has had numerous uses ranging from skin care to the treatment of digestive problems and infections. Several investigators have reported that aloe vera provides anti-inflammatory21-23 and wound-healing benefits.24-26 It is felt that the mode of action of aloe vera stems from its polysaccharide content, principally, glucosamin, Other polysaccharides containing galactose and uronic acids, as well as pentoses, are also present.27

In order to determine in vivo efficacy of Herbal Mouth and Gum Therapy on reducing gingival inflammation and bleeding, a clinical study was conducted.

Materials and Methods

Forty healthy adult volunteers from the Junior Comprehensive Care Clinics at New York University College of Dentistry were accepted as subjects for this three-month, examiner-blinded, parallel-group clinical trial. To be eligible for a baseline clinical examination, subjects had to first indicate that during the previous six months they habitually brushed their teeth two or more times per day, and had noticed "bleeding gums" or "blood in the toothpaste" after brushing or flossing their teeth. At the baseline examination, subjects were enrolled in the study if they were found to have at least five Löe-Silness gingival bleeding sites and 20 natural teeth, including four molars. Subjects were to be excluded if they had rampant caries, obvious periodontal disease, chronic dental neglect or serious medical conditions, as determined by the investigator. Subjects were also excluded if they had taken antibiotics within seven days, or had used a chlorhexidine rinse within three months of the start of the study.

Following the examinations to determine subject qualification and initial levels of gingivitis and gingival bleeding, subjects were given a complete oral prophylaxis, which included the removal of supragingival plaque and calculus deposits. The teeth were then polished, and complete plaque removal was verified by the use of erythrosin. All subjects were then provided with an initial supply of a mouthrinse, Herbal Mouth and Gum Therapy (Woodstock Natural Products, Englewood Cliffs, NJ), or a control mouthrinse (distilled water which contained a dye, FD&C Red No. 3), and instructed to rinse their mouths with one capful for 20-30 seconds twice daily. Subjects were assigned to the two treatment groups by random assignment.

Subjects were instructed to clean their teeth in their usual manner, not to use any other mouthrinses, or oral irrigation products for the duration of the study. Subjects were to return for clinical examinations after three months of product use. At these examinations, gingivitis and gingival bleeding scores were recorded.

Gingivitis and Gingival Bleeding

Gingivitis was measured using the Gingival Index (GI) of Löe and Silness,26 using a 0-3 evaluation scale of gingival tissues adjacent to six surfaces (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual) of all natural teeth, excluding third molars. A probe was inserted about 1 mm into the gingival sulcus and passed from interproximal to interproximal surfaces. The GI was scored according to the following criteria: 0 = Normal gingiva; 1 = Mild inflammation - slight change in color, slight edema. No bleeding on probing; 2 = Moderate inflammation - redness, edema, glazing. Bleeding on probing; 3 = Severe inflammation - marked redness, edema, ulceration. Tendency to spontaneous bleeding.

For gingivitis, results were reported as the mean GI score for each subject, averaged across all surfaces evaluated. For bleeding, results were reported as the number of sites that bled on probing or bled spontaneously (grades 2 or 3).

Statistical Analyses

A one-way analysis of variance (ANOVA) was performed on gingivitis and gingival bleeding scores, comparing scores from baseline and three months with the baseline readings as a covariate. Additionally, an independent t-test was performed before treatment to determine whether any significant differences existed between the baseline evaluations of the two groups. Significance was based on alpha = 0.05.

Results

An independent t-test before treatment indicated that there were no significant differences between the baseline evaluations of the two groups in the study. However, a one-way analysis of variance (ANOVA) comparing gingivitis and bleeding scores from baseline and three months, with the baseline scores as a covariate, indicated that Herbal Mouth and Gum Therapy produced a statistically significant (p < 0.01) effect on both parameters relative to the control mouthrinse (Tables II and III). Comparative percent reductions of the two products against gingivitis and gingival bleeding are presented in Table IV.

Table II

<table>
<thead>
<tr>
<th>Index</th>
<th>Herbal Mouth and Gum Therapy</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingivitis</td>
<td>1.04 ± 0.25</td>
<td>1.06 ± 0.17</td>
</tr>
<tr>
<td>Gingival Bleeding</td>
<td>24.50 ± 3.05</td>
<td>23.40 ± 1.83</td>
</tr>
</tbody>
</table>

Table III

<table>
<thead>
<tr>
<th>Index</th>
<th>Herbal Mouth and Gum Therapy</th>
<th>Control</th>
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</thead>
<tbody>
<tr>
<td>Gingivitis</td>
<td>0.76 ± 0.14</td>
<td>0.94 ± 0.10</td>
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<tr>
<td>Gingival Bleeding</td>
<td>16.61 ± 2.57</td>
<td>21.01 ± 1.41</td>
</tr>
</tbody>
</table>

Table IV

<table>
<thead>
<tr>
<th>Index</th>
<th>Herbal Mouth and Gum Therapy</th>
<th>Control</th>
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<tbody>
<tr>
<td>Gingivitis</td>
<td>26.9</td>
<td>11.3</td>
</tr>
<tr>
<td>Gingival Bleeding</td>
<td>31.4</td>
<td>10.2</td>
</tr>
</tbody>
</table>

Discussion

The results of this study indicate that Herbal Mouth and Gum Therapy improved oral health with regard to gingivitis and gingival bleeding after three months of use. Furthermore, results indicate that the botanical and herbal extracts found in Herbal Mouth and Gum Therapy appear to provide oral health benefits, as well as a natural alternative for those consumers who want to avoid the artificial ingredients contained in some commercial mouthrinse products.
It should also be noted that Herbal Mouth and Gum Therapy does not contain alcohol compared to some currently marketed commercial and prescription products. The purpose of alcohol in mouthrinse preparations is to dissolve active ingredients and not function as an antiseptic. The absence of alcohol in the mouthrinse studied here is a benefit for those consumers who do not want to use alcohol products in any concentration. The absence of alcohol also addresses safety issues, especially for children, considering the number of alcohol-related poisonings in children that are reported each year to poison control centers.

It should also be noted that Herbal Mouth and Gum Therapy contains bloodroot, (*Sanguinaria canadensis*; Table I). Studies with sanguinarian compounds have shown that it has the potential to decrease plaque and gingivivitis. According to the manufacturer, bloodroot is not the primary active ingredient in the herbal product studied here, as is echinacea and goldenseal. It is entirely possible that *Sanguinaria canadensis* contributed to the results obtained in the study. Further research should be contemplated to determine the benefits of each of the ingredients in the herbal mouthrinse.

Lastly, it is interesting to note that the incorporation of herbal ingredients into a mouthrinse product may provide a practical approach toward expanding oral health benefits provided by the routine use of such natural products. At the same time, continuing studies should be undertaken to assess the substantivity of the herbal mouthrinse, and to monitor microbes to ensure that neither resistant nor pathogenic opportunistic organisms emerge.

**Conclusion**

The results of this study support the clinical efficacy of Herbal Mouth and Gum Therapy in reducing gingivitis and gingival bleeding.

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References

sample, the subject rinsed with one of the mouthrinses or the water control for 30 seconds, then waited one hour, at which time a post-test saliva sample was collected. Again, the sample was divided and used to culture the different types of bacteria. Following a 48-hour incubation period, the numbers of microbial colonies on each plate were counted and compared. The results indicated that all of the mouthrinses tested performed significantly better than the water control. Herbal Mouth and Gum Therapy and Peridx did not demonstrate a statistically significant difference in inhibiting aerobic, microaerophilic and anaerobic bacteria. Both Herbal Mouth and Gum Therapy and Peridx were significantly more effective than Listerine in inhibiting the three different types of bacteria. (J Clin Dent 9:43-45, 1998.)

**Introduction**

In the last 10 years, a number of non-alcohol containing mouthrinses, including those containing natural ingredients, have appeared on the over-the-counter market, with the sales of such products showing increases of 20 percent per year. Given the limitations in mechanical plaque control demonstrated by the general public, antibacterial agents that supplement a toothbrushing regimen may be a very desirable adjunct. Initially, claims of anti-gingivitis and antimicrobial efficacy by mouthwash manufacturers were difficult to demonstrate to the dental professional. However, the situation changed markedly with the establishment of formal standards and testing requirements for these claims by the American Dental Association in 1985. To date, the ADA Council on Scientific Affairs has accepted only two brand name mouthrinse categories for gingivitis claims. First, essential oils with products such as Listerine (Warner-Wellcome, Morris Plains, NJ) and other brands with identical formulations to Listerine. The second category is chlorhexidine, represented by Peridx (Zila Pharmaceuticals, Phoenix, AZ) for the control of plaque and gingivitis. The Food and Drug Administration has approved only Peridx for these claims.

Clinical studies with Listerine began in the 1970’s and were initially of short duration, ranging from 7 to 60 days, and indicated statistically significant reductions of about 35 percent in levels of plaque and gingivitis. Further studies of six month duration or longer have subsequently been published supporting its efficacy in plaque reduction and gingivitis. Additionally, comparative studies of Listerine have also shown significant reductions in plaque and gingivitis in multi-cell designs.

Peridx is an effective agent for the reduction of both plaque and gingivitis, with reductions in short-term studies averaging 60 percent, and in long-term studies showing reductions in plaque averaging 55 percent and in gingivitis averaging 45 percent. Peridx also has been shown to function as a broad spectrum antimicrobial agent with activity against a wide range of Gram positive and Gram-negative supragingival and subgingival plaque bacteria.

From time to time, products with naturally occurring ingredients have been introduced to the market. Several of these “natural” products have demonstrated both clinical efficacy as well as antimicrobial properties. More recently, one natural product, Herbal Mouth and Gum Therapy (Woodstock Natural Products, Englewood Cliffs, NJ) has shown antimicrobial activity in vitro, and may have a potential use in reducing gingivitis or gingival bleeding. The product contains several herbal extracts which appear to demonstrate antimicrobial and anti-inflammatory properties. According to the manufacturer, the main active ingredients are echinacea and goldenseal. The mouthrinse also contains other herbal extracts such as calendula, bloodroot, grapefruit seed extract and aloe vera gel. The therapeutic activities of each of these ingredients may be found in Table I.

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The primary ingredient is echinacea, popular in both the United States and Europe. In Germany more than 300 echinacea products are available for consumption, and in 1994 German doctors and pharmacies prescribed echinacea more than 2.5 million times. Echinacea is a native American wildflower belonging to the sunflower family. Of the nine species native to the United States and Canada, the three used medicinally are E. purpurea, E angustifolia and E. pallida. Medicinal uses are for boosting and aiding the immune system. These species of echinacea also possess anti-inflammatory properties. As a result, echinacea has been prescribed for a wide variety of treatments ranging from the common cold to treating gingival inflammation.

Another ingredient is goldenseal, also found as a native American wildflower. Some of its chemical contents include berberine, biotin, canadine, choline and para-aminobenzoic acid. It has been used for its anti-inflammatory and antimicrobial properties and immune-strengthening properties.

This in vivo study compared the antimicrobial properties of Herbal Mouth and Gum Therapy mouthrinse with natural ingredients to Listerine, an ADA-accepted over-the-counter mouthrinse with essential oils, and one prescription mouthrinse with chlorhexidine ingredient (Peridx). These three mouthrinses were tested against a water control.

**Method and Materials**

**Study Protocol**

Twenty healthy adult volunteers from the Junior Comprehensive Care Clinics at New York University College of Dentistry participated in this double-blind study. Neither the subjects nor examiners were aware of the mouthrinses used in each test trial. Each subject used the mouthrinses randomly in a series of four experimental sessions, which were separated by at least 10 days, with each subject serving as his/her own control.

In order to participate in the study, each subject was required to have at least 20 natural teeth. In addition, subjects were not to use antibiotics during the study, or have used antibiotics or chlorhexidine mouthrinses for one month prior to the study. Each of four experimental sessions was conducted in the following manner: Subjects rinsed their mouths with distilled water for 30 seconds. Each subject then chewed paraffin wax for one minute to displace plaque microorganisms. Subjects then collected saliva by expectorating into a sterile test tube that was provided for that purpose. The saliva collected represented a baseline measure for that session. Each subject was then presented with one of the four mouthrinses, and requested to rinse for 30 seconds. At the end of this period, subjects were asked not to introduce any substance into their mouths for one hour.
At the end of one hour, each subject again chewed paraffin wax for one minute and collected another saliva sample. Each of the salivary samples were tested two hours after collection. The microbial testing procedure proceeded as follows: Salivary samples were sonicated using a Branson Sonifier. A 10 ml 1 x 10-5 sample of each salivary specimen was obtained through serial dilution with a 0.9 percent sodium chloride solution. 0.1 ml aliquots of the 1 x 10-5 dilution was delivered with serological pipettes and spread on the surfaces of BHI agar plates utilizing preformed sterile glass spreading rods. Each subject generated six Petri plates per trial (three prior to rinsing their mouths with a mouthrinse and three after rinsing with a mouthrinse). Of each group of three plates, one was incubated aerobically, one in 10 percent CO₂ (microaerophilic, candle jar method) and one anaerobically (BBL Gas Pack System). All plates were incubated for 48 hours at 37°C. Following the incubation period, microbial colonies on each plate were counted with the aid of a colony counter.

Products Tested

In addition to a water control, the herbal mouthrinse trademarked as Herbal Mouth and Gum Therapy with no alcohol was tested. Another product tested was Listerner Original, a combination of the phenol-related essential oils, thymol and eucalyptol, mixed with menthol and methyisalicylate in a 26.9 percent hydroalcoholic vehicle. The third product was a cationic bis-biguanide (0.12 percent chlorhexidine) in an 11.6 percent hydroalcoholic vehicle, known as Perident.

Statistical Analysis

The results were obtained using a general linear model with a repeated measures design. A repeated measures design compares the different scores for each subject and controls for the subject’s initial score. It provides an Analysis of Variance when the same measurement is made several times on each subject or case. The p values, as reported by the general linear model, performs a post hoc item to item comparison of the products in the

<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>Total Mean Pre- and Post-Test Bacterial Counts for Category and Mouthrinse</strong></td>
</tr>
<tr>
<td><strong>Test</strong></td>
</tr>
<tr>
<td>Aerobic Bacteria</td>
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<td>Pre-Test</td>
</tr>
<tr>
<td>Post-Test</td>
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<tr>
<td>Microaerophilic Bacteria</td>
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<tr>
<td>Pre-Test</td>
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<tr>
<td>Post-Test</td>
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<tr>
<td>Anaerobic Bacteria</td>
</tr>
<tr>
<td>Pre-Test</td>
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<tr>
<td>Post-Test</td>
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<table>
<thead>
<tr>
<th>Table III</th>
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<tbody>
<tr>
<td><strong>Percent Reduction of Bacteria Inhibited by Solution</strong></td>
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<tr>
<td><strong>Water</strong></td>
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<td>Aerobic Bacteria</td>
</tr>
<tr>
<td>Microaerophilic Bacteria</td>
</tr>
<tr>
<td>Anaerobic Bacteria</td>
</tr>
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</table>

inhibition of bacteria. The differences in pre- and post-test bacterial counts for each type of bacteria and mouthrinse are also generated. In addition, percentage reductions of the bacterial counts were also calculated.

Results

The results of the study, shown in Tables II and III, indicated that all of the mouthrinises tested performed significantly better than the water control on all tests (p < 0.001). Herbal Mouth and Gum Therapy and Perident did not demonstrate a statistically significant difference in inhibiting aerobic, microaerophilic and anaerobic bacteria (p > 0.05). Both the herbal product and Perident showed statistically significant inhibition of aerobic, microaerophilic and anaerobic bacteria compared to Listerine (p < 0.001). It should be noted that specific bacteria were not identified for the aerobic, microaerophilic or anaerobic groups.

Discussion

All of the mouthrines tested in this study performed significantly better than water. This study indicates that an herbal mouthrinse inhibits bacteria more effectively than Listerine, and not statistically different than Perident. Thus, the herbal mouthrinse may provide oral health benefits, and provide a natural alternative for those consumers who wish to avoid artificial sweeteners, chemicals and alcohol containe in either over-the-counter or prescription mouthrinse products.

With regard to alcohol-containing mouthrines, it should be noted that most commercial products contain alcohol in concentrations ranging from 5 to 27 percent. The products used in this study have the following alcohol concentrations: Listerine, 26.9 percent, and Perident, 11.6 percent. Herbal Mouth and Gum Therapy contains no alcohol. Although alcohol has long been used for its antiseptic properties, to function as an effective germicide, concentrations should range from 50 to 70 percent. Alcohol levels higher than 70 percent and lower than 50 percent lose effective germicidal activity. As a result, alcohol concentrations in commercial preparations are generally below the 50 percent level, and thus are not primary contributors to antimicrobial effects, and mainly function as vehicles that dissolve and deliver mouthrinse ingredients. Thus, the alcohol concentrations noted in Listerine and Perident may raise issues for those individuals concerned about using alcohol-containing products. At the same time, safety concerns have been raised regarding the known number of alcohol-related poisonings in children each year, and the use of alcohol products in individuals who may be chemically dependent.

This study indicates that the non-alcohol product, Herbal Mouth and Gum Therapy, can significantly reduce aerobic, microaerophilic and anaerobic bacteria in saliva for periods up to two hours. It is possible the product may play a role in reducing the discharge of airborne microorganisms if used by patients prior to dental procedures. Further research efforts may be valuable to discern whether such products do indeed reduce viable bacteria during dental procedures.

Conclusion

All of the mouthrines tested performed significantly better than the water control in the tests conducted. Both Herbal Mouth and Gum Therapy and Perident were significantly more
effective than Listerine in inhibiting aerobic, microaerophilic and anaerobic bacteria.

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References

An In Vitro Investigation of the Antimicrobial Activity of an Herbal Mouthrinse

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Abstract
The purpose of this study was to determine the antimicrobial activity of one prescription and two commercially available over-the-counter mouthrinses and positive and negative controls against bacteria commonly found in the oral cavity. The bacteria used in this study were Streptococcus mutans, Streptococcus sanguis and Actinomyces viscosus. Sterile discs, which were treated with the mouthrinses, were placed on agar plates along with the controls. Zones of microbial inhibition were measured in millimeters after 48 hours. The results indicated that all of the mouthrinses demonstrated antimicrobial activity against the bacteria tested. Herbal Mouth and Gum Therapy® mouthrinse, containing natural ingredients, produced the largest zones of microbial inhibition when compared to Listerine® against all three of the bacteria tested. Herbal Mouth and Gum Therapy mouthrinse produced larger zones of microbial inhibition when compared to Periex® against two of the three bacteria tested, and produced similar zones of inhibition against the third bacteria. (J Clin Dent 9:46-48, 1988.)

Introduction
Over-the-counter consumer products, such as mouthrinses, have increased in sales and represent a substantial market share of at-home oral care products. In addition to containing antimicrobial agents, such products often contain many different ingredients including alcohol, detergents, emulsifiers, organic acids and dyes.¹ Mouthrinse products have provided another approach toward expanding the therapeutic benefits provided by routine oral hygiene procedures.² The clinical applications for antimicrobial mouthrinses fall into three broad categories: they are useful as preventive agents, as therapeutic agents and they assist certain professional procedures.

Of the many antimicrobial mouthrinses available commercially, two categories have been accepted as effective by the American Dental Association’s Council on Dental Therapeutics. One is essential oils, an example of which is Listerine (Warner-Lambert, Morris Plains, NJ). Listerine was the first over-the-counter antiseptic and antigingivitis rinse approved by the ADA.³ It has both short- and long-term studies documenting it as an effective antimicrobial rinse.⁴ Artifon and other brands containing identical ingredients to Listerine have appeared on the market with the ADA Seal.

The other category is chlorhexidine-containing products, an example being Periex (Zila Pharmaceuticals, Phoenix AZ). Periex, a prescription mouthrinse, has shown efficacy as an antiseptic agent and is effective in controlling
inflammation as well.\textsuperscript{6,7}

New products containing naturally occurring ingredients have been introduced as both toothpastes and mouthrinses. Several studies have demonstrated the clinical efficacy of using products with naturally occurring ingredients.\textsuperscript{8,9} More recently, a mouthrinse with naturally occurring ingredients has appeared in the marketplace and is marketed as Herbal Mouth and Gum Therapy (Woodstock Natural Products, Englewood Cliffs, NJ). It contains six herbal ingredients, the main being echinacea and goldenseal. Table I lists the herbal ingredients in this product as well as their reported activity.\textsuperscript{10,11}

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</tbody>
</table>

Both echinacea and goldenseal are native American plants and were used by the Plains Indians of the American Midwest to treat a variety of ailments, i.e., external infections and venomous bites.\textsuperscript{10,11} The herbal preparations continued to be widely used in the early part of the 20th Century until their popularity waned with the discovery of penicillin and other manufactured drugs.\textsuperscript{10,11} Investigators have demonstrated that echinacea has the ability to boost the immune system while possessing anti-inflammatory characteristics.\textsuperscript{12,14} It has been prescribed for colds, colic, flu and other infectious diseases.\textsuperscript{11} Goldenseal has also been used for colds, flu and as an anti-inflammatory and antibiotic. The herb contains berberine, biotin, choline, cholegenic acid and inositol among its ingredients.\textsuperscript{11} Several studies have been reported that demonstrate its anti-inflammatory and medicinal benefits.\textsuperscript{13-17}

The purpose of this study was to determine the antimicrobial activity of Herbal Mouth and Gum Therapy mouthrinse, and to compare it to two other mouthrinses with known antimicrobial properties, as well as a positive (penicillin G) and negative (no ingredient) control.

**Materials and Methods**

The zones of microbial inhibition produced by the following mouthrinses were measured and compared:

Herbal Mouth and Gum Therapy is a product containing six herbal extracts (Table I). The percentages of each of the ingredients are proprietary. There is no alcohol in this product.

Listerine Original is a combination of the phenol-related essential oils, thymol, eucalyptol menthol and methysalicylate, in a 26.9 percent hydroalcoholic vehicle.\textsuperscript{2}

Periex is a cationic bis-biguanide (0.12 percent chlorhexidine) in an 11.6 percent hydroalcoholic vehicle.\textsuperscript{2}

The following bacteria, provided by the Department of Microbiology, Division of Basic Sciences, New York University College of Dentistry, were utilized in this study: 1. *Streptococcus mutans*; 2. *Streptococcus sanguis*; and 3. *Actinomyces viscosus*.

*Streptococcus mutans* is the only cariogenic organism of the three tested. The other bacteria tested are considered to be early colonizers in the formation of dental plaque. All bacteria were cultured in yeast-glucose broth overnight at 37°C. After 18 hours, the broth culture was diluted 10-fold, and grown as above until a density of 10\(^6\) cells/milliliter was determined by optical density using a Klett-Summerson photometer. Assays were performed on Brain-Heart Infusion agar sterile Petri dishes (Fisher Scientific Co., Ottawa, Canada). The bacteria were swabbed over the surfaces of the agar plates in two directions. Sterile paper discs measuring 13 mm in diameter were uniformly coated with each mouthrinse and placed with sterile forceps onto the prepared plates. In addition, positive control antibiotic discs (penicillin G—2 Units) and the negative control (sterile untreated discs) were also placed on the prepared plates. These positive and negative controls were used to demonstrate antimicrobial activity or non-activity, and viability of the culture on the agar medium.

Three samples of each material were placed on one plate at a time, along with the control discs. Each material control was tested three times.

*Streptococcus mutans* and *Streptococcus sanguis* were incubated aerobically at 37°C. *Actinomyces viscosus* was grown anaerobically at 37°C in a BBL GasPak Anaerobic System\textsuperscript{R} (Becton Dickinson Microbiology Systems, Cockeysville, MD). Each Petri dish was evaluated after 48 hours, and zones of inhibition measured in millimeters with a caliper (Mitutoyo Mfg. Co., Ltd., Tokyo, Japan).

**Results**

The results of this study are summarized in Table II. The products and zones of inhibition as measured in millimeters are presented. All three mouthrinses demonstrated antimicrobial activity against all three bacteria. Herbal Mouth and Gum Therapy mouthrinse produced larger zones of inhibition than Listerine against all three bacteria tested, and larger zones of inhibition than Periex against *S. mutans* and *A. viscosus*. Herbal Mouth and Gum Therapy mouthrinse produced the same zone of inhibition as Periex against the third bacteria, *S. sanguis*. With two of the three bacteria tested, *A. viscosus* and *S. sanguis*, Herbal Mouth and Gum Therapy produced larger zones of inhibition than the positive control, penicillin G. With *S. mutans*, Herbal Mouth and Gum Therapy produced larger zones of inhibition than the positive control, penicillin G.

<table>
<thead>
<tr>
<th>Product</th>
<th>Streptococcus mutans (mm)</th>
<th>Streptococcus sanguis (mm)</th>
<th>Actinomyces viscosus (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbal Mouth and Gum Therapy</td>
<td>20 (1.6)</td>
<td>16 (4.0)</td>
<td>20 (1.6)</td>
</tr>
<tr>
<td>Periex</td>
<td>20 (1.6)</td>
<td>16 (4.0)</td>
<td>20 (1.6)</td>
</tr>
<tr>
<td>Listerine</td>
<td>15 (4.4)</td>
<td>15 (4.4)</td>
<td>15 (4.4)</td>
</tr>
<tr>
<td>Penicillin G (Positive Control)</td>
<td>17 (3.0)</td>
<td>17 (3.0)</td>
<td>17 (3.0)</td>
</tr>
<tr>
<td>Sterile Disc (Negative Control)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
inhibition than penicillin G, while Peridex and penicillin G produced the same zones of inhibition. Penicillin produced larger zones of inhibition than Listerine against S. mutans and S. sanguis, and the same zones of inhibition against A. viscosus.

Discussion

If people could mechanically remove plaque by brushing and flossing, antimicrobial mouthrinses would have minimal importance to the oral hygiene regimen. Yet, most people brush an average of only 37 seconds, and few use dental floss. Thus, rinsing and/or irrigating with an antimicrobial mouthrinse can be an important adjunct to maintaining oral hygiene. This study indicated that all of the mouthrinses tested produced antimicrobial zones of inhibition against the selected oral organisms. It should be noted that the positive control, penicillin G, was used to demonstrate antimicrobial activity and viability of the culture by producing zones of inhibition to growth. It is not surprising that this antibiotic did not produce the largest zones of inhibition given the different rates of diffusion into the agar medium.

Antimicrobial activity of the mouthrinses can be related to the anionic and cationic potencies of the products, as well as their effect on changing cellular osmotic pressure and cell metabolism. In addition, the antimicrobial ingredients may absorb into bacterial surfaces and disrupt the cell membrane. This is the case with Listerine, whereby the mode of action of phenolics is through cell wall disruption and inhibition of bacterial enzymes. Additionally, there is some evidence that Listerine can also extract the lipopolysaccharide-derived endotoxin from Gram-negative bacteria. The bactericidal effect of Peridex is due largely to disruption of cell integrity and the precipitation of cytoplasmic content. The mode of action of the Herbal Mouth and Gum Therapy mouthrinse is not thoroughly understood. It is thought to be in part due to a cationic effect as well as a disruption in cell metabolism.

Except for Herbal Mouth and Gum Therapy mouthrinse, the other mouthrinses tested contain alcohol in percentages varying from 11.6% to 26.9% (Table III). It should be noted that alcohol has long been used for its antiseptic properties, although in mouthrinse preparations it mainly functions as a vehicle that dissolves and delivers mouthrinse ingredients. At the same time, alcohol in high concentrations has been shown to produce ulceration, inflammation and epithelial peeling. More recently, in order to combat the number of alcohol-related poisonings in children yearly, manufacturers of mouthrinses having over 5% alcohol have been requested to market their products in child-proof packages. Therefore, if one were simply to focus alone on the alcohol-containing issue, a natural and non-alcohol containing mouthrinse may provide an alternative for use in personal oral hygiene regimens.

To date, little information exists regarding the production of opportunistic organisms from the extended use of Herbal Mouth and Gum Therapy mouthrinse. While the results of this study for the antimicrobial effects in vitro appear to be promising, more in vivo research should be conducted with this product in order to assess any untoward clinical implications.

Conclusion

All of the mouthrinses tested demonstrated antimicrobial activity. The Herbal Mouth and Gum Therapy mouthrinse produced larger zones of microbial inhibition than Listerine against all three bacteria tested. This mouthrinse also had larger zones of microbial inhibition against two of the three bacteria tested, and produced similar zones of inhibition against the third bacteria, compared to Peridex.

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References

Clinical Efficacy of an Herbal Toothpaste

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Abstract
The purpose of this three-month, double-blind, parallel-design clinical study was to compare the efficacy of two commercially available dentifrices, Herbal Toothpaste and Gum Therapy® and Colgate Total®, in controlling gingivitis, gingival bleeding, plaque and stain. Forty healthy adult volunteers from the Junior Comprehensive Care Clinics at New York University College of Dentistry were accepted as subjects for this clinical trial. To be eligible for a baseline clinical examination, subjects had to first indicate that during the previous six months they habitually brushed their teeth two or more times per day, and had noticed "bleeding gums" or "blood in the toothpaste" after brushing or flossing their teeth. At the baseline examination, subjects were enrolled in the study if they had at least five Löe-Silness gingival bleeding sites and 20 natural teeth, including all anterior teeth and four molars. An independent t-test before treatment indicated that there were no significant differences between the two groups at baseline. A one-way Analysis of Variance indicated that both dentifrices had a significant effect on gingivitis, gingival bleeding, plaque and dental stain (p < 0.05). No significant statistical differences were observed between Herbal Toothpaste and Gum Therapy and Colgate Total for gingivitis or gingival bleeding. Herbal Toothpaste and Gum Therapy produced statistically significant differences in reducing plaque and stain relative to Colgate Total (p < 0.05). The results obtained in this study support the clinical efficacy of both products in reducing gingivitis and plaque, and demonstrate the efficacy of Herbal Toothpaste and Gum Therapy in maintaining reductions of plaque and stain. (J Clin Dent 9:31-33, 1998.)

Introduction
It is generally agreed that the control of gingivitis and dental caries should provide long-term benefits in the maintenance of a healthy dentition.1,2 Today, an increasing number of commercial products are available containing new ingredients which are positioned, either directly or through inference, as providing added therapeutic or cosmetic benefit compared to conventional toothpastes and mouthwashes.3 One such product, Colgate Total (Colgate-Palmolive Company, New York, NY), was recently approved by the United States Food and Drug Administration and accepted by the American Dental Association for its ability to prevent gingivitis, plaque and caries, and to fight the build-up of calculus.

Recent years have seen an interest in products that are composed of "natural" ingredients. The term "natural," associated with a consumable product, refers to the ingredients being derived from natural sources with minimal processing. Such ingredients, specifically herbal extracts, are made by pressing herbs with a heavy hydraulic press and then soaking them in alcohol or water. The excess alcohol or water is allowed to evaporate, yielding a concentrated extract as an ingredient.

The purpose of this three-month, double-blind, parallel-design clinical study was to determine the efficacy of Herbal Toothpaste and Gum Therapy (Woodstock Natural Products, Englewood Cliffs, NJ), a dentifrice composed of natural ingredients that are all alcohol-—free extracts. Additionally, this dentifrice contains sodium bicarbonate, 0.76% sodium monofluorophosphate in a calcium base, natural domestic spearmint oil, and no dyes, preservatives or artificial flavorings. In this study, Herbal Toothpaste and Gum Therapy was compared to Colgate Total dentifrice for the control of gingivitis, gingival bleeding, plaque and stain.

Materials and Methods
Forty healthy adult volunteers from the Junior Comprehensive Care Clinics at New York University College of Dentistry were accepted as subjects for this three-month, double-blind parallel group clinical trial. To be eligible for inclusion in the study, the subjects had to first indicate that during the previous six months they habitually brushed their teeth two or more times per day, and had noticed "bleeding gums" or "blood in the toothpaste" after brushing or flossing their teeth. At the baseline examination, subjects were enrolled in the study if they had at least five Löe-Silness gingival bleeding sites1 and 20 natural teeth including all anterior teeth and four molars. Subjects were excluded if they had rampant caries, obvious periodontal disease, chronic dental neglect or serious medical conditions as determined by the investigator. Subjects were also excluded if they had taken antibiotics within seven days or had used a chlorhexidine rinse within three months of the start of the study.

Following entrance examinations to determine subject qualification and initial levels of gingivitis, gingival bleeding, supragingival plaque and stain, subjects were given a cleaning to remove existing stain, supragingival plaque and calculus. No subgingival scaling was performed so as not to interfere with bleeding sites present. The teeth were then polished, and complete plaque and stain removal were verified by the use of phosphate buffered disclosing solution (Lactona Plaque Detector Disclosing Solution, Lactona Co., Montgomeryville, PA). All subjects were then randomly assigned to use either the Herbal Toothpaste and Gum Therapy or Colgate Total, and instructed to brush their teeth for one minute twice daily (morning and evening) in their customary manner. Throughout the study, all toothpaste tubes were masked so that neither the subjects nor the examiners knew which products were being used by which subjects. All subjects were given the same brand of new toothbrush to use during the study.

Subjects were instructed not to use any other dentifrices, mouthwashes or oral irrigation products for the duration of the study. Subjects were to return for clinical examinations after three months of product use. At these examinations, gingivitis, gingival bleeding, supragingival plaque and stain scores were recorded.

Indices
Gingivitis and Gingival Bleeding. Gingivitis was measured using the Gingival Index (GI) of Löe and Silness,4 which uses a scale of 0-3 in evaluating gingival tissues adjacent to six surfaces (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual) of all natural teeth, excluding third molar's. A probe was inserted about 1 mm into the gingival sulcus and passed from interproximal to interproximal surface. The GI was scored according to the following criteria:
0 = Normal gingiva
1 = Mild inflammation—slight change in color, slight edema.
No bleeding on probing.
2 = Moderate inflammation—redness, edema, glazing. Bleeding on probing.
3 = Severe inflammation—marked redness, edema, ulceration. Tendency to spontaneous bleeding.

For gingivitis, results were reported as the mean G1 score for each subject averaged across all surfaces evaluated. For bleeding, results were reported as the number of sites that bled on probing or bled spontaneously (grades 2 or 3).

**Plaque.** The plaque thickness at the gingival margin was determined using the Silness and Löe Plaque Index. All teeth were evaluated, with the exception of third molars. For each tooth, plaque was scored at the six gingival areas (distobuccal, buccal, mesiobuccal, mesiolingual, lingual and distolingual) using a 0-3 scale. Examiners evaluated plaque on the cervical thirds of teeth as follows:

0 = No plaque.

1 = A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be recognized only after running the probe across the tooth surface.

2 = Moderate accumulation of soft deposits within the gingival pocket or on the tooth and gingival margin that can be seen with the naked eye.

3 = Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

For plaque, results were reported as the mean plaque score for each subject averaged across all surfaces evaluated.

**Stain.** Extrinsic dental stain was determined using a non-invasive visual assessment technique, in which the labial surfaces of the six maxillary and mandibular anterior teeth were graded. Stain was scored using a 0-4 scale as follows:

0 = No stain present.

1 = Stain covering 0%-25% of surface.

2 = Coverage of 25%-50%.

3 = Coverage of 50%-75%.

4 = Coverage of greater than 75%.

A subject's total stain score was determined by summing the individual scores assigned to each labial surface.

**Statistical Analyses**

A one-way Analysis of Variance (ANOVA) was performed on gingivitis, gingival bleeding, plaque and stain scores comparing scores from baseline and three months, using the baseline reading as a covariate. Additionally, an independent t-test was performed before treatment to determine whether any significant differences existed between the baseline evaluations of the two groups. Significance was based on alpha = 0.05.

**Results**

An independent t-test before treatment indicated that there were no significant differences between the two groups at baseline. A one-way ANOVA indicated that both dentifrices had a significant (p < 0.05) effect on gingivitis, gingival bleeding, plaque and stain (Tables I and II). No significant differences were observed between Herbal Toothpaste and Gum Therapy and Colgate Total for gingivitis or gingival bleeding. Herbal Toothpaste and Gum Therapy produced statistically significant differences in reducing plaque and stain relative to Colgate Total (p < 0.05). Comparative percent reductions for the two products against gingivitis, gingival bleeding, plaque and stain are presented in Table III.

**Table I**

<table>
<thead>
<tr>
<th>Summary of Baseline Means (SD)</th>
<th>Herbal Toothpaste and Gum Therapy</th>
<th>Colgate Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingivitis</td>
<td>0.78 (0.13)</td>
<td>0.79 (0.14)</td>
</tr>
<tr>
<td>Gingival Bleeding</td>
<td>15.28 (1.36)</td>
<td>15.42 (0.33)</td>
</tr>
<tr>
<td>Plaque</td>
<td>0.64 (0.11)</td>
<td>0.65 (0.12)</td>
</tr>
<tr>
<td>Stain</td>
<td>0.19 (0.06)</td>
<td>0.17 (0.04)</td>
</tr>
</tbody>
</table>

**Table II**

<table>
<thead>
<tr>
<th>Summary of Three Month Means (SD)</th>
<th>Herbal Toothpaste and Gum Therapy</th>
<th>Colgate Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingivitis</td>
<td>0.58 (0.08)</td>
<td>0.60 (0.09)</td>
</tr>
<tr>
<td>Gingival Bleeding</td>
<td>8.89 (1.36)</td>
<td>9.33 (1.09)</td>
</tr>
<tr>
<td>Plaque</td>
<td>0.51 (0.07)</td>
<td>0.55 (0.09)</td>
</tr>
<tr>
<td>Stain</td>
<td>0.13 (0.05)</td>
<td>0.12 (0.04)</td>
</tr>
</tbody>
</table>

**Table III**

<table>
<thead>
<tr>
<th>Percent Reductions of Gingivitis, Gingival Bleeding, Plaque and Stain Scores after Three Months</th>
<th>Herbal Toothpaste and Gum Therapy</th>
<th>Colgate Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingivitis</td>
<td>25.6</td>
<td>24.0</td>
</tr>
<tr>
<td>Gingival Bleeding</td>
<td>41.7</td>
<td>39.6</td>
</tr>
<tr>
<td>Plaque</td>
<td>20.3</td>
<td>15.3</td>
</tr>
<tr>
<td>Stain</td>
<td>31.5</td>
<td>29.4</td>
</tr>
</tbody>
</table>

**Discussion**

The results of this study indicate that both dentifrices improved oral health after three months of use. Colgate Total has been approved for claims against gingivitis by the FDA and has gained the American Dental Association Seal of Acceptance for protection against plaque, gingivitis and caries. Colgate Total contains 0.243% sodium fluoride in a silica base and is the only dentifrice in the United States to contain the antibacterial ingredient triclosan (0.3%). However, it should be noted that the FDA approval of Colgate Total did not attribute gingivitis efficacy to the antibacterial effect of triclosan. The FDA paper stated that triclosan, an ingredient found in many soaps, is a disinfectant. Its antibacterial properties in the mouth have not been determined. The activity of triclosan appears to depend upon its local concentration in the oral mucosa and dental plaque. As a result, in order to prolong its action, Colgate Total is formulated with 2.0% of a copolymer of polyvinylmethy ether/maleic acid (PVM/MA).

Herbal Toothpaste and Gum Therapy, when compared to Colgate Total, demonstrated no significant statistical difference in reducing both gingivitis and gingival bleeding. At the same time, Herbal Toothpaste and Gum Therapy demonstrated that it was significantly better in maintaining the reductions in plaque and stain that were obtained after the initial baseline prophylaxis compared to Colgate Total. Herbal Toothpaste and Gum Therapy contains several botanical and herbal extracts which may, in addition to being natural ingredients, provide medicinal benefits for those consumers who want to avoid artificial sweeteners and chemicals. Table IV lists herbal ingredients found in the Herbal Toothpaste and Gum Therapy formulation and their proposed
Table IV
Herbal Ingredients Found in Herbal Toothpaste and Gum Therapy

<table>
<thead>
<tr>
<th>Herbal Ingredient</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinacea</td>
<td>analgesic, anti-inflammatory, antibiotic</td>
</tr>
<tr>
<td>Goldenseal</td>
<td>antiseptic, antibiotic</td>
</tr>
<tr>
<td>Calendula</td>
<td>anti-inflammatory</td>
</tr>
<tr>
<td>Bloodroot</td>
<td>antibiotic, anti-inflammatory</td>
</tr>
<tr>
<td>Grapefruit Seed Extract</td>
<td>anti-fungal, antibiotic</td>
</tr>
<tr>
<td>Aloe Vera Gel</td>
<td>analgesic, anti-inflammatory, moisturizer</td>
</tr>
<tr>
<td>Bee Propolis</td>
<td>anti-inflammatory, antibiotic</td>
</tr>
</tbody>
</table>

benefits. It is unclear from this study why the Herbal Toothpaste and Gum Therapy dentifrice significantly maintained the reductions of plaque and stain formation compared to Colgate Total. The results certainly warrant long-term clinical study to determine continued effects of Herbal Toothpaste and Gum Therapy and its impact on oral organisms with prolonged use.

Conclusion

The results obtained by this study support the clinical efficacy of both products in reducing gingivitis and plaque, and demonstrate the efficacy of Herbal Toothpaste and Gum Therapy in maintaining reductions of plaque and stain.

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References