An in-vitro analysis of the antimicrobial efficacy of herbal toothpastes on selected primary plaque colonizers

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Abstract

Plaque associated oral disease affects a considerable portion of the population and is considered one of the major causes of tooth loss. In most cases toothbrushing only removes a limited amount of dental plaque and other chemical agents are required to reduce the bacterial load. **Aims & objectives:** The purpose of study was to determine whether there was any significant difference in the antimicrobial activity of 4 herbal toothpastes against cultures of 3 primary plaque colonizers. **Methods:** A total of 5 toothpastes were tested for their antimicrobial efficacy against *Streptococcus mutans* (NCTC 10920), *Streptococcus sanguinis* (NCTC 10904) and a non-specific *α-heamolytic streptococcus* by agar diffusion method. The data were collected and analysed using one way ANOVA and Tukey's multiple comparison test significant at $p<0.05$. **Results**: Dentazyme® herbal toothpaste showed the greatest ability to inhibit bacterial growth for all the tested organisms ($p<0.05$). Nature Fresh had the lowest potential for antimicrobial activity. **Conclusions:** Dentazyme® Herbal toothpaste was the only herbal toothpaste to inhibit the growth of all the bacteria tested and had similar antimicrobial efficacy to a triclosan containing toothpaste (Colgate® Total®).

KEYWORDS: Plaque, Herbal toothpaste, antimicrobial activity, bacteria

Introduction

Plaque associated oral disease affects a considerable portion of the population and is considered one of the major causes of tooth loss (1). In most cases, the chronic accumulation of dental plaque often leads to caries and periodontal disease (in genetically susceptible individuals), that may not only affect the patient's oral health, but may also contribute to a number of chronic systemic diseases (2). It is now well recognized that toothbrushing alone only removes 50% of dental plaque and that additional mechanical and antimicrobial measures are required to further reduce the bacterial load (3). Although antimicrobials such as chlorhexidine and other chemicals in the form of gels, mouthrinses and varnishes have been proposed as plaque control agents, studies indicate that when dental plaque has formed a mature biofilm, the efficacy of these agents are significantly reduced (4). An effective plaque control measure may therefore be to target plaque formation before the mature biofilm is established.

Several recent studies show that plant and herbal extracts have significant antimicrobial effects against plaque bacteria. As a result, a number of these agents have been incorporated into dentifrices, including toothpastes and mouthrinses (5-7). However only a limited amount of published data exists regarding the efficacy of these products on the initial plaque forming bacteria (6). Therefore the aim of this study was to determine whether there was any significant difference in the antimicrobial activity of four herbal toothpastes against specific primary plaque colonizers.

MATERIALS AND METHODS

Four herbal toothpastes were purchased from a large local retail store in Cape Town, South Africa (Table 1). Colgate® Total® (Colgate-Palmolive Company, Guateng, South Africa) served as the positive control, and a 9 mm sterile filter disc (Munktell & Filtrak, Bärenstein, Germany) as the negative control.

Three different oral microorganisms were obtained from the Department of Medical Bioscience, University of the Western Cape, South Africa (Table 2).

The toothpastes were tested at full strength by placing dentifrice samples of approximately 50 milligrams on the sterile filter discs and then positioning these discs onto the surfaces of Columbia Blood Agar Base (CM0331) (Oxoid Limited, Hampshire, United Kingdom) previously inoculated with the test microorganisms. The Mcfarland Standard 3 was used to ensure that each test organism had a similar concentration. This corresponded to 900 × 10^6/ml of bacteria for each of the species tested. The agar plates were incubated together with toothpaste impregnated discs at 37°C for 24 hours in a temperature controlled room. All tests were conducted in triplicate. The antimicrobial activity of the test toothpastes were represented by a clear zone (zone of inhibition) surrounding the test discs (Figure 1).

In order to compare the efficacy of the various products tested, the diameters of the zones of inhibition were measured and compared 24 hours after initial incubation. The mean diameters of inhibition zones were determined for all toothpastes. The data were analyzed with one-way ANOVA followed by a Tukey's multiple comparison test for differences amongst the different groups (significance level was $p<0.05$).

Results

After 24 hours of incubation, all four test toothpastes displayed observable zones of inhibition (Table 3). Colgate® Total®, produced inhibition zones for all three microorganisms cultured, whereas the sterile filter disc produced no observable inhibitory effect for any of the bacteria.

Only three of the tested toothpastes i.e. Colgate® Herbal, Aquafresh® Herbal and Dentazyme® Herbal, produced zones of inhibition against all the microorganisms. Nature Fresh Antimicrobial toothpaste showed inhibitory zones only for the non-specific *α-haemolytic streptococcus* and was ineffective against the other two bacteria.

Statistical analysis revealed that Dentazyme® Herbal had a statistically significantly greater ability in inhibiting the growth of *S. mutans* and *S. sanguinis* compared to all other toothpastes tested ($p<0.05$). However for the *α-haemolytic streptococcus* species, its inhibitory effect was not significantly different to that of Aquafresh® Herbal and Colgate® Total® ($p>0.05$). Colgate® Herbal produced inhibitory zones that were significantly smaller than all other toothpastes tested except for Nature Fresh ($p<0.05$).

Discussion

Dental plaque is a significant risk factor for the development of dental and periodontal disease (1). Recent evidence indicates that rather than being an accumulation of bacteria, it is a complex biofilm consisting of a variety of bacteria embedded in a polysaccharide matrix. This structure allows for bacteria to exhibit pathogenic characteristics not normally seen in a planktonic state (8).

One of the most commonly used anti-plaque chemical agents is chlorhexidine digluconate (3). Even though this agent has well documented antimicrobial efficacy against bacteria associated with dental plaque, Zaura-Arite et al (4) showed that when chlorhexidine was applied to a 48-hour old plaque biofilm, it was only effective on the outer layer of the structure, whereas most of the middle and core layers were resistant to its effects. Chlorhexidine therefore requires multiple applications in order to be clinically effective. The chronic use of chlorhexidine has also been associated with oral epithelial desquamation, altered taste sensation and staining of the teeth and oral prosthesis (3).

These limitations together with that of other chemical plaque controlling agents, has prompted researchers to search for alternative antimicrobial sources (9-20). One of these includes the wide variety of herbs and plant-derived products that are currently marketed for therapeutic use. Tea tree oil, propolis, garlic, neem and other herbs and spices have all proven antimicrobial effects against a range of oral bacteria, including the periodontal pathogens (9-15). Many of these have been successfully incorporated into a number of dentifrices, including toothpastes, mouthrinses, and gels (5-7).

Relatively few published studies in the literature have compared the effect of herbal based toothpastes on plaque associated bacteria (5,7,13,14). Most previous studies have tested individual herbal derivatives on oral pathogens or have used herbal based mouthrinses as their test material (5-20). The substantivity of toothpaste makes it an ideal mechanism for delivering antimicrobials to the oral cavity, and was therefore chosen as the dentifrice of choice for this study. It also allows for the direct application of antibacterial chemicals to the tooth surface, thereby potentially preventing the colonization of the tooth structure by oral bacteria. In a recent controlled clinical trial it was shown that certain herbal-based toothpastes had a similar clinical effect as toothpastes containing triclosan and fluoride (14). Netuschil et al (14) showed that Meliamint®

Table 1: Ingredients of various toothpastes used in the study

Toothpastes	Ingredients as stated on package
Colgate® Herbal® **Colgate-Palmolive Company, Gauteng, South Africa**	Sodium Monoflourophosphate 1.1%, Aqua, Calcium Carbonate, Sorbitol, Sodium Lauryl Sulphate, Sodium Monoflourophosphate, Cellulose gum, Sodium silicate, Aroma, Sodium Bicarbonate, Sodium Saccharin, Eucalyptus Globulus Leaf Oil, Xanthan Gum, Methylparaben, Commiphora Myrrh Extract, Chamomilla Recutita (Matricaria) Extract, Propylparaben, Metaleuca Alternifolia (Tea Tree) Leaf Oil, Salvia Officinalis (Sage) Oil, Eugenol, CI 74260
Nature Fresh Herbal **Nature Fresh Health Products, South Africa**	Olive leaf, Aloe Ferox bitters, Cloves, Tea Tree, Mint and Aniseed oil with calcium, magnesium, sea salt, zinc and baking soda
Dentazyme® Herbal **Amka Products, Pretoria, South Africa**	Sorbital, Hydrated Silica, Water (Aqua), Sodium Lauryl Sulphate, Peg-32, Flavour, Sodium Flouride, Cellulose Gum, Sodium saccharine, Methylparaben, Tea Tree (Melaleuca Alternifolia) leaf oil, CI 19140, CI 42090, Eugenol, Limonene, Linalool
Aquafresh® Herbal **GlaxoSmithKline, Gauteng, South Africa**	Aqua, Hydrated Silica, Sorbitol, Glycerin, Peg-6, Sodium lauryl sulphate, Aroma, Carrageenan, Xanthan Gum, Sodium Flouride, Sodium Saccharin, Titanium Oxide, Eucalyptus Globulus, Mentha Arvensis, Salvia Officinalis, Anthemis nobilis, CI 77492, CI 74260, CI 73360

Table 2: Microorganisms used in the study

NAME	National Collection of Type Cultures (NCTC), Strain number
Streptococcus mutans	10920
Streptococcus sanguinis	10904
non specific *α-heamolyitic viridans streptococci*	This specific strain was obtained from standard culture stock and has not officially been named yet.

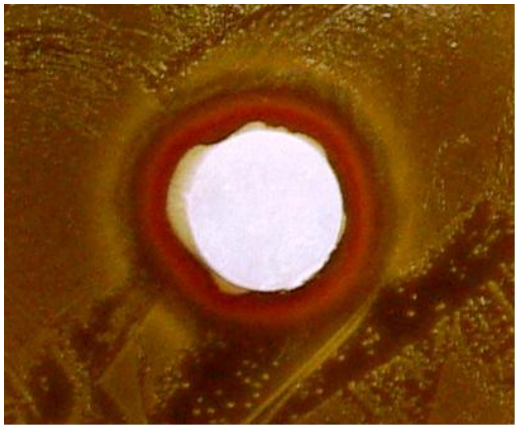

Figure 1: Zone of inhibition around toothpaste impregnated disc

Table 3: Anti-microbial activity of toothpastes against various bacteria

Toothpastes and Controls	Mean diameter and standard deviations of inhibition zones in millimetres		
	Streptococcus mutans	***Streptococcus sanguinis***	***α-haemolytic streptococcus***
Positive Control (Colgate® Total®)	12.50 ± 2.50	10.00 ± 0.00	35 00± 5.00
Negative Control (Sterile Filter Disc)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Dentazyme® Herbal	19.67 ± 5.50	25.38 ± 10.70	37.14 ± 6.11
Colgate® Herbal	7.76 ± 2.60	13.84 ± 3.62	27.14 ± 8.02
Aquafresh® Herbal	11.00 ± 3.38	15.00 ± 4.76	34.64 ± 6.92
Nature Fresh Herbal	0.00 ± 0.00	0.00 ± 0.00	23.92 ± 6.25

(WalaVita Bad Boll, Germany), a fluoride-free herbal based toothpaste that contains peppermint, clove, chamomomile, mastic gum, propalis wax and chlorophyll, was more effective than Colgate Total in the management of gingivitis.

Several herbal toothpastes are now readily available to the public (7). Many are found in retail stores and are available without prescription. Some of the proposed active ingredients include myrrh, sage, eucalyptus, tea tree oil, mallow, matricariae and propolis. To our knowledge, this is the first study conducted using the herbal based toothpastes mentioned. The results of the present study indicate that all of the test toothpastes had some degree of antimicrobial ability, the exact nature of which remains unknown. Dentazyme® Herbal together with Colgate® Herbal are the only products used in the study that contain both eugenol and tea tree oil, both of which have documented anti-bacterial activity (20). However, Dentazyme® Herbal has significantly stronger antimicrobial potential when compared to Colgate Herbal and therefore the antimicrobial effect cannot solely be attributed to the presence of these two ingredients. The effect of other factors such as the concentrations of these two ingredients, the presence, type and concentration of fluoride (which in itself has antimicrobial properties) and the exact formulation of the different toothpastes warrants further investigation.

Nature Fresh was the only non-fluoridated toothpaste that was used in the study. Even though it contained tea tree oil, it proved to be significantly weaker than most of the other toothpastes in its ability to inhibit bacterial growth. The efficacy of Nature Fresh as a potential therapeutic agent in the prevention or management of plaque associated oral disease therefore needs to be questioned.

Although the above study clearly indicates that the tested toothpastes have antimicrobial properties, the results need to be interpreted with caution. The oral

cavity is a complex environment that is characterized by temperature variations in various parts of the mouth, the presence of saliva, and variation in local supra and subgingival bacteria. Any of these changes may have a significant effect on the clinical efficacy of the toothpastes tested.

Conclusions

The results indicate that there was a significant difference in the ability of the various herbal toothpastes to inhibit the growth of certain plaque bacteria. Dentazyme® herbal toothpaste was the only herbal toothpaste to inhibit the growth of all the bacteria tested and had similar antimicrobial efficacy to a triclosan containing toothpaste (Colgate® Total®).

Conflict of interest

The authors declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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