ENAMEL FLUORIDE UPTAKE AND ANTIMICROBIAL EFFECTIVENESS OF AN HERBAL FLUORIDE MOUTHRINSE

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INTRODUCTION

Current epidemiological data have suggested a rise in the prevalence of dental caries in both children and adults. The use of fluoride rinse in combination with water fluoridation, proper brushing with fluoride dentifrice as well as flossing, a proper diet and regular dental office visits may all work in conjunction to reverse the current trend.[2] In addition to fluoride for the prevention of dental caries, many rinses also deliver antimicrobial ingredients which may in turn help to prevent plaque and gingivitis.[3,4] Rinsing cannot replace proper brushing and flossing, but has the added advantage of accessibility to surfaces in the mouth, including interproximal hard and soft tissues, and, depending on their composition, remain active for extended periods of time.[6,10]

While there are many mainstream brands which offer fluoride mouth rinse products with antimicrobial benefits, there are not many products that offer these same benefits in the "natural" channel. While the FDA does not have a definition for "natural", generally, "natural" products limit the use of synthetic colors, flavors, sweeteners, stabilizers, preservatives and active ingredients. These products tend to use natural chemicals in combination with plant-based ingredients to deliver therapeutic benefits. The potential beneficial or detrimental effects of these ingredients on active ingredients in rinses are mostly unknown.

Natural oral health products can only be considered alternatives if they demonstrate comparable or greater effectiveness as compared to conventional products. The Natural Dentist Healthy Teeth Anticavity Fluoride Rinse (Natural Dentist, Inc., Medford, MA) is an ADA-accepted herbal fluoride rinse currently on the market and contains no alcohol, artificial sweeteners, dyes or preservatives.

OBJECTIVES

The objectives of the study were to determine the Enamel Fluoride Uptake (EFU) of The Natural Dentist Anticavity Fluoride Rinse (TND) and to determine its antimicrobial effectiveness as measured by its Minimum Inhibitory Concentration (MIC) against predominant oral pathogens.

METHODS for EFU

- Methods followed a modification of FDA Test #40
- 3mm diameter disks of human teeth embedded in acrylic with exposed enamel were prepared. Specimens were ground with 600 grit wet/dry paper for 10 minutes and polished with micro-fine Gamma Alumina for 45 minutes.
- Enamel specimens were demineralized in 0.5 ml of 1M $HClO^4$ for 15 seconds under agitation and immediately rinsed with deionized water. Fluoride content was measured for buffered samples of each solution by comparison to a standard curve. The amount of enamel removed was determined by measuring the calcium content of the solution by atomic absorption. From the resulting fluoride and calcium levels, the indigenous fluoride level of each specimen prior to treatment was calculated.
- The demineralized layer was removed and an incipient, caries-like lesion was formed by immersion of the samples in 0.1M lactic acid/0.2% Carbopol 907/HAP solution for 24 hours, at 37°C.
- Specimens were immersed in 25 mls of the test product with constant stirring for 30 minutes at room temperature and then rinsed with deionized water.
- Specimens were again demineralized into 0.5 ml of 1M $HClO^4$ for 15 seconds and the solutions were analyzed for fluoride and calcium content. From these data, the fluoride uptake in each specimen after treatment was calculated.

METHODS for MIC[8]

- An agar dilution method was employed.
- Basal medium consisted of Trypticase soy agar, 5 µg/ml hemin, 10 µg/ml N acetyl muramic acid, 0.5 µg/ml menadione & 5% sheep blood.
- Test agents were prepared to provide a final conc^n of 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 µg/ml.
- Test species were grown anaerobically for 3 days. Growth was harvested, suspended in sterile Mycoplasma broth & OD adjusted to MacFarlane # 0.5 standard.
- An MIC 2000 inoculator was used to transfer strain suspensions onto the surface of plates containing the test agents.
- Plates were incubated anaerobically and evaluated daily. MIC was the lowest concentration of the agent that completely inhibited growth of the test species.

RESULTS

- There was no significant difference in EFU between TND Anticavity Fluoride Rinse and Phos- Flur Anticavity Fluoride Rinse (Colgate-Palmolive Co., Piscataway, NJ) (Table 1 and Fig. 1).
- Both TND Anticavity Fluoride Rinse and Phos-Flur Anticavity Fluoride Rinse were more effective in promoting fluoride uptake than ACT Anticavity Fluoride Rinse: Mint Flavored (Chattem, Inc., Chattanooga, TN) (Table 1 and Fig. 1).
- TND Anticavity Fluoride Rinse effectively inhibited the growth of the 42 test species including the caries-pathogen, *S. mutans* (Table 2 and Fig. 2).
- TND Anticavity Fluoride Rinse had comparable MICs as compared to the positive control (TND Healthy Gums Mouth Rinse[8]) (Table 2 and Fig. 2).

Table 1.
EFU for Test Fluoride Rinses

Product	n		Pre-Tx F Level (ppm)	Post-Tx F Level (ppm)	Fluoride Uptake (ppm)
ACT Anticavity Fluoride Rinse: Mint Flavored (pH 6.2)	11*	Mean	58.32	1498.37	1440.05
		SD	18.94	114.59	120.2
		SEM	5.71	34.55	36.24
The Natural Dentist Anticavity Fluoride Rinse (pH 4.0)	11*	Mean	62.12	2534.59	2472.47
		SD	22.58	324.91	313.25
		SEM	6.81	97.97	94.45
Phos-Flur Anticavity Fluoride Rinse (pH 4.0)	12	Mean	61.11	2523.52	2462.42
		SD	23.94	328.03	318.42
		SEM	6.91	94.69	91.92

Groups within rows were not significantly different ($p>0.05$)
*Data from one specimen were rejected as outlier data

Figure 1. EFU for Test Fluoride Rinses

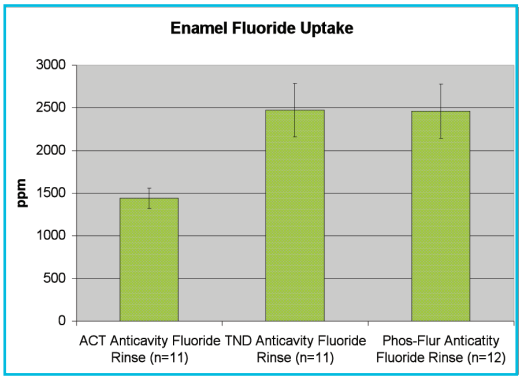

Table 2. MIC values for TND Fluoride Rinse vs. TND Healthy Gums Mouth Rinse

Species	ATCC #	TND Healthy Gums Mouth Rinse	TND Anticavity Fluoride Rinse
Aggregatibacter actinomycetemcomitans	29253	128	32/64
Actinomyces gerencseriae	23860	64	64
Actinomyces israelii	12102	32	32
Actinomyces naeslundii 1	12104	32	32
Actinomyces naeslundii 2	43146	64	64
Actinomyces odontolyticus	17929	32	32
Campylobacter gracilis	33236	128	256
Campylobacter rectus	33238	64	128
Campylobacter showae	51146	128	128/256
Capnocytophaga gingivalis	33624	64	64
Capnocytophaga ochracea	33596	64	128
Capnocytophaga sputigena	33612	128	128
Eikenella corrodens	23834	32	32
Eubacterium saburreum	33271	32	32/64
Fusobacterium nucleatum ss nucleatum	25586	64	8/32
Fusobacterium nucleatum ss polymorphum	10953	16/32	16/32
Fusobacterium nucleatum ss vincentii	49256	64	16/32
Fusobacterium periodonticum	33693	64	64
Gemella morbillorum	27824	64	64
Leptotrichia buccalis	14201	64	64
Neisseria mucosa	19696	64	128
Parvimonas micra	33270	32	16/128
Porphyromonas gingivalis	33277	32	16/64
Prevotella intermedia	25611	16/32	32
Prevotella melaninogenica	25845	32	16/32
Prevotella nigrescens	33563	32	32
Proprionibacterium acnes	11827	16/32	64/128
Selenomonas noxia	43541	64	128
Streptococcus anginosus	33397	128	64
Streptococcus constellatus	27823	64	64
Streptococcus gordonii	10558	64	128
Streptococcus mitis	49456	64/128	32
Streptococcus salivarius	27945	32	32/64
Streptococcus mutans	25175	32	32/64
Streptococcus oralis	35037	64	64
Streptococcus sanguinis	10556	64	64
Tannerella forsythia	43037	16/32	32
Veillonella parvula	10790	64	64
Prevotella denticola	33185	64	32/128
Porphyromonas endodontalis	35406	32	32
Prevotella loescheii	15930	64	64
Prevotella tannerae	51259	16/32	16/32

Table 2: Tests were conducted in duplicate. Single numbers indicate that duplicate runs were identical, while 2 numbers indicate that the runs differed. A two-fold difference was considered marginal (e.g. 64 vs. 128), whereas a > 2 fold differences (e.g. 64 vs 256) was considered significant. In instances of pairs of values for duplicates, the higher value should be used to err on the conservative side. The species in red represent odor-producing bacteria.

Fig 2. MIC values for the 2 tested mouth rinses

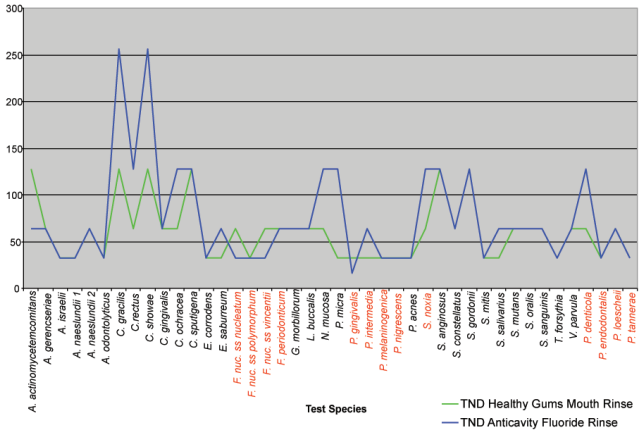

Table 3. Ingredients of The Natural Dentist Anticavity Fluoride Rinse

INGREDIENT	PURPOSE
Water	Base
Vegetable Glycerin	Soothing, Protective Barrier
Aloe Vera Gel[1,11]	Soothing, Moisturizer
Sodium phosphate monobasic	pH buffer
Xylitol	Non-cariogenic sweetener
Olivamidopropyl Betaine	Emulsifier
Natural Flavors	Flavor, Breath Freshening
Citric Acid	Preservative
Grapefruit Seed Extract[1,5]	Cleansing
Menthol	Breath Freshening
Active: Sodium Fluoride (0.05%)	**Anticavity**

CONCLUSION

The data from these *in vitro* studies indicate the effectiveness of The Natural Dentist Anticavity Fluoride Rinse in terms of fluoride uptake and antimicrobial activity. The pH of the test product appeared to have an impact on the fluoride uptake with a lower pH increasing levels of fluoride uptake. TND Anticavity Rinse was shown to be as effective as Phos-Flur in terms of fluoride uptake and showed superior fluoride uptake as compared to ACT. For consumers looking for a natural alternative to conventional rinses on the market, the ADA-accepted TND Anticavity Fluoride Rinse would be just as effective as these leading brands.

In addition, the ingredients in TND Anticavity Fluoride Rinse (Table 3) were effective in inhibiting the growth of oral bacterial species as compared to a positive control (TND Healthy Gums Mouth Rinse[7,8,9]). Therefore, when used by patients, the herbal fluoride mouth rinse may provide oral health benefits by inhibiting the growth of periodontal and cariogenic pathogens in the mouth. Further, it may serve as a natural antimicrobial mouth rinse alternative for those patients who want a product that does not contain artificial ingredients.

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