A COMPARATIVE STUDY OF THE EFFECTIVENESS OF MOUTHWASH, TOOTHPASTE AND CHEWING STICK AS INHIBITORS OF BACTERIAL GROWTH

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SUMMARY
Based on in vitro findings from previous work done on chewing stick, toothpaste and mouthwash a reduction in oral flora is expected after using each of them. A study was therefore conducted on the changes in oral bacteria of five (5) individuals who each of these agents for a week. Oral rinses from subjects at various intervals were collected and inoculated onto culture media. Chewing stick, a mouthwash and toothpaste regularly available on the Ghanaian market were tested. The effectiveness of each product was determined by the percentage reduction in bacterial counts with respect to counts before using the index agents.

Antibacterial activity was evident for all three agents. The findings of this study indicated that mouthwash is the effective antibacterial agent. Although chewing stick showed a slightly higher percentage reduction in bacterial counts than toothpaste a 2-tail sample-test showed that this difference was insignificant even at a 25% significance level. Further studies may have to be performed with a larger sample size and a longer duration of use to establish the results of this preliminary study. As to whether these levels of inhibition are healthy for an individual is also a matter worth investigating.

Keywords: Mouthwash, Toothpaste, Chewing stick, Oral bacteria.

INTRODUCTION
Our mouths are inhabited by millions of bacteria usually referred to as normal flora of the mouth since they can be beneficial to the health of the individual. To these microbes, the mouth is a warm place with abundance of food, conditions, which enables them, reproduce rapidly. When uninter-
rupted the situation normally leads to an ecological imbalance where the bacteria could cause damage to the teeth and gum.

Two major dental diseases are periodontal diseases and dental caries. Plaque formation contributes to their causation. Plaque is a mixture of bacteria and salivary materials that form on the teeth. It is a complex microbial community present on virtually every tooth surface and is surrounded by and embedded in a complex matrix of host-derived salivary glycoproteins and bacterial extra-cellular polymers. The bacterial composition of plaque varies according to the age of the individual, caries status, age of plaque, composition of diet and many others. The predominant dental plaque genera are Streptococci, Actinomyces, Fusobacterium, Neisseria, Haemophilus, Veillonella, Porphyromonas and Treponema.

If allowed to accumulate, the plaque causes inflammation of the gums resulting in periodontal diseases. Some of these bacteria in plaque convert sugar in the buccal cavity to acid. This acid decalcifies and dissolves the hard tissues of the tooth initiating the process of dental caries. Avoiding sugary diet is not enough to control plaque as oral bacteria grow at undiminished rate by using salivary glycoproteins as substrate. Good plaque control is difficult to achieve and sustain, therefore continual efforts are being made to find chemical methods of plaque control that would be less arduous.

Much work has concentrated on devising toothpaste and mouthwash preparations to facilitate tooth cleaning, since these are likely to be accepted by the population. In Ghana, “chewing sticks and sponges” derived from local plants of various kinds, have been widely used and accepted as tradi-

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tional methods of plaque control. There has been proof of the antimicrobial properties of these “chewing sticks and sponges”\(^5\). Also the addition of antimicrobial agents to toothpaste has been suggested as one possible method of aiding improvement of oral hygiene\(^3\). Studies on toothpastes indicate that a number exerted antimicrobial action against a range of microorganisms\(^4\).

The above *in vitro* findings may not result in reduction of salivary oral flora *in vivo*. On this basis, this study was conducted to find out which of these three products is the most effective antibacterial agent so as to provide a basis for educating Ghanaians on which mouth cleaning agent to adopt. Pepsodent toothpaste and corsodyl mouthwash commonly available on the Ghanaian market were evaluated. These were compared with chewing stick.

**METHODS**

**Cleaning Agents**

Splints measuring about (7 x 0.5) cm are cut from logs of the “Nsokodua” tree and used chewing sticks. The wood is soft, pale and slightly bitter. The name “Nsokodua” is given to two closely related species *Garcinia spp. G afzelii* which occurs in the forest savannah boundary of Ghana and *G epunctata* which is restricted to the wet forest\(^7\). One end of the stick is chewed and made into short bristles, which are then brushed across the surface of the teeth.

Pepsodent toothpaste (Unilever Ghana Ltd.), is next to the Nsokodua, in terms of popularity as agent for oral hygiene. It is a brand of toothpaste manufactured locally and used in the urban areas of Ghana. This toothpaste contains fluoride and some antibacterial agents though the specific agents have not been revealed for commercial purposes. Fluorides are known to have some bacteriostatic activity against plaque forming bacteria although their major function is caries control.

The mouthwash, Corsodyl (ICI Pharmaceuticals Ltd., England), contains 0.2% w/v chlorhexidine gluconate, an antimicrobial agent, which is equivalent to 1% w/v chlorhexidine gluconate solution B.P. Chlorhexidine is a cationic compound, which as a result of its electrical charge binds to oral surfaces, especially teeth and is slowly released in an active form into saliva\(^8\). It is suspected to interfere with the adherence of plaque causing bacteria to the teeth, thus reducing the rate of plaque accumulation\(^6\).

**Subjects and Specimen collection**

Students (2 males and 3 females) aged between 14-20 years, attending the SOS-GHIC School volunteered for this study. They are from the same social class with similar oral hygiene practices and all participated in the three tooth cleaning regimes. Subjects refrained from all forms of oral hygiene procedures the night before the study began.

During the first week of the experiment, the subjects used only chewing stick for mouth cleaning. On waking up in the morning, 10ml of phosphate buffered saline (PBS) was used to rinse the mouth and collected in sterile Falcon centrifuge tubes. Chewed stick, to produce bristles, was used to clean the teeth thoroughly for five minutes. The mouth was rinsed with water and thirty minutes after cleaning the mouth, a second rinsing with 10ml of PBS was carried out and collected to serve as a second source of oral microbes. The process was repeated every other day for three days, however, the initial rinsing with PBS before mouth cleaning, was done only on the first day.

The process was repeated using the toothpaste as the cleaning agent and the push/brush up standard method of tooth brushing was used.

A process similar to that of the first week was used but this time the mouthwash was used as the mouth-cleaning agent. As directed by its manufacturer, 10ml of the mouthwash was used to rinse the mouth for a period of one minute before being discarded. All samples were transported on ice to the laboratory and examined within two hours of collection.

**Laboratory Methods**

The number of bacteria in each sample was estimated using the spread plate method after ten-fold serial dilutions of the sample. The PCA plates were incubated anaerobically using anaerobic jars and gas paks (BBL). All the plates were incubated at 37°C for 22-24 hours. The plates with visible and countable bacteria colonies (usually 300 or less), from each sample were selected and counted with an illuminated Gallenkamp colony counter.

A second set of PCA plates, were incubated aerobically. The microbes were identified using Gram stain and API identification kits. Media such as blood agar, chocolate gar and MRS media were used to isolate specific bacteria for identification.
Date Analysis
In order to obtain a normal distribution, the total bacterial counts were converted to \(\log_{10}\) colony forming units (CFU) values. The mean bacterial counts were calculated and 0.9 confidence intervals were determined for each value. The 0.9 confidence level indicates that in very large number of samples, 90% of the intervals calculated are expected to contain the population mean. The \(\log_{10}\) CFU values obtained by the five subjects were compared using the percentage reduction in mean bacterial counts obtained on the third day of using each product. Finally a 2-tail sample test was carried out to find out if the percentage reductions in bacterial counts were significant. The significance test and the confidence intervals were calculated by using a graphical calculator (Casio TI-83).

RESULTS
The bacterial counts from PCA plates that were incubated aerobically were similar. Each of the three cleaning agents has inhibitory effects on bacterial growth on plates incubated anaerobically. A decrease in colony counts was observed after the subjects had used each product for one week. The means calculated fall within a 90% confidence interval.

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<th>Table 1 Changes in levels of buccal bacteria of subjects A-E after chewing stick for one week</th>
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The results are shown in Tables 1 to 3. The oral organisms identified include; *Clostridium spp.*, *Streptococcus spp.*, *Neisseria spp.* and *Actinomyces spp.*

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<th>Table 2 Changes in buccal bacteria of subjects A-E after using toothpaste for one week</th>
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DISCUSSION
A study was carried out on the changes in oral micro flora on five individuals, who used each of the following mouth cleaning agents; chewing stick, toothpaste and mouthwash for three consecutive weeks, using one each week. Previous work done on chewing stick and toothpaste involved growing microbes in media inoculated with extracts from these agents of oral hygiene. However, in this work, oral samples collected from subjects who used these cleaning agents were cultured instead. This was done in order to observe the effect these mouth-cleaning agents have on bacteria in the mouth.

The composition of dental plaque varies with age and diet. In order to ensure consistency in the oral flora being worked with, the subjects chosen belonged to the same age group (adolescents) and feed on the same diet. In spite of this, the micro flora of each individual is unique, therefore, each subject had to undergo all three mouth-cleaning regime, in order to observe the effect of each cleaning agent on a particular oral flora. A repeated measures cross-over design was therefore used. Also, for social reasons, it was impossible for the subjects to live through a week without using any form of mouth cleaning agent. This would have served as the base line, nevertheless, samples collected before each mouth-cleaning regime, served this purpose.

All subjects showed a fall in bacterial counts 30 minutes after using the chewing stick on the first day. The highest reduction was a 26% fall in initial counts by Subject D. Subject E registered a 20% increase of initial counts. Subsequent days also showed drops in counts as compared to the previous day except for C, who showed a minimal increase on day 3 as compared to day 2. On the last day of sample collection, day 3, there were percentage reductions ranging from 12% to 32% of initial counts. Considering the mean counts each day, a clear trend can be observed in which there is a con-
timous drop in counts till the third day. The final average count was about a 23% decrease of the initial average count.

This goes to support previous studies conducted on chewing sticks. In Ghana, out of five chewing stick species tested all had marked antibacterial activity. Similar results were obtained in Nigeria. Later investigations established that, chewing sticks possess antibacterial activity against Escherichia coli, Bacillus, Pseudomonas aeruginosa and Staphylococcus aureus. It suggested that the antibacterial activity of the chewing sticks was due to the presence of phenolic compounds. When toothpaste was compared to chewing stick, it was found that chewing stick was superior to toothpaste as an antibacterial agent. However, in this study there is not enough statistical evidence to make a similar conclusion. This could mean that the in vitro activity of chewing stick does not accurately reflect its activity in vivo or the sample size was not large enough. Also, considering the progressive rise in percentage reduction of bacterial counts with time, it is tempting to suppose that the effectiveness of chewing stick increase with prolonged usage.

Other factors apart from the fact that chewing sticks contain antibacterial compounds may have contributed to the percentage reduction in bacterial counts observed in this study. The slightly bitter taste of Nsokodua also serves as a stimulant for salivary flow. Saliva contains a potent enzyme, lysozyme, which kills bacteria by digesting the polysaccharides in their cell walls. Lysozyme is very effective even in low concentration so an increase in the secretion of saliva during chewing enhances the action of the chewing stick. Also, the abrasive actions of the stick fibres exert a mechanical cleaning effect on the teeth. It is also possible, that since chewing is done for a relatively longer period than tooth brushing, it allows for thorough cleaning and its antibacterial effect lasts longer. Available epidemiological evidence, however, would suggest that this mechanical cleansing action, even if combined with some antibacterial action, is not sufficient to prevent dental caries in the presence of a cariogenic diet.

The data for toothpaste also showed a fall in counts for all subjects on day 1. The highest percentage decrease in bacterial count on day 1, was about 28% by E. On day 2 however, with the exception of subjects C and D who showed falls in counts as compared to day 1, the rest showed some increase in counts as compared to day 1. Nevertheless, all the counts on day 2 showed substantial decrease as compared to the total count before using toothpaste. All subjects but E registered falls in counts on day 3 as compared to day 2. Subject E showed a minimal increase of 0.01 log$_{10}$ CFU. On day 3, the last day of sample collection, there were reductions in counts ranging from 3.6%-32% of initial count. On the average, there was a fall in counts as compared to initial counts. However, counts did not show a progressive fall as was shown by chewing stick due to a slight rise in mean counts on day 2. Nevertheless, the final average count showed a 20% fall with respect to the initial average count (Table 2). Toothpaste may prevent plaque formation by mechanism such as, interfering with bacterial adhesion to the tooth surface. The toothpaste being studied showed reduction in bacterial counts, which could be due to the action of an antibacterial agent in the paste. However, the abrasives and detergents in this product could have enhanced this. In addition toothpaste also makes brushing a pleasant experience, encouraging thorough cleaning.

For mouthwash, there was also a reduction in buccal counts for all subjects, with subject B, registering the greatest reduction of about 51% of initial counts, on day 1. There was progressive reduction in counts on days 2 and 3 except B and D on day 2 who showed counts quite higher than day 1. Also E showed counts on day 3 that was slightly higher than day 2. On the last day of sampling for the week, that is day 3, subjects registered decreases ranging from 12%-42% of initial count (i.e. before using mouthwash). On the average, a progressive fall in counts was observed from day 0 to day 3 with the reduction in final average count being 30% of initial average count (Table 3).

Mouthwash showed rapid reduction in bacterial counts 30 minutes after usage on Day 1. Though the percentage reduction rose in subsequent days, the rate of reduction however fell. Toothpaste also showed quite a high percentage reduction in bacterial counts on Day 1, but there was no significant rise afterwards. Although, chewing stick showed the least percentage reduction on the first day, it showed a progressive rise in percentage reduction afterwards.

Mouthwash has a much higher antibacterial activity as compared to toothpaste. This is due to presence of Chlorhexidine in the mouthwash, which has inhibitory effects on Gram negative and Gram positive bacteria by interfering with their metabolism. Whatever, the antibacterial agent in the toothpaste, the study has showed that it has less antimicrobial effect. Finally, these results obtained take into ac-
count how each mouth-cleaning agent is used. Mouthwash for example, is used without the aid of a toothbrush. Tooth brushing does not only clean the mouth mechanically, but it also increases the surface area for the action toothpaste. The mouthwash may therefore contain a more effective antibacterial agent than these results show, but due to the mode of usage (rinsing) its effect may have been limited.

In conclusion, all three mouth-cleaning agents commonly used in Ghana have antibacterial effects. Mouthwash is the most effective anti bacterial mouth-cleaning agent. Chewing stick showed a higher percentage reduction in bacterial counts than toothpaste. However, this difference is statistically insignificant. Chewing stick and toothpaste are therefore rated as equals in inhibiting bacterial growth.

Further studies with a larger sample size and longer period of usage are recommended to verify these findings. Further developments to enhance the antimicrobial action of the toothpaste in question, may be of benefit in Ghanaians. Also, higher concentrations of the active ingredient in chewing stick could be incorporated in toothpaste or mouthwash. In addition, whether the observed levels of bacterial inhibition are healthy for an individual is an issue worth investigating in later studies.

REFERENCES


