

# ***In-vitro* Assessment of the Antibacterial Quality of Some Commonly Used Herbal and Non-herbal Toothpastes on *Streptococcus mutans***

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**Abstract** In-vitro assessment of the antibacterial quality of four commonly used brands of herbal toothpastes in comparison with their corresponding non-herbal toothpastes on *Streptococcus mutans* was examined. Eight brands of commercially available toothpastes comprising of four herbal and non-herbal brands respectively were purchased in a local market in Awka South, Anambra State, Nigeria. The herbal brands selected were designated AH, BH, CH and DH and their respective non-herbal brands designated ANH, BNH, CNH and DNH served as positive controls. The use of distilled water served as negative control. The antibacterial activity evaluation was performed using agar well diffusion method. Different concentrations of toothpastes were made by 2-fold serial dilution method using sterile distilled water as diluents. Inhibition zones were measured in millimeters after 24hrs. Among the non-herbal toothpastes, triclosan containing toothpaste gave the highest zone of inhibition compared to non-triclosan toothpastes. The herbal toothpaste of each brand gave better inhibition result in comparison with their corresponding non-herbal brands. BH toothpaste gave maximum inhibition followed by AH, CH and lastly DH toothpastes. The result of this study indicates that herbal toothpastes are marginally better in inhibiting the growth of *Streptococcus mutans*; although both herbal and non-herbal toothpastes were equally effective in reducing pathogenic plaque and maintaining oral hygiene.

**Keywords** *In-vitro* Assessment, Antibacterial Quality, Herbal, Non-herbal Toothpastes, *Streptococcus mutans*

In Nigeria, as in other developing countries, very significant proportion of dental problems are due to dental biofilms generally known as dental plaque. Dental bio-films are a complex community of microorganisms found on the tooth surface embedded in a matrix of polymers of bacterial and salivary origin. There are several strategies for managing bio-films [1]. It has been reported that more than 500 bacterial strains which are opportunistic may be found in dental plaque [2]. Poor oral hygiene is one of the reasons for accumulation of these microbes and their harmful activities [3]. Tooth brushing with toothpaste is the most widely practiced form of oral hygiene in most countries [4]. The most common oral hygiene aid used to improve the oral health of an individual is the toothbrush [5]. Daily personal oral hygiene (tooth brushing and flossing) is recommended in the interest of good hygiene and for the control of dental plaque [6].

The success of any toothpaste, in part, lies in its ability to eliminate pathogenic oral microflora and deliver fluoride to the teeth which most effectively protects both deciduous and permanent teeth from dental caries [7]. However, fluorides are not the only active ingredients in toothpastes for overall test performance in oral hygiene. Also important are the cleaning abilities of toothpaste provided by abrasives, as the antibacterial qualities, which, in turn, are provided by a variety of substances with different abilities to inhibit the growth of microorganisms in the oral cavity, as well as a number of excipients which are incorporated with specific aims to solve specific problems [8].

The term surfactant is a blend of "surface active agents". Surfactants are usually organic compounds that are amphiphilic, that is, they contain both hydrophobic tails and hydrophilic heads. The hydrophobic and hydrophilic

## **1. Introduction**

moieties partition at the interface between fluid phases with different degrees of polarity and hydrogen bonding such as oil/water or air/water interfaces. Chemically synthesized surfactants are commonly used in petroleum, food and pharmaceutical industries as emulsifiers, wetting agents, cleaning agents and foaming agents. Alkyl sulfates are surfactants used in the production of some tooth pastes [9,10]. Among the many substances that inhibit growth of germs in toothpastes are triclosan, xylitol and flourides [11,12]. Nowadays, toothpastes are produced to serve multiple purposes at the same time and, thus, possess a complex chemical composition [13].

Natural toothpastes are those without triclosan or fluoride. They usually contain natural ingredients such as special mineral salts e.g. sodium fluoride and sodium chloride, and plant extracts like lemon, eucalyptus, rosemary, chamomile, sage and myrrh [14]. 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 mouth rinsers [15].

Hence, the aim of this study was to compare whether there was any significant difference in the antibacterial action between some herbal and non-herbal toothpastes used in Nigeria.

## 2. Materials and Methods

### 2.1. Isolation of Bacterial Culture

*Streptococcus mutans* was isolated from patients with dental caries attending Federal Dental Clinic, Enugu using sterile swab stick and dental probe. They were inoculated on sodium azide blood agar and identified through various standard microbiological and biochemical procedures [16-18].

### 2.2. Source of Toothpastes

Eight toothpastes comprising of four brands of non-herbal toothpastes and their corresponding herbal toothpastes were purchased from *Eke-Awka* local market, Awka South of Anambra State, Nigeria. They were designated AH, ANH, BH, BNH, CH, CNH, DH and DNH. The labeled compositions of the toothpastes are shown in Table 1.

### 2.3. Sample Preparation

Non-herbal and herbal toothpastes were liquified by equimolar mixing of the toothpaste in warm, sterile distilled water at the ratio (1:1 w/v). Thereafter, the solubilized toothpastes were subjected to two-fold serial dilutions and four working concentrations of 1.0, 0.5, 0.25, 0.125, 0.063g/ml were obtained.

### 2.4. Antibacterial assay

The antimicrobial activities of different concentrations of the toothpastes were determined using agar well diffusion method described by, *Agu et al.* (2013) and *Adindu et al.* (2016) [19,20]. Exactly 0.5ml of overnight culture of *Streptococcus mutans* adjusted to 0.5 Macfarland Nephelometer standard as described by *Cheesbrough* (2010) [18], was seeded on Mueller Hinton agar. The plates were allowed to dry for 1hr. Six wells each 6 mm diameter were made using sterile cork borers in the already seeded Mueller Hinton agar media. Aliquots (0.2ml) of the respective serially diluted concentrations were introduced into each of the five wells. Water was used as the negative control and was introduced into the sixth well. The assay plates were held at 4°C for 1 hour to repress the growth of the seeded culture while promoting diffusion of the oral agents. The plates were incubated at 37°C for 24hrs. The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition in millimeter.

**Table 1.** Ingredients of the Coded Toothpastes used in this Study

Coded Brands	Ingredients
AH	Sodium fluoride, Sorbitol, Aqua, Hydrated Silica, Sodium Lauryl sulfate, Aroma (flavour), Cellulose Gum, Sodium Saccharin, Aloe Barbadensis leaf extract, Limonene, Eucalyptus, Peppermint, Sage, Thyme, Aloe Vera Leaf Extract.
ANH	Sodium fluoride, Zinc sulfate, Sorbitol, Aqua (water), Hydrated Silica, Sodium Lauryl sulfate, Aroma (flavour), Cellulose Gum, Sodium Saccharin, Mica, Sodium Hydroxide, Glycerin, Eugenol.
BH	Sodium Monofluorophosphate, Calcium Carbonate, Aqua, Sorbitol, Sodium lauryl Sulphate, Hydrated Silica, Flavor, Cellulose Gum, Magnesium Aluminium Silicate, Sodium Saccharin, Sodium Bicarbonate, <i>Eucalyptus globulus</i> oil, Chamomilla recutita (Matricaria) flower extract, Metaleuca Alternifolia, (Tea tree) leaf oil, <i>Salvia officinalis</i> (sage) oil, Eugenol.
BNH	Sodium Monofluorophosphate, Calcium Carbonate, Aqua, Sorbitol, Sodium lauryl Sulphate, Aroma, Cellulose Gum, Sodium Bicarbonate, Tetrasodium Pyrophosphate, Benzyl Alcohol, Sodium Saccharin, Sodium Hydroxide, Limonene, No sugar.
CH	Sodium Fluoride, Aqua, Hydrated silica, Sorbitol, Glycerine, PEG-6, Aroma/flavor, Carrageenan, Xanthan Gum, Sodium Fluoride, Sodium Saccharin, Titanium Dioxide, <i>Eucalyptus globulus</i> , <i>Mentha arvensis</i> , <i>Salvia officinalis</i> , <i>Anthemis nobilis</i> .
CNH	Sodium Fluoride, Aqua, Hydrated Silica, Sorbitol, Glycerine, PEG-6, Sodium Lauryl Sulphate, Sorbitol, Glycerine, PEG-6, Sodium Lauryl Sulphate, Flavor, Xanthan gum, Sodium Saccharin.
DH	Sodium monofluorophosphate, Sorbitol, Silica, Sodium Lauryl Sulphate, Binder, Sodium Saccharin, Aqua, Calcium, Carbonate, Natural Colour, Flavour with Natural herbs extracts, preservatives.
DNH	Sodium monofluorophosphate, Sorbitol, Silica, Sodium Lauryl Sulphate, Binder, Sodium Saccharin, Aqua, Calcium, Carbonate, triclosan, Natural Colour

**Table 2.** Antibacterial activity of herbal and non-herbal toothpaste against concentration

CONC (mg/ml)	AH	ANH	BH	BNH	CH	CNH	DH	DNH
1	20	14	30	16	19	12	12	9
0.5	17	12	26	14	15	9	11	8
0.25	14	10	22	13	12	0	0	0
0.125	11	0	15	10	10	0	0	0
0.063	0	0	13	0	0	0	0	0

### 3. Results

The antibacterial effect showed that all the toothpastes used had growth inhibitory effect on *Streptococcus mutans*, and the negative control showed no activity. BH toothpaste brand gave the maximum inhibition zone compared to other toothpastes brand. This was followed by AH, CH and DH. The herbal toothpastes of each brands used gave higher inhibitory activity compared to their corresponding non-herbal toothpastes. The herbal toothpaste (BH) was effective even at 0.063g/ml giving 13mm than others (Table 2).

### 4. Discussion

*Streptococcus* spp are the most dominant bacterial species in dental plaque and the major pathogens of dental infection [4]. Amongst the Streptococci, *Streptococcus mutans* is recognized as the main opportunistic pathogen of dental caries which can demineralize the enamel. Poor oral hygiene is considered as a major reason for the accumulation and emergence of this harmful effect [3]. Equally, maintenance of good oral hygiene is the key to the prevention of dental diseases [3].

The most widely practiced oral hygiene method is tooth-brushing with toothpaste [21]. Toothpastes have been widely reported to have different antimicrobial components like triclosan, bromochlorophene, sodium lauryl sulfate (SLS), sodium mono-fluorophosphate (MPF), sodium fluoride (SF), xylitol [4,12,22]. Conversely, herbal toothpaste may not contain all these antimicrobial agents. However, irrespective of this property, herbal toothpastes have been in use for a fairly long period of time and are fast gaining popularity in most parts of Africa, Asia and Europe mainly because they are derived naturally and do not possess harsh chemicals present in most commercially available toothpastes [18]. Tea tree oil, propolis, garlic, neem lemon, eucalyptus, rosemary, chamomile, sage and myrrh and other herbs and spices have all shown proven antimicrobial effects against a range of oral bacteria, including the periodontal pathogens such as [14,23-24]. Many of these have been successfully incorporated into a number of dentifrices, including toothpastes, mouth-wash, mouth gargle, and gels.

Data from the present study show that all the herbal and non-herbal toothpastes used demonstrated wide variations in their activity against the test organism, a feature which may

have been largely due to their different antimicrobial active ingredients (Table 1). Evidently, Moran et al, 1988 [22] and Okpalaugo et al.2009 [14] evaluated the *in-vitro* antibacterial properties of many dentifrices and concluded that not even 50% of the dentifrices available commercially had antibacterial properties to benefit dental health or antiplaque action. This is contrary to the present study. This may be due to the changes in the manufacturing guidelines that were set after the study was conducted and the addition of newer substances in the dentifrices with better antibacterial and anti-plaque properties [1,25].

The toothpastes having the largest microbial inhibition zone and probably the strongest antimicrobial properties may not be necessarily superior to those found to have smaller zones of inhibition [15]. From the present study, the herbal brands gave higher zones of inhibition than the non-herbal toothpastes. This can be related to the presence of triclosan, sodium MPF and SLS in CNH products (Table 1). Triclosan [5-chloro-2-(2,4-dichlorophenoxy) phenol] has been used for more than 30 years as a general antibacterial [3]. Triclosan has been accepted by the FDA as an antiplaque-antigingivitis therapeutic additive to toothpastes [26]. Hence, toothpastes with triclosan and sodium mono-flouro-phosphate are more effective than toothpastes containing only sodium mono-flouro-phosphate or sodium fluoride as an active antibacterial ingredient. This was confirmed by research done in other comparative studies of non-herbal toothpastes [3-4,7,14].

CH brand gave better effectiveness against the test organism than the other brands of herbal toothpastes may be because of the greater range of different herbs contained in CH (Table 1).

Recently, several studies have shown 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 mouth-rinsers [15]. The herbal toothpastes used in this study gave better effectiveness against *Streptococcus mutans* than their corresponding non-herbal toothpaste brands. Shetty et al. (2015) [27] carried out a study of a comparative evaluation of the effect of a herbal dentifrice and a regular dentifrice on beneficial oral microflora and reported that herbal dentifrices are more effective in preservation of oral microflora when compared to regular dentifrices, although both were equally effective in inhibiting the pathogenic microorganisms. Some researchers Ozaki et al., 2006 [25], Tatikonda et al., 2014 [28], George et al., 2009 [29] had

reported that herbal dentrifice was as effective as non-herbal dentrifices in the control of plaque and gingivitis. Saxena et al. (2011) [30] found in an *in-vitro* study that herbal dentrifices showed a maximum inhibition zone.

Nevertheless, a contrary report by Peck et al. (2011) [23] on the study of an *in-vitro* analysis of antimicrobial efficacy of herbal toothpaste on selected primary plaque colonizers showed that non-herbal toothpaste gave better inhibition zone than herbal toothpastes used except for Dentazyme herbal toothpastes which have similar antimicrobial efficacy with the CH. This present study showed that all the herbal toothpaste used were more effective against the test organism than their corresponding non-herbal toothpastes brands. The difference in reports of these two studies may be due to different herbal compositions and ingredients of the toothpastes used in the two studies, as well as geographical environmental factors.

## 5. Conclusions

From the data obtained in the study, it could be deduced that herbal toothpaste show better inhibitory activity against the growth of *Streptococcus mutans* than the non-herbal counterpart in reducing pathogenic plague and maintaining oral hygiene. It is recommended that further in-vitro and in-vivo studies be carried out on commercially available toothpastes widely used in Nigeria.

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