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Astringency as Antisensitivity Marker of Some Nigerian Chewing Sticks

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Astringents contract the tissues and canals of the body. Chewing sticks are used for oral hygiene both as an antibacterial and desensitizing agent. Astringency of cold water extracts of *Afzelia africana* Sm. ex Pers. (Caesalpiniaceae), *Dialium guineense* Wild. (Fabaceae), *Masularia acuminata* (G.Don) Bullock ex. Hoyle, *Rauwolfia vomitoria* Afz. (Apocynaceae), *Terminalia glauscens* Planch. (Combretaceae), *Vernonia amygdalina* Del.(Asteraceae) and *Zanthoxylum zanthoxyloides* (Lam.) Waterman (Rubiaceae) was measured by precipitating extracts with hemoglobin, centrifugation and loss of absorbance measured spectrophotometrically at 578 nm relative to tannic acid as Tannic Acid Equivalents (TAE). Total Tannin (TT) was determined using the protein tannin precipitation method and Total Phenols (TP) with Prussian blue. Relative Astringency (RA) was astringency of tannin present relative to tannic acid. Activity was in the order of *Afzelia>Terminalia>Zanthoxylum>Masularia>Vernonia>Rauwolfia>Dialium.* TT ranged from 106.92±0.03 mg/100 g dry plant in *Dialium* to 632.86±0.42 mg/100 g dry plant in *Afzelia*. TAE was 27.37±0.07 mg/100 g dry plant in *Dialium* to 148.11±0.07 mg/100 g dry plant in *Afzelia*. RA correlated positively with TAE (R^2 = 0.8763) and TT (R^2 = 0.9493). Desensitization may be due to the astringent activity as these extracts will form a protective layer on the exposed dentine; contract/block the tube like channels that pass through teeth and connect to nerves thereby reduce the ability of the nerves to transmit pain.

Key words: Teeth sensitivity, chewing sticks, astringency, antisensitivity marker

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INTRODUCTION

Gum disease may be an indication of poor general health or weak immune system which allows bacteria to thrive. People have teeth sensitive to acid from eating citrus fruits, air, heat, cold water and some unexplained factors. Bacterial invasion of dentin may be one of the causes of hypersensitivity and sensitive teeth may also develop as a result of inflammation induced sensitization of the nerves in the pulp-dentin border in teeth with open tubules. The open dentinal tubules associated with sensitivity invite penetration of bacteria (Narhi *et al*., 1992). At least 250 million adults suffer from sensitive teeth around the world: that's about 40% of the population in the areas studied More women notice sensitive teeth, than men; 48% female/38% male (GST, 2002). This may be because women brush their teeth more often and possibly more vigorously, leading to gum recession. The study also found sensitivity is not just a problem of old age-all ages groups can have sensitive teeth. In fact, with changing diets, especially the growing popularity of snack foods and soft drinks, sensitivity is increasingly felt by younger age groups. Dentine may also be exposed as a result of gum recession or gum disease. Stress, as a result of teeth grinding will wear away enamel. Unfortunately some people grind their teeth unconsciously while asleep. Acidic food and drink can cause erosion of tooth enamel and this is often exacerbated by over-enthusiastic tooth brushing. This can expose the underlying dentine which is sensitive to hot and cold.

Astringents contract the tissues and canals of the body. Teeth sensitivity is manifested by occasional discomfort or pain when eating or drinking cold, hot or acidic food, or brushing the teeth. As a result of dentine exposure, the nerve in the centre of the tooth becomes susceptible to stimuli and responds with a short sharp pain. The earliest known reference to a toothpaste is in a manuscript from Egypt in the 4th century A.D., which prescribes a mixture of powdered salt, pepper, mint leaves and iris flowers. The Romans used toothpaste formulations based on human urine. All these were in use before Colgate began marketing the first commercial brand in 1873.

The Babylonians recorded the use of chewing sticks in 7000 BC and its use ultimately spread throughout the Greek and Roman Empires. Chewing sticks were also used by Egyptians, Jews and in the Islamic Empires (Almas, 2001). Chewing sticks are now widely used in Africa, South America, the Middle East and Asia as a means of maintaining oral hygiene. They are made from the roots, twigs, or stem of a plant. The appropriate parts depending on the plant material are cleaned with water to remove dirt, cut to a convenient length which varies from 15-30 cm long. The user holds one end directly in his mouth and chews it into a fibrous brush-like fringe, which is used to scrub the surfaces of the teeth. A combination of vertical and horizontal strokes of the brush like end on tooth surfaces removes plaque and water soluble active constituents of the chewing sticks act as the cleansing agent. The tongue is scrubbed as well. Cleansing movement is directed away from the gingival margin to avoid induced recession and undue damage to the gums. Chewing sticks are used in the mornings before breakfast and at night after supper for daily oral hygiene maintenance (El-Said *et al*., 1971; Almas and Al-Lafi, 1995; Aderinokun *et al*., 1999; Almas, 2001; Ndukwe *et al*., 2005; Elujoba *et al*., 2005). In certain parts of West Africa e.g. Senegal, chewing sticks are used frequently during the day as well. *Zanthoxylum zanthoxyloides* (Lam.) Waterm. root imparts tingling and peppery taste sensations and numbness in the mouth; *Masularia acuminata* (G. Don) Bullock ex Hoyle stem, produces a strong bitter taste and frothing; an initial bitterness and later sweet taste by *Vernonia amygdalina* Del. root (Tella, 1976) while *Terminalia glaucescens* Planch. root gives a discolouration of the mouth after use (Sofowora, 1993). Other oral/dental conditions treatable with plants are toothache/decay, teeth sensitivity, gingivitis, ulcerative gingivitis, angular stomatitis, mouth ulcers, swollen tonsil, oral thrush, tonsillitis and black tongue (Hollist, 2004).

Several studies have been reported on the antibacterial/antifungal effects of chewing sticks on cariogenic bacteria inhibitory action on dental plaque formation, showing that all of them were active against the oral microbial flora in varying degrees (El-Said *et al*., 1971; Fadulu, 1975; Akpata and Akinrimisi, 1977; Odebiyi and Sofowora, 1979; Wolinsky and Sote, 1983; Rotimi and Mosadomi, 1987; Sofowora, 1993; Sote and Wilson, 1995; Almas and AL-Lafi, 1995; Sathananthan *et al*., 1996; Wolinsky *et al*., 1996; Aderinokun *et al*., 1999; Taiwo *et al*., 1999; Almas and Al-Zeid, 2004; Ndukwe *et al*., 2005).

However, a review of literature did not show any previous investigation into the use of chewing sticks for teeth sensitivity despite the changing diets in Nigeria, especially the growing popularity of snack foods and soft drinks. The present study assessed the potential of using astringency as antisensitivity marker of chewing sticks.

MATERIALS AND METHODS

Collection of plant materials and preparation of extracts: The plant materials *A. Africana*, *D. guineense*, *M. acuminata*, *R. vomitoria*, *T. glauscens*, *V. amygdalina* and *Z. zanthoxyloides* were purchased as chewing sticks

samples tied into small bundles from sellers in Mushin open market, Lagos, Nigeria in January 2003. The Forest Research Institute of Nigeria (FRIN) herbarium confirmed the species identity. The samples were cut into small pieces and dried at 50°C. Further disintegration was achieved with household wooden mortar and pestle. One hundred gram of sample was weighed and extracted by pulverizing with distilled water in a blender. Extracts were filtered and filtrate centrifuged. The pH of the supernatant was determined and kept at -4°C.

Determination of Total Tannin (TT): The amount of tannin present was determined using standard protein precipitation method as in the African Pharmacopoeia, 1986.

Determination of Total Phenols (TP): The original Prussian blue assay for total phenols (both tannin phenol and non tannin phenol) as described by Price and Butler (1977) was used with Ferric chloride as the first reagent and distilled water as blank.

Determination of astringency as Tannic Acid Equivalent (TAE): This was determined spectrophotometrically as earlier described by Odukoya *et al.* (2001). In brief, fresh blood collected from a cow at slaughter was diluted 1:50 with distilled water. The hemoglobin solution was centrifuged free of debris. One milliliter of the extract was added to a centrifuge tube, 1 mL of distilled water was added and mixed. One milliliter of the diluted haemoglobin solution was also added, mixed for 15 sec and then centrifuged for 10 min. A blank was prepared without any plant extract. Absorbance of the supernatant solution was recorded at 578 nm. The extracts were replaced with solutions of known concentrations of tannic acid and absorbance also recorded at 578 nm. The standard curve obtained from this data was used to determine the equivalent amount of tannic acid present in the chewing sticks extracts obtained from three replicate assays. Results were reported as mean TAE±SEM.

Determination of Relative Astringency (RA): This represents the astringency of tannin present relative to tannic acid.

RESULTS AND DISCUSSION

Activity was in the order of *Afzelia>Terminalia>Zanthoxylum>Masularia>Vernonia>Rauwolfia>Dialium*. TAE correlated with TT (R^2 = 0.8130), RA correlated positively with TAE (R^2 = 0.8763) and TT (R^2 = 0.9493) respectively. *D. guineense* has the highest TAE, lowest TT, TP and RA, while *A. africana* had the highest TT, TP and Moderate TAE and highest RA. The higher the extracts are in tannins, the lower its acidity (Table 1). A high RA is an indication of strong astringency. The less tannic an extract is, the more acidity it can support, the combination of high acid and high tannins gives the most astringent. High astringent extracts will produce greater desensitization, thus astringency could be an index for antisensitivity. Extraction ratio (tannin to total phenols) was agreeable (Ahmed *et al.*, 2005).

The assay for total phenols measures phenolic acids, flavonoids and tannins and is based on the reducing power of the phenolic hydroxyl groups (Hann *et al.*, 1984). While astringency measures protein precipitating phenols in terms of Tannic Acid Equivalents (TAE), this assay depends on measurement of phenol and depends on both tannin content and structure (Martin and Martin, 1983). Phenolic compounds undergo characteristic reactions due to the chemistry of the phenolic functional group. There is tendency for the phenolic group to be oxidized thus the reduction of hemoglobin for quantitative analysis.

Tannins contribute to astringency (most significantly) and bitterness. Bitter perception is quite well understood, since it is one of the five primary tastes and is sensed by a specific receptor found in taste buds on the tongue and soft palate. Astringency perception is much less well understood: the common understanding is that it is actually mediated by the sense of touch rather than by taste. Tannins taste astringent because they bind with salivary proline-rich proteins and precipitate them out. This leads to a reduction in permeability of cell membranes and hardening of the cement substance of the capillary endothelium. Muscle contraction and tissue wrinkling accompanies this effect, with increased friction between mouth surfaces and a sense of dryness or roughness.

Table 1: Analysis of chewing sticks extracts

Extract	TT mg/100 g	TP mg/100 g	TAE mg/100 g	TT/TP Ratio	RA
A. africana[r]	632.86±0.42	846.02±0.03	148.11±0.07	1.3	4.3
T. glaucens[r]	368.52±0.23	426.77±0.21	118.42±0.63	1.2	3.1
Z. zanthoxyloides[r]	321.61±0.11	391.46±0.08	121.51±0.26	1.2	2.6
M.acuminata[s]	296.98±0.72	342.94±0.28	139.21±0.01	1.2	2.1
V. amygdalina[s]	188.59±0.47	256.33±0.17	94.86±0.18	1.4	1.9
R. vomitoria[r]	169.36±0.33	206.83±0.30	242.83±0.67	1.2	0.7
D. guineense[s]	106.92±0.03	132.44±0.13	277.37±0.07	1.2	0.3

r = root; s = stem

In dentine hypersensitivity, lesions exhibit patent tubules at the exposed dentine surface and appropriate stimuli trigger pulpal nerves via a hydrodynamic mechanoreceptor mechanism to produce a typically short, sharp, painful response (Martin, 2002). Desensitizing toothpastes work in one of two ways, to relieve the pain of sensitive teeth, depending upon its active ingredient: Potassium Nitrate or Strontium Chloride. Potassium works by calming nerves to stop the sensitivity associated with trigger activities while Strontium Chloride helps block the tubules, preventing the nerve from being stimulated. It is proposed that the desensitization may be due to the astringent activity, as these extracts will form a protective layer on the exposed dentine; contract/block the tube like channels that pass through teeth and connect to nerves thereby reduce the ability of the of the nerves to transmit pain as with strontium chloride. Alum a mineral astringent was used as a toothpaste ingredient in 1800. Extracts of these chewing sticks can be incorporated into toothpastes or used as mouth washes.

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