

The effect of crude extracts of nine African chewing sticks on oral anaerobes

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Summary. Chewing sticks are widely used in Nigeria for dental and oral hygiene. In-vitro susceptibility tests were done with crude extracts from nine popular sticks on four species of *Bacteroides*. *Serindeia warneckei* chewing stick had the greatest and most consistent inhibitory effect on the four species; extracts from bark and pulp were bactericidal at concentrations of $\leq 1\%$. Extracts of other sticks, when inhibitory, were only so at higher concentrations—in the range 2–30%. All the black-pigmented oral anaerobes were very susceptible to eight of the nine chewing-stick extracts but non-pigmented anaerobes showed variable susceptibilities.

Introduction

Dental plaque formation and gingivitis are associated with the early stages of development of periodontal disease (Löe *et al.*, 1965; Syed and Loesche, 1978). There are numerous reports of studies on the microflora of the gingival crevice, dental plaque and chronic destructive periodontal disease. Most stress the importance of anaerobes in the aetiology of periodontal disease (Gibbons *et al.*, 1963; Loesche *et al.*, 1972; Kelstrup and Theilade, 1974; Slots, 1977; Duerden, 1980; Rotimi and Mosadomi, 1983). Clinical studies by Hine (1950) showed that the acquisition and maintenance of this microflora are associated with the accumulation of debris, leading to inflammation of the gingiva and subsequent infections. Others have corroborated this observation and shown that when bacterial deposits are removed the gingival inflammation subsides (Ramfjord and Kiester, 1954; Waerhaug, 1955).

The two methods employed by Nigerians to remove this debris are by tooth brush and paste, or by use of parts of various plants native to West Africa, referred to as “African Chewing Sticks”. About 80–90% of the Nigerian population use chewing sticks, mainly because they are readily available, cheap and efficacious. A few use a combination of the two methods.

Medicinal properties associated with gum healing, analgesia, antisickling, haemostasis and astringence have been attributed to chewing sticks, as well as the possession of antimicrobial and plaque inhibiting effects (El-Said *et al.*, 1971; Isaacs-Sodeye

et al., 1975; Wolinsky and Sote, 1983 and 1984; Rotimi *et al.*, in press). Saliva-extracted “factors” obtained by chewing the end of the sticks produce an inhibitory effect on certain oral pathogens associated with the development of dental caries, gingivitis and other periodontal diseases (Enwonwu, 1974; Akpata and Akinrimisi, 1977; Rotimi *et al.*, in press). In an earlier study on the anaerobic bacterial flora of the gingival crevice of adult Nigerians we reported that some African chewing sticks removed the black pigmented *Bacteroides* spp. of the normal flora (Rotimi and Mosadomi, 1983); other *Bacteroides* spp. appeared to be unaffected. This suggests the presence of antimicrobial substances specifically active against black-pigmented anaerobes. We report here the in-vitro susceptibility of some oral *Bacteroides* spp. to crude extracts of nine different chewing sticks. The different parts of each stick were also investigated to determine where the inhibitory substances may reside.

Materials and methods

Bacterial strains

Three reference strains of *Bacteroides* spp. and five strains isolated in our laboratory from clinical specimens were used as the test strains. The reference strains were *B. oralis* VPI 9958, *B. melaninogenicus* WPH 125 and *B. gingivalis* ATCC 33277; the local isolates were one *B. oralis* (gingival crevice), two *B. melaninogenicus* (gingival crevice) and two *B. asaccharolyticus* (gingival crevice).

Culture media

Kanamycin-blood agar (Blood Agar Base No. 2,

Table I. Chewing sticks commonly used in Nigeria

Botanical names	*Yoruba native name	Plant part used as chewing sticks
<i>Serindeia warneckeii</i>	Meyinro	Stem
<i>Fagara zanthoxyloides</i>	Orin Ata	Root
<i>Distemonanthus benthamianus</i>	Ayan	Root and Stem
<i>Massularia accuminata</i>	Pako Ijebu	Stem
<i>Anogeissus leiocarpus</i>	Pako dudu	Root
<i>Vernonia amygdalina</i>	Ewuro	Root and Stem
<i>Bytyrospermum paradoxum</i>	Igi Emi	Root
<i>Terminalia glaucescens</i>	Pako pupa	Root
<i>Nauclea latifolia</i>	Egbo Egbesi	Root

* Yoruba is one of the three languages spoken in Nigeria.

Oxoid, with lysed human blood 5% and kanamycin 75 µg/ml), blood agar, steamed Brain Heart Infusion Broth (BHI; Oxoid) to which menadione 1 µg/ml and L-cysteine hydrochloride 75 µg/ml were added, and steamed Robertson's cooked-meat broth, were used.

Chewing sticks

Nine chewing sticks commonly used in Nigeria are shown in table I, together with their native Yoruba (largely South-West Nigeria) names and the plant parts used as chewing sticks. All were bought in the open Nigerian market.

Preparation of crude extracts

Extracts were obtained from the bark, the pulp and the whole stick (i.e., the bark and pulp). The bark was peeled with a sharp knife and chopped into small pieces. The pulp and the whole stick were similarly chopped. Each part was weighed and stored separately in 10-g amounts in clean wide-mouthed 250-ml screw-capped bottles. Extracts were made by grinding the contents of a bottle in a pestle and mortar, adding 100 ml of sterile deionised distilled water to the resultant fibres and allowing them to soak for 48 h in a cold room (4°C), before decanting the fluid and centrifuging it at 2000 g for 10 min. The supernate was passed through a 0.45 µm membrane filter (Millipore Corp., Bedford, MA, USA). Extracts were stored in 5-ml portions at -20°C for no longer than 1 week.

Tests for inhibitory action

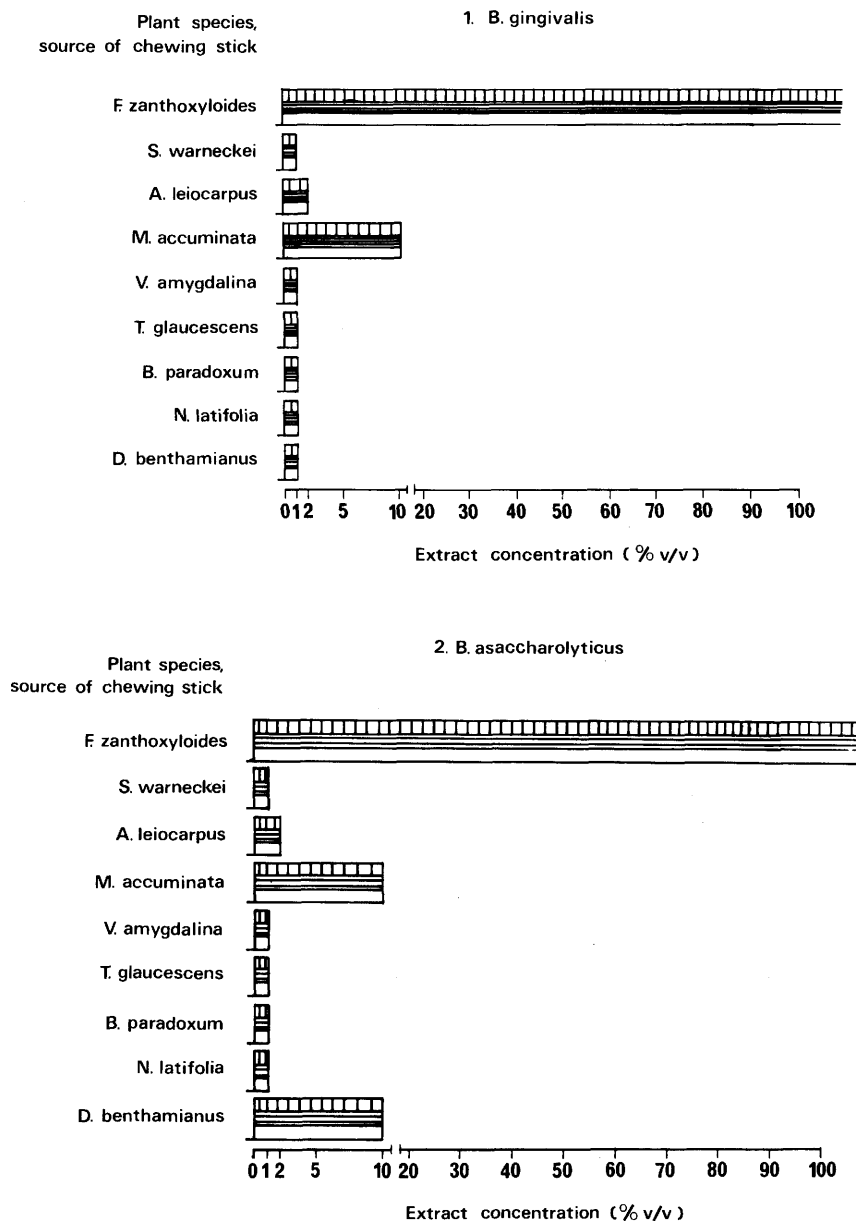
Bactericidal activity was determined for each set of extracts against reference and clinical isolates of the four *Bacteroides* spp. Each extract was diluted with pre-reduced BHI to give final concentrations of 80%, 50%, 10%, 2% and 1% (v/v). Each extract dilution was seeded with 0.1 ml of a BHI-broth culture of each test strain diluted in sterile distilled water to give $c.10^5$ cfu/ml. The

bottles were incubated in anaerobic jars with a gas generating kit system (Oxoid) which generated approximately H₂ 90%, CO₂ 10%. The jars were controlled biologically by including in each jar a culture of *Pseudomonas aeruginosa* seeded on to a slant of Simmon's citrate agar. Incubation was at 37°C for 24 h. *B. fragilis* NCTC 9343 was included in each experiment as a control. Bottles containing broth-free extracts and another containing broth only were seeded with the control strain and incubated along with each batch of tests. After incubation, the highest dilution that prevented visible growth of the test strains was taken as the inhibitory concentration. All tubes that showed no growth were subcultured on kanamycin-blood agar and plain blood agar and the bactericidal concentration was determined by the highest dilution at which there was no visible growth on solid media.

Results

Figs. 1-4 summarise the various effects of crude extracts of different components of nine chewing sticks on *Bacteroides* spp. found in the oral cavity. These effects were measured by relative inhibitory concentrations and relative bactericidal concentrations; both values were the same in most of the tests and, in the few instances when they were not, there was only a difference of one dilution. All the local isolates tested had the same susceptibility as their corresponding reference strains to each extract.

The relative bactericidal effects of the different extracts against *B. gingivalis* are shown in fig. 1. Extracts of eight of the nine sticks were bactericidal at concentrations of $\leq 10\%$. However, *F. zanthoxyloides* did not inhibit the growth of the organism after incubation for 24 h. Essentially similar effects were produced by extracts of the same sticks on the other black-pigmented species, *B. asaccharolyticus* and *B. melaninogenicus* (figs. 2 and 3). However, the

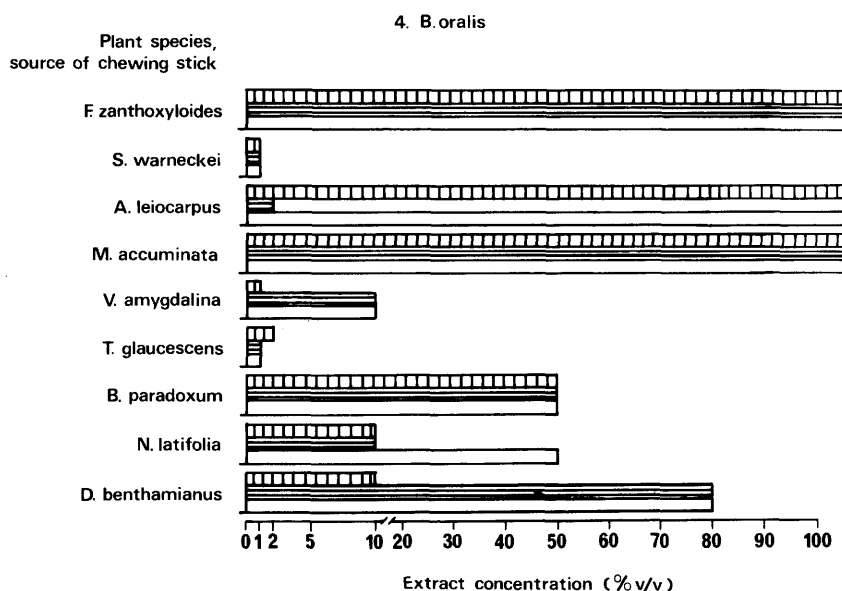
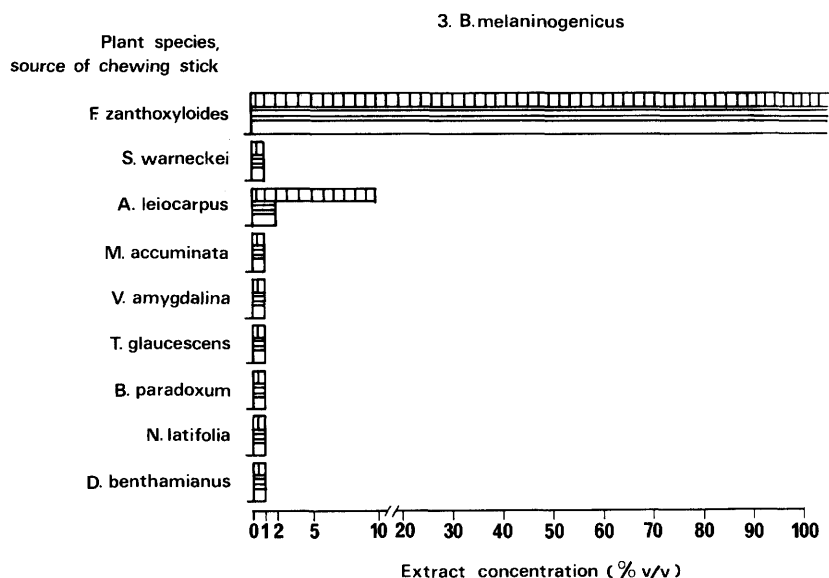


Figs. 1-4. Growth of oral *Bacteroides* strains in the presence of crude extracts of chewing sticks. Bars indicate growth up to the concentrations shown: ■ whole-stick extract; ▒ bark extract; □ pulp extract.

extracts of the whole stick and of the pulp of *A. leiocarpus* killed *B. melaninogenicus* at concentrations of 2%, whereas the bark extract was bactericidal only at 10%. Extracts of *F. zanthoxyloides* had no effect on the growth of either species.

Fig. 4 shows the effects of the chewing-stick extracts on another related but non-pigmented oral anaerobe, *B. oralis*. Extracts of *S. warneckei* and *T. glaucescens* and of the bark of *V. amygdalina* were

capable of killing *B. oralis* at concentrations of $\leq 2\%$. This species was also inhibited by *B. paradoxum* extract at a concentration of 50%. Separate extracts of the bark and pulp of *A. leiocarpus* were not inhibitory whereas an extract of the whole stick killed the organism at a concentration of 2%. *B. oralis* was completely resistant to extracts of *F. zanthoxyloides*, *M. acuminata* and only killed by *D. benthamianus* at a concentration of 80%.



Discussion

Nigerians use chewing sticks for their mechanical cleansing effect. The choice of stick depends largely on traditional preference rather than clinical effectiveness. The lower incidence of dental caries amongst users of chewing sticks (compared to non-users) had been attributed to the superior mechanical cleansing action on the teeth (Enwonwu, 1974). A few earlier reports, however, demonstrated the

antimicrobial properties of some of these sticks (El-Said *et al.*, 1971; Akpata and Akinrimisi, 1977). Our results confirm and extend these reports and show that *S. warneckei* has the most consistent inhibitory effect on all the *Bacteroides* spp. tested.

Extracts of *F. zanthoxyloides* were completely inactive against all the anaerobes tested. This observation was in agreement with that of Wolinsky and Sote (1983) who reported on the lack of effect of *F. zanthoxyloides* on the adherence and growth of

the oral opportunist pathogen, *Streptococcus mutans*. Although this stick is used by Nigerians for dental hygiene its medicinal property such as the anti-sickling effect on red blood cells (Isaacs-Sodeye *et al.*, 1975) has wider implications and has popularised its use, particularly among those with sickle-cell disease.

The high degree of sensitivity demonstrated by the black-pigmented anaerobes to all the sticks except *F. zanthoxyloides* is interesting. The patterns of inhibition of the three species were essentially identical; when there was any variation it was usually not more than one dilution. In contrast, *B. oralis*, a non-pigmented oral anaerobe, had a different sensitivity pattern; it was moderately sensitive to extracts of only three sticks. This may explain why black-pigmented strains are not commonly found in the gingival crevice of adult Nigerians who use chewing sticks for oral hygiene (Rotimi and Mosadomi, 1983) and may also partly explain the rarity of dental caries and gingivitis amongst the users of native chewing sticks for oral hygiene (Manley *et al.*, 1975).

Until recently the active principle of these chewing sticks was not known. However, results of previous studies have shown that it is heat stable (Rotimi *et al.*, in press) and soluble in methanol (Wolinsky and Sote, 1984). The recent report by Wolinsky and Sote (1984) has identified the active principles as tannin-like substances belonging to a large group of non-dialysible polyphenols of varying molecular weights. These tannins are said to be similar in quality but not necessarily in quantity in all chewing sticks.

Tannin-like substances are present in the bark and pulp of dicotyledonous plants and earlier reports have shown that they inhibit bacterial growth and are capable of protecting certain plants against bacterial infection (Uritani, 1971; Van Sumere *et al.*, 1975). Our studies described here confirm the presence of material possessing antibacterial activity in extracts of such plants and we believe that the heat-stable polyphenol tannins reported earlier to be present in African chewing sticks are responsible for this activity. However, we believe that the quantities of the tannins in the bark and pulp of each species may vary.

The varying susceptibility of each anaerobic species may be a function of the available binding sites on the bacterial cell walls; these are probably bacterial surface proteins (Gibbons and Qureshi, 1979). Tannins have been shown to form irreversible complexes with proline-rich proteins (Hagerman and Butler, 1981) which would lead to inhibition of cell-wall-protein synthesis, a property that may explain the mode of action of these chewing-stick extracts. We recommend, as an alternative to either the use of extracts as mouth rinses or the traditional use of the stick for oral hygiene, the incorporation of tannins and tannin-like substances extracted from some of the potent African chewing sticks, e.g., *S. warneckei*, into toothpaste. A double blind trial of conventional toothpaste versus that containing chewing-stick tannins should be done.

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