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Antimicrobial Activities of some Nigerian Chewing Sticks

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ABSTRACT

The antimicrobial activities of the ethanolic extracts of three Nigerian chewing sticks, namely, *Terminalia glaucescens*, *Anogeissus leiocarpus* and *Pseudocedrela kotschyi* were investigated. Results from this study showed that the antimicrobial activities of the tested chewing sticks vary and are target-microbe specific. Of the tested chewing sticks, *A. leiocarpus* showed a significantly higher antibacterial activity ($P<0.05$) against Staphylococcus *aureus* and *Streptococcus pyogenes*; and this was closely followed by *T. glaucescens*, while *P. kotschy*i virtually had no activity against these two organisms. However, the activity of *T. glaucescens* against *Streptococcus mutans* was significantly higher ($P<0.05$) than that exhibited by *A. leiocarpus*. The extracts of the three chewing sticks had no activity against *Candida albicans*. The antibacterial activities of these two potent chewing sticks made them suitable for better dental care.

INTRODUCTION

Utilization of non-timber forest products (NTFP) is gaining importance in the tropical world because of their commercial importance to the host community (Akande and Hayashi, 1998). These authors further stated that chewing sticks are important NTFP widely used for dental cleaning in the tropical West Africa.

Buada and Boakye-Yiadom (1973) stated that these chewing sticks impact varying taste sensation; a tingling peppery taste, a bitter taste and numbness is provided. Enwowu (1997) posited that, chewing sticks, in addition to providing mechanical stimulation of the gums, also destroy microbes; these advantages of the chewing sticks over the conventional toothpaste and brush has been attributed to the strong teeth of Africans (Ugoji *et al*, 2000).

The choice of chewing sticks to be used in most cases depends on its cleansing action of the teeth; the therapeutic value, or preferred taste or flavour. The sticks (which may be stem or root with bark removed or retained) are cut to convenient lengths and washed thoroughly with fresh water to get rid of the earth or any dirt. The diameter should afford good grip, say between 0.5-1.30 cm. Akande and Hayashi (1998) reported that some of the chewing sticks being used are obtained from the following plants: *Garcinia manni, Masularia accuminita*, *Terminalia glaucescens*, *Anogeissus leiocarpus, Pseudocedrela kotschyi*, *Xanthoxyllum gilletti* and *Azadiracta indica.*

Investigations carried out on some of these chewing sticks showed that they posses antimicrobial activity against oral microbial flora such as *Staphylococcus aureus* and *S. auricularis* (Akande and Hayashi ,1998), *Candida albicans, Aspergillus flavus, Microsporium gypseum* and *Trichophyton metagrophytes* (Adekunle and Odukoya, 2006).

The whole of Nigerian landscape is blessed with an abundant supply of the plants from which chewing sticks are obtained. These indigenous raw materials need to be processed and packaged for local consumption and perhaps for export. This become more imperative as Indian product-Darbur herbal toothpaste has become a household name in Nigeria.

It must be stressed that the development of virile herbal toothpaste is consequent upon the bioactivity of the constituent chewsticks against a wide range of oral pathogens. Hence the aim of this paper is to report the antimicrobial activity of the ethanolic extracts of three Nigerian chewing sticks; *Terminalia glaucescens, Anogeissus leiocarpus* and *Pseudocedrela kotschyi* on oral pathogens such as *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus mutans* and *Candida albicans*.

MATERIALS AND METHODS

The experiment involved three types of chewing sticks, four different concentration of extracts, three bacteria and one fungus, and three trials.

Source of plant materials

The chewing sticks selected were obtained from the roots of *Terminalia glaucescens* and *Anogeissus leiocarpus* and the branch of *Pseudocedrela kotschyi*. They were all collected from Saki in Oke- Ogun area (a woody savanna vegetation) of Oyo state, Nigeria. The plant parts were authenticated by a Botanist at the Department of Biology, The Polytechnic, Ibadan. Nigeria.

Source of microorganisms

Pure cultures of *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus mutans* and *Candida albicans* isolated from patient with dental diseases were obtained from the Medical Microbiology Department of the University College Hospital (U.C.H) Ibadan, Nigeria. Bacterial cultures were maintained on Blood agar slant and the fungus on Potato dextrose agar slant.

Extract preparation

The root and branch of the test plant were well dried and then grinded to powder. About 200g of the powder were separately soaked in 400ml of 95% ethanol in a 500ml reagent bottle and stoppered. This was allowed to stand for 14 days to permit full extraction of the active ingredients. The fluids were then filtered using Whatman No1 filter paper. The extracts were rotary dried to obtain the concentrate. It was then kept in fridge prior to use. A 2.0g/l solution of each extract was prepared and fractionated into 0.4g/l, 0.2g/l and 0.1g/l concentrations needed for the bioassay.

Bioassay

The method reported by Akande and Hayashi (1998) was employed for the antimicrobial testing. The bacterial strains were subcultured three times in the fresh media (Bacto *Staphylococcus* base medium) stocks to obtain a more vigorous population. However, potato dextrose agar was used for the assay on *Candida albicans*. Fresh stocks were also used to seed the 20ml no-agar medium and incubated at optimal temperature of 35^0C for 60 h. A 0.2-0.5ml portion of the new culture was

aseptically transferred into Pyrex plates containing melted soft medium, gently agitated and poured as overlay on assay-plate containing the base medium. The preparation was left to gel and dry under a hood. Spots where extracts were to be introduced into the plates were carefully marked using sterile cork borer (6mm diameter) and small drops of extract of different concentrations were added. The plates containing bacteria were incubated at 35^0C and fungal plates at 28^0C, and the zone of inhibition measured in mm after 24h, 48h and 72 h growth. A control experiment was set up by using drops of sterile distilled water in place of different extract concentrations.

Preliminary Phytochemical Studies

Basic phytochemical screening were performed on the chewing sticks extract as described by Harbone (1998)

RESULTS

The antimicrobial activity of the tested chewing sticks extracts against *Staphyloccus aureus* is shown in Table 1. Among the three tested chewing sticks, *A. leiocarpus* showed a significantly higher anti-staphylococcal activity ($P< 0.05$) and this was followed by *T. glaucescens*; while *P. kotschyi* virtually had no activity against *S. aureus*. A two way ANOVA test revealed that for the tested chewing sticks, there was no significant difference ($P>0.05$) between the length of incubation, but a significant difference exists ($P<0.05$) between extract concentrations.

The sensitivity of *Streptococcus pyogenes* to the ethanolic extracts of the tested chewing sticks is shown in Table 2. *P. kotschyi* had no activity against *S. pyogenes*. The activity of the extract of *A. leiocarpus* was significantly higher ($P<0.05$) than that exhibited by *T. glaucescens*. The result of two way ANOVA test is similar to that obtained for *S. aureus* in Table 1.

Table 3 shows the activity of the tested chewing sticks extracts against *S. mutans*. *T. glaucescens* showed a significantly higher ($P<0.05$) activity than *A. leiocarpus*. A two way ANOVA test revealed that for both *T. glaucescens* and *A. leiocarpus* there was significant difference($P<0.05$) between extract concentrations. However, for *T. glaucescens* there was no significant difference ($P>0.05$) between the length of incubation, while there was for *A. leiocarpus*. From Table 4 it is obvious that none of the ethanolic extracts of the tested chewing sticks had activity against *Candida albicans*.

Table 5 shows the phytochemicals present in the ethanolic extracts of the test chewing sticks. Alkaloid, Glycoside, Steroid, Phenol, Tannins, Saponin were present in the three samples investigated. Anthraquinone was present in all the samples except *T. glaucescens*. Fluoride was present in all except *P. kotschyi*, while *A. leiocarpus* is the only sample that contained calcium.

DISCUSSION

The results presented in this study showed that the tested chewing sticks contained antibacterial compounds. In addition, the results revealed that the antimicrobial activities of the tested chewing sticks vary and are target-microbe specific. Of the three extracts, that of *T. glaucescens* was the most active against Staphylococcus *aureus*. This is in agreement with the findings of Akande and Hayashi (1998). Inference from two way ANOVA test depicts that the antibacterial activities of *A. leiocarpus* and *T. glaucescens* against *S. aureus*, *Streptococcus pyogenes* and *Streptococcus mutans* were still potent within 72h incubation; except for *A. leiocarpus* against *S. mutans*. The exhibited

bioactivities of these chewing sticks would be consequent upon the phytochemicals they contained. The low to none activity of the extract of *P. kotschyi* against the bacteria tested compares favourably with the results reported by Akande and Hayashi (1998) even though it contained most of the phytochemicals present in the other test chewing sticks. The fact that this plant species is being used as local chewing stick may be suggesting that the level of these phytochemicals might be very low, or that the adoption of other solvent for extraction may yield higher concentration of phytochemicals in the extract that could have potency against the tested oral pathogens.

The no activity of all the extracts of the tested chewing sticks against *Candida albicans* may be suggesting that these plant species contained no phytochemical active against *Candida albicans* or that the method of extraction did not yield any anti-candida compounds. This result compares favourably with a more recent report of Adekunle and Odukoya (2006). The results obtained from this study compliment earlier observations by Rotimi et al (1988), Akande and Hayashi (1998) and Adekunle and Odukoya (2006) and thus giving the reasons why these plants are suitable for better dental care. Conclusively, the quantification of the phytochemicals in these test chewing sticks should be the object of further investigation.

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Table 1. Inhibition of *Staphylococcus aureus* by different concentrations of ethanolic extracts of three tropical chewing sticks.

Chewing sticks	Time of Incubation (hours)	Extract concentrations (g/l) 0.1	0.2	0.4	2.0

Terminalia glaucescens	24	1.33±0.47	3.00±1.41	3.67±0.47	5.33±0.47
	48	1.17±0.24	2.33±0.85	3.33±0.24	4.67±0.62
	72	1.16±0.24	2.33±0.85	3.33±0.24	4.67±0.62
Anogeissus leiocarpus	24	2.67±0.94	3.50±1.08	5.50±1.08	7.00±1.63
	48	2.50±1.08	3.33±1.18	5.00±1.41	6.50±1.87
	72	2.50±1.08	3.33±1.18	5.00±1.41	6.50±1.87
Pseudocedrela kotschyi	24	-	-	-	1.00±0.00
	48	-	-	-	0.9±0.10
	72	-	-	-	0.9±0.10
Control	24	-	-	-	-
	48	-	-	-	-
	72	-	-	-	-

Values in mm [mean ± S.D (n=3)]

- implies no detectable inhibition

Table 2. Inhibition of *Streptococus pyogenes* by different concentrations of ethanolic extracts of three tropical chewing sticks.

Chewing sticks	Time of Incubation (hours)	Extract concentrations (g/l) 0.1	0.2	0.4	2.0
Terminalia glaucescens	24	-	1.00±0.00	2.17±0.24	3.67±0.47
	48	-	1.00±0.00	2.00±0.40	3.50±0.71
	72	-	1.00±0.00	2.00±0.40	3.50±0.71
Anogeissus leiocarpus	24	2.33±0.47	3.50±0.41	4.17±0.62	4.67±0.47
	48	2.00±0.41	3.17±0.62	3.83±0.62	4.17±0.23
	72	2.50±0.41	3.17±0.62	3.83±0.62	4.33±0.24
Pseudocedrela kotschyi	24	-	-	-	-
	48	-	-	-	-
	72	-	-	-	-
Control	24	-	-	-	-
	48	-	-	-	-
	72	-	-	-	-

Values in mm [mean ± S.D (n=3)]

- implies no detectable inhibition

Table 3. Inhibition of *Streptococus mutans* by different concentrations of ethanolic extracts of three tropical chewing sticks.

Chewing sticks	Time of Incubation (hours)	Extract concentrations (g/l) 0.1	0.2	0.4	2.0
Terminalia glaucescens	24	2.33±0.47	4.00±0.00	5.00±0.00	6.33±0.47
	48	1.33±0.47	3.00±0.00	4.00±0.00	6.33±0.47
	72	1.00±0.00	2.00±0.00	2.67±0.47	4.33±0.47
Anogeissus leiocarpus	24	-	1.00±0.00	2.33±0.47	4.00±0.00
	48	-	-	2.00±0.00	3.00±0.00
	72	-	-	-	1.00±0.00
Pseudocedrela kotschyi	24	-	-	-	-
	48	-	-	-	-
	72	-	-	-	-
Control	24	-	-	-	-
	48	-	-	-	-
	72	-	-	-	-

Values in mm [mean ± S.D (n=3)]

- implies no detectable inhibition

Table 4. Inhibition of *Candida albicans* by different concentrations of ethanolic extracts of three tropical chewing sticks.

Chewing sticks	Time of Incubation (hours)	Extract concentrations (g/l) 0.1	0.2	0.4	2.0
Terminalia glaucescens	24	-	-	-	-
	48	-	-	-	-
	72	-	-	-	-
Anogeissus leiocarpus	24	-	-	-	-
	48	-	-	-	-
	72	-	-	-	-
Pseudocedrela kotschyi	24	-	-	-	-
	48	-	-	-	-
	72	-	-	-	-
Control	24	-	-	-	-
	48	-	-	-	-
	72	-	-	-	-

- implies no detectable inhibition

Table 5. Result of the qualitative analysis of the phytochemicals present in chewing sticks samples.

SAMPLE	Alkaloid	Glycoside	Steroid	Anthraquinone	Phenol	Tannins	Saponin	Calcium	Fluoride
Pseudocedrela kotschyi	+	+	+	+	+	+	+	ND	ND

Anogeissus leiocarpus	+	+	+	+	+	+	+	+	+
Terminalia glaucescens,	+	+	+	ND	+	+	+	ND	+

+ implies present
ND implies not detected