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Full Length Research Paper

In vitro assessment of aqueous and ethanolic extracts of some Nigerian chewing sticks on bacteria associated with dental infections

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The antimicrobial efficacy of aqueous and ethanolic extracts of ten Nigerian chewing sticks was investigated using standard methods. These extracts were tested against six bacteria associated with dental infections; they are *Actinomyces vercosus, Neisseria sicca, Streptococcus mutans, Staphylococcus aureus, Lactobacillus salivarius* and *Moraxella catarrhalis.* Generally, ethanolic extract had higher activity on the test organisms than the aqueous extracts. The zone of inhibition of *Viellaria paradoxical* (Gaertn. F) ranged between 5 and 13 mm. *M. catarrhalis* was most susceptible bacterium to the extracts. *A. vercosus* was resistance to the extract of *P. guineense* (Schum & Thonn), *A. indica* (A. Juss) and *M. accumunata* (G. Don).

Key words: Chewing sticks, oral hygiene, pathogens, antibacterial activity

INTRODUCTION

The use of plant as oral cleansing agent in addition to it being used as food is age long (Odebiyi and Sofowora, 1980; Oyagade, 1999). In developing countries, thousands of rural communities still depend mainly on locally available plants for oral hygiene. These plants, like other medicinal plants, are cheap and handy to most of the peasant people (Oluma et al., 2004). As a result of proximity, availability, reliability and age long practice, people still largely practice the use of chewing sticks for oral hygiene. These herbs contain many useful compounds which are antibacterial. Many reports confirmed the potentials of medicinal herbs in prevention of some infectious diseases (Ung et al., 2007; Ayogu and Amadi, 2009).

The use of chewing sticks is a common practice in the Middle East, Asia and Africa for hygienic, religious and social purposes (Akpata and Akinrinmisi, 1987). They are cheap and readily available to the low socio-economic class. Apart from the antibacterial activity which prevents formation of dental plaque, they serve as natural toothbrush. Chewing sticks are inexpensive and readily available to people in the rural areas (Hemler, 2003).

Chewing sticks are made from either the root or the stem of local trees and shrubs. The types of tree used for chewing sticks differ from one community to the other (Oyagade, 1999; Igoli et al., 2000) . The effectiveness of these plants is as a result of their antibacterial activities present in them (Hemler, 2003).

The antibacterial activities of selected chewing sticks used in the south western Nigeria were therefore investigated in this study.

MATERIALS AND METHODS

Collection of plant materials and preparation of extracts

Fresh twigs of screened plants were obtained from Ado- Ekiti, Nigeria. The samples were identified and confirmed in the herbarium unit of the department of plant science and forestry, University of Ado-Ekiti, Nigeria. Extracts were obtained from the air-dried bark, pulp and the whole stick by peeling the bark and chopping it into small pieces. The plants were ground and milled into smaller particles. The finely pulverized sample was soaked in water or ethanol each at the ratio of 1:10 in a conical flask. This

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Table 1. The scientific and common names of the plants used for oral cleansing.

Botanical names	Families	Local name
Viellaria paradoxical (Gaertn.F)	Sapotaceae	Emi gbegiri
Distermonanthus benthamianus (Baill)	Fabaceae	Ayan
Anogcisus leiocaspus (DC Guill & Perr)	Combretaceae	Ayin
Piper guineense (Schum & Thonn)	Piperaceae	Orin iyere
Fagara zanthoxyloides (Lam)	Rutaceae	Orin Ata
Azadirachta indica (A. Juss)	Meliaceae	Dongoyaro
Guarania kola (Heckel)	Clusiaceae	Orin orogbo
Terminalia schimperianah (Flanch)	Combretaceae	Idi ogangan
Sarcocerphalus latifolia (Bruce)	Rubiaceae	Egbesi
Massularia accumunata (G. Don)	Rubiaceae	Pako ijebu

Table 2. Antibacterial activities of water and ethanolic extracts of *Viellaria paradoxical* (zone of inhibition in millimeter).

Test organisms	Aqueous extract (mg/ml)					Ethanolic extract (mg/ml)				
	12.5	25.0	50.0	100.0	150.0	12.5	25.0	50.0	100.0	150.0
Actinomyces vercosus	-	-	5	6	7	5	5	7	8	9
Neisseria sicca	-	-	5	5	5	3	3	6	7	8
Streptococcus mutans	-	2	6	7	8	2	4	7	8	9
Staphylococcus sp	-	-	5	6	6	2	2	6	8	8
Lactobacillus salivarius	2	2	5	6	7	-	4	6	7	9
Moraxella catarrhalis	-	-	-	-	-	-	5	6	12	13

was covered and shaken at intervals of 30 min for 6 h. The suspension was allowed to stand for 48 h. The solution was shaken and filtered using Whatman filter No 1. The filtrate was evaporated to dryness at 40°C and the concentrated extract of each plant was stored at 4°C until used.

Source and process of bacteria

The microorganisms used for this study were isolated from patients with different dental infections at dental unit of the University of Ado-Ekiti, Teaching Hospital, Ado-Ekiti, Nigeria. The isolates were identified using the methods of Fawole and Oso (2001).
The isolates were grown at 37°C in Mueller-Hilton (Oxoid) broth for 18 h with shaking and diluted to an optical density of 0.1 (0.5 McFarland Standard) at optical activity of 625 nm with Mueller-Hilton (Oxoid) broth and stored at 4°C.

Determination of antibacterial activity of chewing sticks

The methods of CLSI (2005) and Oyagade et al. (1999) were used to determine the resistance of the isolates to different plant extracts.

RESULTS AND DISCUSSION

The medicinal plants were screened and quite a lot of them were confirmed to contain antibacterial activity as earlier reported by (Rio and Reeio, 2005). Table 1 shows the list of plants, local names and the parts used in this study. The antibacterial activities of aqueous and methanolic extracts of the screened medicinal plants are shown in Table 2.

The aqueous and the ethanolic extracts of *Viellaria paradoxical* (Gaertn. F) showed microbial activities against *Actinomyces vercosus, Neisseria sicca, Streptococcus mutans* , *Staphylococcus* sp and *Lactobacillus salivarius.* This result agrees with the findings of Zhao et al. (2002) and Akujobi et al. (2006). *Distermonanthus benthamianus* (Baill), *Anogcisus leiocaspus* (DC Guill & Perr) and *Terminalia schimperianah* (Flanch) inhibited the growth of all the test organisms (Tables 3, 4 and 9). The aqueous extract of *Piper guineense* inhibited the growth of *N. sicca, S. mutans* and *M. catarrhalis* whereas *A. vercosus, Staphylococcus* sp and *L. salivarius* were resistant to the extract (Table 5). The varied susceptibility of each organism to the plant extract may be related to the physiological process of individual bacterial species as stated by Garret et al. (2000) and difference in the quantity and quality of the active ingredients (Cowan, 1999; Adetuyi et al., 2004), extraction methods employed and the level of concentration of such an extract (Ogueke et al., 2006).

The aqueous extract of *F. zanthoxyloides* inhibited the growth of the test organisms except *M. catarrhalis* whereas *N. sicca, Str. mutans* and *Staphylococcus* sp were inhibited by the ethanolic extracts (Table 6). Likewise, *G. kola, A. indica, M. accumunat* and *S. latifolia*

Table 4. Antibacterial activities of water and ethanolic extracts of *Anogcisus leiocaspus* (DC Guill & Perr) (zone of inhibition in millimeter).

Test organisms	Aqueous extract (mg/ml)					Ethanolic extract (mg/ml)				
	12.5	25.0	50.0	100.0	150.0	12.5	25.0	50.0	100.0	150.0
Actinomyces vercosus	4	5	5	6	8	-	-	6	10	11
Neisseria sicca	5	5	6	7	9	-	-	7	8	8
Streptococcus mutans	-	-	-	5	6	-	-	-	7	8
Staphylococcus sp	-	-	6	7	7	-	-	-	8	9
Lactobacillus salivarius	5	5	6	7	8	-	-	-	6	7
Moraxella catarrhalis	-	5	5	7	9	4	5	7	8	10

Table 5. Antibacterial activities of water and ethanolic extracts of *Piper guineense* (zone of inhibition in millimeter).

Test organisms	Aqueous extract (mg/ml)					Ethanolic extract (mg/ml)				
	12.5	25.0	50.0	100.0	150.0	12.5	25.0	50.0	100.0	150.0
Actinomyces vercosus	-	-	-	-	-	-	-	6	7	8
Neisseria sicca	-	-	5	6	6	-	-	-	6	7
Streptococcus mutans	5	6	7	8	9	-	5	6	7	9
Staphylococcus sp	-	-	-	-	-	5	5	6	7	8
Lactobacillus salivarius	-	-	-	-	-	-	-	-	-	-
Moraxella catarrhalis	-	-	5	6	7	-	-	-	7	8

Table 6. Antibacterial activities of water and ethanolic extracts of *Fagara zanthoxyloides* (zone of inhibition in millimeter).

Test organisms	Aqueous extract (mg/ml)					Ethanolic extract (mg/ml)				
	12.5	25.0	50.0	100.0	150.0	12.5	25.0	50.0	100.0	150.0
Actinomyces vercosus	-	-	5	5	5	-	-	-	-	-
Neisseria sicca	-	-	5	5	5	-	-	6	7	8
Streptococcus mutans	5	6	6	7	8	-	-	6	7	8
Staphylococcus sp	4	6	6	7	8	-	-	6	7	8
Lactobacillus salivarius	-	-	5	7	8	-	-	-	-	-
Moraxella catarrhalis	-	-	-	-	-	-	-	-	-	-

Table 7. Antibacterial activities of water and ethanolic extracts of *Azadirachta indica* (zone of inhibition in millimeter).

Test organisms	Aqueous extract (mg/ml)					Ethanolic extract (mg/ml)				
	12.5	25.0	50.0	100.0	150.0	12.5	25.0	50.0	100.0	150.0
Actinomyces vercosus	-	-	-	-	-	5	5	8	9	10
Neisseria sicca	-	-	-	5	6	5	5	6	7	8
Streptococcus mutans	-	-	6	6	7	6	5	7	8	8
Staphylococcus sp	-	-	5	6	7	-	6	7	8	9
Lactobacillus salivarius	-	-	5	6	6	5	5	7	7	9
Moraxella catarrhalis	-	-	5	6	7	5	5	7	8	8

possessed antibacterial activity against the test organisms at varied susceptibilities (Tables 7, 8, 10 and 11). Extracts from these chewing sticks are related to the maintenance of good oral health and also for the removal of dental carries (Sote, 1991). The results of this study indicate that the tested chewing sticks are reliable,

Table 8. Antibacterial activities of water and ethanolic extracts of *Guarania kola* (zone of inhibition in millimeter).

Test organisms	Aqueous extract (mg/ml)					Ethanolic extract (mg/ml)				
	12.5	25.0	50.0	100.0	150.0	12.5	25.0	50.0	100.0	150.0
Actinomyces vercosus	-	-	5	5	5	-	-	-	6	7
Neisseria sicca	-	-	6	6	7	-	5	7	8	8
Streptococcus mutans	-	4	7	8	9	-	5	7	8	9
Staphylococcus sp	-	-	7	7	7	-	-	-	-	-
Lactobacillus salivarius			5	6	7	-	-	-	-	-
Moraxella catarrhalis	-	-	-	5	6	-	-	-	-	-

Table 9. Antibacterial activities of water and ethanolic extracts of *Terminalia schimperianah* (zone of inhibition in millimeter).

Test organisms	Aqueous extract (mg/ml)					Ethanolic extract (mg/ml)				
	12.5	25.0	50.0	100.0	150.0	12.5	25.0	50.0	100.0	150.0
Actinomyces vercosus	5	6	7	8	9	-	5	8	9	10
Neisseria sicca	-	-	5	6	7	5	5	7	8	10
Streptococcus mutans	-	5	8	9	10	5	5	7	9	10
Staphylococcus sp	-	-	-	-	7	6	6	6	8	9
Lactobacillus salivarius	-	-	-	5	6	5	5	8	9	10
Moraxella catarrhalis	-	-	5	6	7	6	7	7	9	11

Table 10. Antibacterial activities of water and ethanolic extracts of *Sarcocerphalus latifolia* (zone of inhibition in millimeter).

Test organisms	Aqueous extract (mg/ml)					Ethanolic extract (mg/ml)				
	12.5	25.0	50.0	100.0	150.0	12.5	25.0	50.0	100.0	150.0
Actinomyces vercosus	-	-	-	5	6	-	-	-	6	7
Neisseria sicca	-	-	-	6	7	-	-	-	-	-
Streptococcus mutans	3	5	7	9	11	-	-	-	6	7
Staphylococcus sp	-	-	5	6	7	-	-	-	7	8
Lactobacillus salivarius	-	-	5	5	5	-	-	-	-	-
Moraxella catarrhalis	-	-	-	-	-	-	-	-	-	-

Table 11. Antibacterial activities of water and ethanolic extracts of *Massularia accumunata.* (zone of inhibition in millimeter).

Test organisms	Aqueous extract (mg/ml)					Ethanolic extract (mg/ml)				
	12.5	25.0	50.0	100.0	150.0	12.5	25.0	50.0	100.0	150.0
Actinomyces vercosus	-	-	-	-	-	-	-	6	7	8
Neisseria sicca	-	5	7	8	9	-	-	-	-	-
Streptococcus mutans	-	5	8	9	10	-	-	-	7	8
Staphylococcus sp	-	-	-	6	7	-	-	6	7	7
Lactobacillus salivarius	-	5	7	7	9	-	-	-	-	-
Moraxella catarrhalis	-	-	8	9	10	-	-	6	7	8

effective and economical compared to imported tooth pastes

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