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Antibacterial Properties of Extracts of Some Chewing Sticks Commonly Used in Southwestern Nigeria.

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The antibacterial activities of six commonly used chewing sticks – *Terminalia glauscens, Veronia amygdalina, Mascularia acuminata, Afzelia africana, Rauwolfia vomitoria* and *Nauclea latifolia* - were tested against five bacterial species using agar dilution technique and zones of inhibition were measured for each of the extracts. Extraction was done using distilled water and ethanol. The chewing sticks were all screened for their phytochemical components. They all showed different antibacterial activities against test organisms with zones of inhibition ranging from 1.0mm-14.0mm. The ethanol extracts showed higher activities than the aqueous extracts. *N. latifolia* was the most active of all the chewing sticks (2.0mm-14.0mm) while *M. acuminata* showed the least activity (1.0mm-6.0mm). *Escherichia coli* was the least sensitive of all the test organisms. *Staphylococcus aureus,* as well as *Klebsiella pneuminiae, Proteus vulgaris* and *Pseudomonas aeruginosa* showed varied sensitivity to the extracts. The phytochemical screening showed that tannins and phenolics, saponins, reducing compounds and alkaloids are the active components. This study has justified the use of these chewing sticks for the maintenance of oral hygiene in rural communities.

Keyowrds: Antibacterial properties, Chewing Sticks, Nigeria.

Chewing sticks are parts of higher plants, which are cut to suitable lengths and used for maintenance of oral hygiene. Due to their low costs and availability , they are commonly used by the people of the South Western Nigeria where majority of the people live in rural areas where dental infections are prevalent (Odebiyi, 1980).

Although, people living in urban areas have embraced the use of toothbrushes and toothpastes for maintenance of oral hygiene, many are favourable to the use of chewing sticks for prevention of dental caries and other endemic oral/dental infections. Some even combine pastes and toothbrushes with chewing sticks (Elsaid, 1991).

The heterogeneous and luxuriant vegetation in the Southwestern part of Nigeria provides various options of parts of higher trees that could be used for making chewing sticks. The commonly used plants include: *Terminalia glauscens, Faraga zanthoxyloides, Acadia arabica, Serindei wernecker, Mascularia acuminata, Veronia amygdalina* and *Anogeissus shimperi* (Asuquo, 1987; Akpata and Akinremisi, 1987; Fadulu, 1987). Some investigations have been carried out on the antimicrobial activities of commonly used chewing sticks on common oral flora and pathogens. Such studies have, however, reported the susceptibility to chewing sticks, of commensal bacteria in the oral cavity (Akpata and Akinremisi, 1987).

This study is aimed at investigating the antibacterial activity of commonly used chewing sticks against some bacteria - *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris* - which are not residents in the oral cavity.

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MATERIALS AND METHODS

Plant Materials

The following chewing sticks - *Terminalia glauscens, Veronia amygdalina, Mascularia acuminata, Afzelia africana, Rauwolfia vomitoria* and *Nauclea latifolia* - were purchased at the central market in Ado –Ekiti, Ekiti State, Nigeria and identified at the herbarium unit, Department of Plant Science, University of Ado- Ekiti, Ekiti State, Nigeria, where voucher specimens were deposited.

Preparation of Extracts

The chewing sticks were crushed and air-dried. Extraction was carried out by addition of sterile distilled water (300ml) and 70% ethanol (300ml) to 100g of powdered air-dried plant materials. (Alani et al., 2005).

Clinical Strains

The test organisms were all clinical strains of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris.* They were all obtained from the stock culture collection of the Department of Microbiology, University of Ado-Ekiti, Ekiti-State, Nigeria.

Antibacterial Assay

Antibacterial activity was measured using agar dilution technique as described by Alanis *et al,* (2005). The aqueous and ethanol extracts were reconstituted in dimethyl sulfoxide (DMSO, Merck) and serially diluted in molten Muller Hinton agar (MHA, Sigma) in petridishes (100mm x 15mm) to obtain final concentrations: 125, 62.5, 31.25 and 15.625 µg/mL. The solvent did not exceed 1% concentration and did not affect the growth of the test organisms. They were grown in Muller Hinton broth (MHB, Sigma) for 4h at 37°C. Bacterial suspensions with 0.5 McFarland standard turbidity, which is equivalent to 10^8 cfu/mL, were prepared by dilution with sterile Mueller Hinton broth. The diluted inoculum was added to a Steer's replicator calibrated and incubated for 24h at 37°C. After incubation, all dishes were observed for zones of inhibition and the diameters of these zones were measured in millimeters. The minimum inhibitory concentration (MIC) was determined by the lowest concentration that completely inhibited macroscopic growth of the organisms.

Phytochemical Screening

The chewing sticks were all screened for some phytochemicals such as saponin, tannin and phenolics, reducing compounds and alkaloids. The method described by Sofowora (1992) was used for the screening.

RESULTS

It was observed in this study that the chewing sticks showed different antibacterial activity against the test organisms. For all extracts, highest activities, based on the zones of inhibition, were observed at concentration of 125µg/ml, while the lowest activities were recorded at 15.625µg/ml with zones of inhibition raging from 1.0mm–14.0mm (Table 1-6).

Table 1. Effect of *Terminalia glauscens* extracts on test organisms

Test Organisms	Aqueous extract				Ethanolic extract			
	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml
E. coli	-	-	-	-	-	-	-	-
S. aureus	14.0	12.0	8.0	4.0	10.0	9.0	4.0	3.0
K. pneumoniae	-	-	-	-	5.0	4.0	3.0	2.0
P. aeruginosa	12.0	9.0	4.0	2.0	9.0	5.0	3.0	-
P. vulgaris	16.0	12.0	9.0	3.0	11.0	6.0	3.0	1.0

Zone of inhibition in mm.

Table 2. Effect of *Veronia amygdalina* extracts on test organisms

Test Organisms	Aqueous extract				Ethanolic extract			
	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml
E. coli	-	-	-	-	-	-	-	-
S. aureus	11.0	8.0	6.0	4.0	10.0	9.0	5.0	3.0
K. pneumoniae	-	-	-	-	7.0	5.0	2.0	1.0
P. aeruginosa	10.0	9.0	3.0	2.0	12.0	5.0	3.0	1.0
P. vulgaris	-	-	-	-	8.0	6.0	5.0	2.0

Zone of inhibition in mm.

Table 3. Effect of *Mascularia acuminata* extracts on test organisms

Test Organisms	Aqueous extract				Ethanolic extract			
	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml
E. coli	-	-	-	-	-	-	-	-
S. aureus	4.0	3.0	2.0	1.0	-	-	-	-
K. pneumoniae	-	-	-	-	5.0	4.0	3.0	2.0
P. aeruginosa	-	-	-	-	5.0	3.0	2.0	1.0
P. vulgaris	6.0	4.0	3.0	2.0	9.0	6.0	3.0	2.0

Zone of inhibition in mm.

Table 4. Effect of *Afzelia africana* extracts on test organisms

Test Organisms	Aqueous extract				Ethanolic extract			
	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml
E. coli	-	-	-	-	-	-	-	-
S. aureus	-	-	-	-	10.0	6.0	4.0	2.0
K. pneumoniae	8.0	5.0	3.0	1.0	8.0	7.0	5.0	3.0
P. aeruginosa	5.0	4.0	3.0	2.0	9.0	5.0	4.0	3.0
P. vulgaris	8.0	8.0	5.0	4.0	11.0	6.0	5.0	4.0

Zone of inhibition in mm.

Table 5. Effect of *Rauwolfia vomitoria* extracts on test organisms

Test Organisms	Aqueous extract				Ethanolic extract			
	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml
E. coli	-	-	-	-	-	-	-	-
S. aureus	8.0	6.0	4.0	3.0	10.0	8.0	4.0	3.0
K. pneumoniae	-	-	-	-	9.0	7.0	5.0	3.0
P. aeruginosa	7.0	5.0	3.0	2.0	9.0	5.0	4.0	2.0
P. vulgaris	6.0	4.0	3.0	2.0	-	-	-	-

Zone of inhibition in mm.

Table 6. Effect of *Nauclea latifolia* extracts on test organisms

Test Organisms	Aqueous extract				Ethanolic extract			
	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml
E. coli	11.0	6.0	6.0	4.0	12.0	8.0	4.0	3.0
S. aureus	10.0	5.0	4.0	2.0	14.0	11.0	3.0	2.0
K. pneumoniae	15.0	9.0	3.0	2.0	9.0	5.0	4.0	2.0
P. aeruginosa	12.0	8.0	3.0	2.0	-	-	-	-
P. vulgaris	-	-	-	-	10.0	6.0	3.0	1.0

Zone of inhibition in mm.

Table 7. Phytochemical screening of chewing stick extracts

Tests	*P. africana*	*T. glauscens*	*M.acuminata*	*V. amygdalina*	*N. latifolia*	*R..vomitoric*
1. Tannins & Phenolics	+	+	+	+	+	+
2. Saponins	-	+	-	-	+	-
3. Reducing cpds	-	-	-	+	-	-
4. Alkoloids	-	+	+	+	+	+

Generally, the ethanol extracts of all the chewing sticks showed higher antibacterial activity against the test organisms than the aqueous extracts (Table 1-6). This is evident in the enhanced zones of inhibition shown by the ethanol extracts. Of all the aqueous extracts, *N. latifolia* showed the highest activity against the test organisms (Table 6). This is immediately followed by aqueous extracts of *T. terminalia* and *A. africana* respectively while the aqueous extracts of *M. acuminata* was least active (Table 3). The ethanol extracts of *V. amygdalina, A. africana, R. vormitoria* and *N. latifolia* all showed high activity against the bacteria (Tables 2, 4, 5, 6), although slight variations were noted in their activities. Ethanol extract of *M. acuminata* showed the least activity (Table 3).

The individual susceptibilities of the organisms revealed that *E. coli* was least sensitive to all extracts with the exception of *N. latifolia* (Table 6). *S. aureus* showed a very high sensitivity to *T. glauscens, V. amygdalina* and *R. vormitora. K. pneumoniae* was sensitive mainly to ethanol extracts of the chewing sticks while *P. aeruginosa* as well as *P. vulgaris* showed varied sensitivity to both aqueous and ethanol extracts.

The phytochemical screening of the sticks revealed that tannins and phenolics were present in all the chewing sticks. Alkaloids were detected in all except *A. africana*. Reducing compounds were found only in *V. amygolalina, T. glauscens* as well as *N .latifolia* had saponins. The active compounds present in the chewing sticks are shown in Table 7.

DISCUSSION

The chewing sticks tested in this study are widely used for maintenance of oral hygiene by people living in the rural areas in southwestern part of Nigeria. This study has revealed that they possessed inhibitory effect on some other common organisms; apart form those commonly found in the oral cavity. This finding agrees with other studies that have reported the inhibitory effect of chewing sticks (Sote and Wilson, 1995; Oyagade 1999). This further justifies their widespread use for maintenance of oral hygiene - particularly by those living in rural areas (Elsaid, 1991).

Generally, the higher antibacterial activity exhibited by the ethanol extracts suggest that ethanol could be considered as a better solvent for extraction, which increases the potency and spectrum of activity of the extracts. However, other explanations could be given to this important observation.

N. latifolia showed the highest and widest spectrum of antibacterial activity followed by extracts of *T. teminalia* and *A. africana* respectively, while *M. acuminata* was the least. The variation in activity among the chewing sticks tested could be attributed to the different concentrations and constitution of the various active agents – tannins, reducing compounds, alkaloids and phenols – present in the chewing sticks. This correlates with the findings of other studies (Akpata and Akinrimisi, 1987; Odebiyi and Sofowora 1980).

The effects of extracts of some chewing sticks on oral anaerobes have been reported. In this study, *E. coli* was the least sensitive organism to all the extracts. This agrees with the finding of Rotimi and Mosadomi (1987) who reported the insensitivity of *E. coli* to plant extracts used for oral treatment. The variation in the sensitivity among the bacteria could be attributed to the phenotypic differences in the configuration of the cell envelopes. The unique metabolic and physiological processes of the individual bacterial species could be a better explanation for this variation.

Finally, this study has confirmed that some bacteria are susceptible to commonly used chewing sticks and their use could be as effective as toothbrushes and pastes in the future.

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