Antimicrobial Activities and Phytochemical Screening of two Tropical Nigerian Chewing Sticks

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Abstract

Massularia accuminata "Pako Ijebu" G. Don Bullock ex. Hoyle and Distemonanthus benthamianus "Orin Ayan" Baillon (Movingui) are chewing sticks widely used for oral and dental care among the rural natives in the tropical regions of Nigeria. Antimicrobial activity two tropical Nigerian chewing stick was carried out against Aspergillus niger (ATCC 23867), Saccharomyces cerevisiae (ATCC 2601), Proteus mirabilis (ATCC 27673), Pseudomonas aeruginosa (ATCC 27853), and Bacillus subtilis (ATCC 23873) using agar well diffusion method. It was discovered that Massularia accuminata showed a great antibacterial and antifungal effects than Distemonanthus benthamianus. In vitro studies showed that all the test organisms were susceptible to the inhibitory properties of both the aqueous and ethanolic extracts of the two chewing sticks. These inhibitory properties had been attributed to the presence of phytochemicals (glycosides, alkaloids, saponin, tannins, flavonoids anthocyanin, anthraquinone and phlobatannin) in the chewing sticks. The Minimum Inhibitory Concentration (MIC) ranged from 0.1g/l – 0.3g/l for the two chewing sticks. The research has demonstrated the fact that Massularia accuminata is better for orodental care and hygiene than Distemonanthus benthamianus.

Keywords: Antimicrobial, Phytochemical, Chewing sticks, Agar-well diffusion

1.0 Introduction

Chewing sticks are important Non Timber Forest Product (NTFP) widely used for dental cleaning in the tropical West Africa (Akande and Hayashi, 1998). Plants from which chew sticks are derived are abundant and diverse in Nigeria rural communities. Almost the entire rural population of Nigeria uses chewing sticks for orodental hygiene. Chewing sticks are recommended for oral hygiene by the World Health Organization, and some of them, or their extracts, are also used in the ethnomedical treatment of oral infections (Ndukwe, *et al.* 2005).

Previous studies have demonstrated the antiplaque and antibacterial actions of extracts of these Nigerian chewing sticks (NCS) against oral bacteria, such as *Streptococcus mutans* (Wolinsky and Sote, 1984), *Streptococcus mitis* and oral anaerobes (Rotimi and Mosadoni, 1987), which are the organisms commonly implicated in dental caries and orodental infections. All surfaces in the mouth are colonised by a resident microflora that is highly diverse in composition (Marsh, 2003). The largest numbers of microorganisms are found on the tooth surfaces, especially at stagnant sites and are termed dental plaque, the composition of which varies at distinct surfaces (e.g. fissures, approximal surfaces, and the gingival crevice) due to the prevailing biological properties of the site.

Dental plaque has been defined as the diverse microbial community embedded in a matrix of host and bacterial polymers, growing on teeth as a biofilm (Marsh, 2003). 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 (Kareem *et al.,* 2012).

Massularia acuminata (G Don) Bullock belongs to the family Rubiaceae (Burkill, 1997). It is commonly known as "Pako Ijebu" especially among the rural communities in Western Nigeria. It grows as a shrub or small tree to 9 m high; of the understorey of the closed-forest; common from Guinea to Western Cameroons and extending into Congo. The wood is hard and strong. *Distemonanthus benthamianus* Baillon (Movingui) is one of the perennial trees of the evergreen, semi-deciduous and secondary forest of West Africa tropics mainly in the Cameroon, Ghana and Nigeria (Adeniji *et al*., 2011). It belongs to the family Fabaceae (Caesalpiniaceae) (Ngulefack *et al*., 2005). It is commonly called "Orin Ayan" among the Yoruba speaking natives of Western Nigeria. It grows up to 40m high or more with trunk of 1.20m or slightly smaller. *D. benthamianus* is used in traditional Africa medicine to treat bacterial, fungal and viral infections (Ngulefack *et al*., 2005) and it is used as chewing stick for oro-dental hygiene (Aiyegoro *et al*., 2008; Ndukwe *et al*., 2005). Hence, this research is aimed at studying the antimicrobial activities and phytochemical screening of the two tropical Nigerian chewing sticks, their antimicrobial potency and efficiency in cleaning the teeth and eliminating orodental infections in Nigeria.

2.0 Materials and Methods

2.1 Sample Collection

Chewing sticks

Fresh roots and stems of *Massularia acuminata* "Pako Ijebu" and *Distemonanthus benthamianus* "Orin Ayan" respectively were bought from local herb sellers in Ago Iwoye, Ogun State, Nigeria. The samples were identified and authenticated with the previously collected herbarium specimens available in the Department of Botany, University of Lagos, Akoka, Lagos State, Nigeria. The chewing sticks samples were washed under running tap water to remove dirt. The samples were air-dried for 7 days to curb distortion in the composition of the active principle in the chewing sticks. The dried samples were well pulverized into a fine powder with a mixer grinder. The powder was stored in air tight aseptic containers (28ºC±2) for subsequent use.

2.2 Fungal and Bacterial Strains

Pure isolates of *Aspergillus niger* (ATCC 23867), *Saccharomyces cerevisiae (*ATCC 2601), *Proteus mirabilis* (ATCC 27673), *Pseudomonas aeruginosa (*ATCC 27853), and *Bacillus subtilis* (ATCC 23873) were sourced from the Clinical laboratory, Clinical Sciences Department, Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos State, Nigeria. The typed isolates were stored on Nutrient agar (NA) slants in the refrigerator at 4^0C prior to use.

2.3 Preparation of Extracts

The ethanolic and aqueous extracts were prepared using the method of Adekunle and Odukoya (2006) but with slight modifications. About 20g of the powder were separately soaked in 120ml of 95% ethanol in a 250ml reagent bottle and stoppered. This was allowed to stand for 3 days to permit full extraction of the active principles in the powdered chewing sticks. The fluids were then filtered using Whatman No1 filter paper. The extracts were rotary dried to obtain the concentrate. It was then kept in the fridge (4ºC) prior to use. A 2.0g/l solution of each extract was prepared and fractionated into 0.1g/l, 0.3g/l and 0.5g/l, 0.7g/l concentrations needed for the bioassay.

2.4 Antimicrobial Sensitivity Bioassay

The antimicrobial assay was performed by using the agar well diffusion method (Habamu *et al.,* 2010; Perez *et al*., 1990). Wells of 10 mm in diameter were made into previously seeded Nutrient agar plates. Each well was filled with (1.0 ml) of the extract. The same quantity of sterile distilled water and 50% ethanol both without plant extract served as controls. The plates were pre- incubated for 2 hours to allow diffusion of extract before incubating overnight at 37ºC. However, same was repeated for the fungal assay. The diameter of clear zone was measured in mm using a well calibrated veneer calliper. Triplicate plates were prepared for each extract and controls.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts were determined using the method described by Vinothkumar *et al.* 2010 by diluting the extracts double fold with nutrient broth in a series of test tubes and to each of the tubes, equal volume of the test organism was added and incubated at 37ºC for 24 hours except for *Aspergillus niger* and *Saccharomyces cerevisiae* which were incubated at (28ºC ± 2). Controls were prepared by inoculating tubes without the extracts but with the cell suspensions. The tubes were then examined for the presence of turbidity after the incubation period. The least concentration of no observable bacterial and fungal growth when compared with the control was considered as the Minimum Inhibitory Concentration (MIC).

2.6 Phytochemical analysis of Chewing Stick Extracts

Qualitative screening of the phytochemical components of the chewing sticks was carried out using the method outlined by Harborne (1991) to detect the presence of glycosides, alkaloids, saponin, tannins, flavonoids anthocyanin, anthraquinone and phlobatannin

2.7 Statistical Analysis

The Statistical Package for Social Scientists (SPSS, version 19.0) was used for the analysis of the data obtained. Two way ANOVA test was used to determine the level of significance of the test organisms.

3.0 Results

Both aqueous and ethanolic crude extracts of each of the chewing sticks exhibited growth inhibition against all the bacteria and fungi isolates. Both ethanolic and aqueous extract of *Distemonanathus benthamianus* showed a higher antifungal activity ($P<0.05$) on subjection to two way ANOVA test than the aqueous and ethanolic extract of *Massularia acuminata* as shown in Table 1, 2, 3 and 4. A significantly higher antibacterial activity ($P<0.05$) was exhibited by both aqueous and ethanolic extracts of *Massularia acuminata* than the extracts of *Distemonanthus benthamianus* as shown in Tables 1, 2, 3 and 4. The ethanolic crude extracts of the chewing sticks had a greater pharmaceutical effect in comparison with the aqueous extract, which might be because the bioactive components were more soluble in ethanol (Rotimi and Mosadomi, 1987). The control set up did not show any antimicrobial activity. The Minimum Inhibitory Concentration (MIC) ranges from 0.1g/l to 0.3g/l for both aqueous and ethanolic extracts of *Massularia acuminata* and *Distemonanthus benthamianus* as shown in Tables 5 and 6. In Table 7, phytochemical analysis showed that Tannin, Anthocyanin and Phlobatanin were present in the aqueous extract of *Massularia acuminata* while Glycoside, Saponin, Tannin, Flavonoid, Anthocyanin and Anthraquinone were present in the ethanolic extract of *Massularia acuminata.* Also, Alkaloid, Tannin, Anthraquinone and Phlobatanin were present in the aqueous extract of *Distemonanthus benthamianus* while in its ethanolic extract sizeable amount of Saponin, Tannin, Flavonoid, Anthocyanin and Anthraquinone were found present.

Table 1: Sensitivity of the test organisms to aqueous extract of *Massularia acuminata*

	Concentration (g/l)			
	0.1	**0.3**	**0.5**	**0.7**
Organisms				
Aspergillus niger (ATCC 23867)	11±1.9	13±2.5	16±2.7	23±3.2
Saccharomyces cerevisae (ATCC 2601)	15±2.1	19±1.3	22±3.4	25±4.0
Proteus mirabilis (ATCC 27673)	27±3.4	28±1.9	32±1.9	35±2.8
Pseudomonas aeruginosa (ATCC 27853)	29±1.3	31±2.6	32±1.9	34±1.8
Bacillus subtilis (ATCC 23873)	28±2.7	30±0.6	31±1.2	33±3.7
Control	00±00	00±00	00±00	00±00

Means of triplicate zones of Inhibition± Standard Error of Mean (SEM) = √S.D/n
S.D=Standard Deviation
n=Number of test plates (3)

Table 2: Sensitivity of the test organisms to ethanolic extract of *Massularia acuminata*

	Concentration (g/l)			
	0.1	**0.3**	**0.5**	**0.7**
Organisms				
Aspergillus niger (ATCC 23867)	22±2.1	23±2.9	26±2.7	31±3.0
Saccharomyces cerevisae (ATCC 2601)	25±3.2	27±5.3	29±3.1	33±3.0
Proteus mirabilis (ATCC 27673)	27±3.4	31±1.3	33±1.6	35±1.8
Pseudomonas aeruginosa (ATCC 27853)	28±1.9	29±2.3	31±1.5	32±1.6
Bacillus subtilis (ATCC 23873)	31±1.3	32±4.6	32±1.8	34±2.4
Control	00±00	00±00	00±00	00±00

Means of triplicate zones of Inhibition± Standard Error of Mean (SEM) = √S.D/n
S.D=Standard Deviation
n=Number of test plates (3)

Table 3: Sensitivity of the test organisms to aqueous extract of *Distemonanthus benthamianus*

	Concentration (g/l)			
	0.1	**0.3**	**0.5**	**0.7**
Organisms				
Aspergillus niger (ATCC 23867)	13±1.4	15±2.3	16±3.3	20±4.1
Saccharomyces cerevisae (ATCC 2601)	15±2.1	15±1.3	18±2.4	21±4.3
Proteus mirabilis (ATCC 27673)	25±2.6	28±2.9	29±1.2	3±1.9
Pseudomonas aeruginosa (ATCC 27853)	26±1.9	17±2.6	19±1.9	21±1.0
Bacillus subtilis (ATCC 23873)	22±2.7	23±0.6	25±1.2	28±3.7
Control	00±00	00±00	00±00	00±00

Means of triplicate zones of Inhibition± Standard error of Mean (SEM) = √S.D/n
S.D=Standard Deviation
n=Number of test plates (3)

Table 4: Sensitivity of the test organisms to ethanolic extract of *Distemonanthus benthamianus*

	Concentration (g/l)			
	0.1	**0.3**	**0.5**	**0.7**
Organisms				
Aspergillus niger (ATCC 23867)	15±2.9	17±1.5	18±2.2	21±3.7
Saccharomyces cerevisae (ATCC 2601)	16±2.4	19±3.3	21±2.1	24±3.7
Proteus mirabilis (ATCC 27673)	24±2.2	27±2.0	32±2.9	36±3.7
Pseudomonas aeruginosa (ATCC 27853)	18±1.3	19±2.9	19±3.2	22±2.6
Bacillus subtilis (ATCC 23873)	24±3.9	27±3.6	27±1.4	29±2.7
Control	00±00	00±00	00±00	00±00

Means of triplicate zones of Inhibition± Standard Error of Mean = √S.D/n
S.D=Standard Deviation
n=Number of test plates (3)

Table 5: Minimum Inhibitory Concentration (MIC) of Aqueous and Ethanolic extracts of *Massularia acuminata* on the organisms

Organisms	Aqueous (g/l)	Ethanolic (g/l)
Aspergillus niger (ATCC 23867)	0.1	0.3
Saccharomyces cerevisae (ATCC 2601)	0.1	0.1
Proteus mirabilis (ATCC 27673)	0.3	0.1
Pseudomonas aeruginosa (ATCC 27853)	0.1	0.1
Bacillus subtilis (ATCC 23873)	0.1	0.1

Table 6: Minimum Inhibitory Concentration (MIC) of Aqueous and Ethanolic Extracts of *Distemonanthus benthamianus* on the organisms

Organisms	Aqueous (g/l)	Ethanolic (g/l)
Aspergillus niger (ATCC 23867)	0.3	0.1
Saccharomyces cerevisae (ATCC 2601)	0.1	0.3
Proteus mirabilis (ATCC 27673)	0.3	0.1
Pseudomonas aeruginosa (ATCC 27853)	0.1	0.1
Bacillus subtilis (ATCC 23873)	0.1	0.1

Table 7: Phytochemical Compounds found in the crude extracts of the two tropical Nigerian Chewing Sticks

Sample extract (0.5g/l)	Glycoside	Alkaloid	Saponin	Tannin	Flavonoid	Anthocyanin	Anthraquinone	Phlobatanin
Massularia Accuminata								
Aqueous	-	-	-	+	-	+	-	+
Ethanol	+	-	+	+	+	+	+	-
Distemonanthus benthamianus								
Aqueous	-	+	-	+	-	-	+	+
Ethanol	-	-	+	+	+	+	+	-

4.0 Discussion and Conclusion

This research showed that the extracts of the two tropical Nigerian Chewing sticks; *Massularia accuminata* and *Distemonanthus benthamianus* exhibited a great antifungal and antibacterial effects against the test organisms. *Massularia accuminata* showed a high inhibitory activity against the bacteria species tested, since it exhibited the highest antibacterial inhibition among the plant extracts. This was in conformity with the work by Barnabas and Nagarajan (1998). Results obtained showed that ethanol was a better and more powerful solvent than water. This is in agreement with the report of Kareem *et al*. (2008) which stated that active components of plants are more soluble in organic solvent. The high potency of the ethanol extract may be attributed to the dissolving power of alcohol over water (Majorie, 1999). The antifungal and antibacterial inhibitory effects showed by the chewing stick extracts can be attributed to the presence of phytochemical compounds (glycosides, alkaloids, saponin, tannins, flavonoids anthocyanin, anthraquinone and phlobatannin) present in them.

This is also in agreement with the work reported by Cowan (1999) and Draughon (2004). Antimicrobial potency of the two chewing sticks has been the major reason why they are been employed in tropical Nigeria for dental cleaning to guide against dental caries, gingivitis and dental plaque. From the result obtained, it was observed that Minimum Inhibitory effect was vividly shown at 0.1g/l and 0.3g/l concentrations (MIC) of extracts of the two chewing sticks. The great antibacterial effect showed by the chewing sticks was as a result of tannin present in them (Kareem *et al.,* 2012). Hagerman and Butler (1981) have reported that tannins have been shown to form irreversible complexes with proline-rich proteins which would lead to inhibition of cell-wall-protein synthesis, a property that may explain the mode of action of these chewing stick extracts. This research has great shown the antifungal and antibacterial potency of the two chewing sticks in orodental hygiene but more research still needs to be carried out in the quantification of the active phytochemical compounds, purification and eventual patenting of the active principles in the chewing sticks.

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