

Antibacterial Effect of *Distemonanthus benthamianus* Extract Against Some Oral Pathogens.

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Abstract

Chewing sticks are widely used in Nigeria for dental and oral hygiene. In-vitro susceptibility test was done with aqueous extracts of *Distemonanthus benthamianus* on three test isolates (*Staphylococcus aureus* ATCC 8043, *Saccharomyces cerevisiae* ATCC 2601 and *Pseudomonas aeruginosa* ATCC 27853) using agar well diffusion technique. *Staphylococcus aureus* ATCC 8043 produced highest zone of inhibition to water extract of the plant with a value of 35.0 mm at 200 mg/ml. All organisms were susceptible to the ethanolic extract with *Staphylococcus aureus* ATCC 8043 and *Saccharomyces cerevisiae* ATCC 2601 exhibiting highest zone of inhibition with a range of 23.0-35.0 mm and 30.0-35.0 mm respectively. Phytochemical analysis of the plant revealed no significant difference in the concentration of alkaloids and saponin. Flavonoids, phenol, steroid and tannin were among the active principles present in the plant.

Key words: Chewing sticks, active principle, extract, inhibition, concentration.

1.0 Introduction

Distemonanthus benthamianus 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 (Adeniyi *et al.*, 2011). It belongs to the family Leguminosae (Ngulefack *et al.*, 2005). It is commonly known as 'Anyan' in Yoruba language. 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). Extracts from the stem bark of the plant exhibit significant bactericidal effect on *Bacteroides gingivalis* and *Streptococcus mutans* which are implicated in oro-dental infections (Ogundiya *et al.*, 2006).

Recent interest in chewing sticks and their extracts has focused on their effects on organisms that are involved in oral infections. Africans that use chewing sticks have fewer carious lesions than those that use toothbrush and their use has been encouraged by the World Health Organization (Ndukwe *et al.*, 2005). All surfaces in the mouth are colonised by a resident microflora that is highly diverse in composition (Marsh, 2003). The largest number 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, 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 biofilm mode of growth can have clinical significance, since the organisms growing on surfaces become inherently less sensitive to antimicrobial agents (Gilbert *et al.*, 2002), while the properties of a microbial community are greater than the sum of the component species (Marsh and Bowden, 2000). Although dental plaque forms naturally on teeth, in the absence of oral hygiene it can accumulate beyond levels compatible with oral health and at susceptible sites, lead to dental caries or periodontal diseases (Marsh *et al.*, 1995).

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 (Wolinsky and Sote, 1983 and 1984). 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 (Akpata and Akinrimisi, 1977). This study aims at evaluating the antimicrobial effect of aqueous chewing stick extracts of *Distemonanthus benthamianus* on some pathogens while comparing its potentials with those of other tooth cleaning formulae for maximum effectiveness.

2.0 Materials and Methods

2.1 Sample Collection

Chewing stick

Fresh roots and bark of *Distemonanthus benthamianus* (Pako Ayan) were bought in Lafenwa market, Abeokuta, Nigeria. The samples were identified and confirmed with the authentic herbarium specimen available in the Biological Sciences Department, Crescent University Abeokuta. The chewing stick samples were washed under running tap water to remove dirt. The samples were dried at 60°C for 2 days in an oven. The dried samples were ground well into a fine powder with a mixer grinder. The powder was stored in air tight containers at room temperature for further use.

2.2 Bacterial Strains

Isolates used for this study (*Staphylococcus aureus* ATCC 8043, *Saccharomyces cerevisiae* ATCC 2601) were obtained from Fidson Healthcare Plc, Lagos State while *Pseudomonas aeruginosa* ATCC 27853 was obtained from Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State.

2.3 Extraction from chewing stick

Plant extracts of *Distemonanthus benthamianus* were prepared according to the method of Alade and Irobi (1993) and as adopted by Alagesabooopathi (2011) but with little modifications. Fixed weights (0.5 g, 1 g, 1.5 g, 2 g) of powdered plant material were soaked separately in 10 ml of distilled water and ethanol (50%) for 72 h to obtain the following concentrations; 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml respectively. Each mixture was stirred at intervals using a sterile glass rod. At the end of the extraction, each extract was passed through Whatman No. 1 filter paper and the filtrate obtained was concentrated in vacuum using rotator evaporator. Then the extracts were used for antimicrobial assay.

2.4 Preparation of test organisms

Sterilized molten media (Nutrient agar) were separately inoculated with about 1.5×10^5 cfu/ml of inoculum from overnight broth cultures of the different test organisms and poured into petri dishes and then allowed to solidify. For *S. cerevisiae*, Potato Dextrose Agar (PDA) was used.

2.5 Sensitivity test

The antimicrobial assay was performed by using the agar well diffusion method (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 37⁰C. The diameter of clear zone was measured in mm. Triplicate plates were prepared for each extract and controls.

2.6 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts were determined by diluting the extracts double fold (2:2) 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 37°C for 24 hours except for *Saccharomyces cerevisiae* ATCC 2601 which was incubated at 27°C. 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 that did not permit any visible growth when compared with the control was considered as the minimum inhibitory concentration.

2.7 Phytochemical Properties of Chewing Sticks Extracts

The extracts were examined according to the method of Harborne (1991) for the presence of alkaloids, tannins, glycosides, saponin, flavonoids, phenol, phytate, oxalate, glycoside, steroids, hemagglutin, and trypsin inhibitors.

2.8 Statistical Analysis

The Statistical Package for Social Scientists (SPSS, version 16.0) was used for the analysis of the data obtained. Duncan's multiple range test was used to determine the level of significance of the organisms.

3.0 Results

3.1 Sensitivity test

All the pathogens used in this investigation were susceptible to water extract of *Distemonanthus benthamianus*, the only exception was *Staphylococcus aureus* which was resistant to the water extracts at concentrations of 50 mg/ml and 100 mg/ml. However, at 150 and 200 mg/ml, water extract of the plant produced the highest significant zone of inhibition on *Staphylococcus aureus* while the least zone of inhibition was observed on *Pseudomonas aeruginosa* with an average value of 21.0 mm (Table 1).

Table 1: Sensitivity of the Pathogens to water extract of *Distemonanthus benthamianus*

Organisms	Concentration (mg/ml).			
	50	100	150	200
<i>S. aureus</i> ATCC 8043	N.I	N.I	32.0±1.7 ^a	35.0±2.6 ^a
<i>P. aeruginosa</i> ATCC 27853	14.0±1.0 ^b	16.0±3.6 ^b	18.0±1.7 ^{ab}	21.0±1.0 ^a
<i>S. cerevisiae</i> ATCC 2601	22.0±2.7 ^a	25.0±0 ^a	25.0±1.7 ^a	27.0±4.0 ^a
Control	N.I	N.I	N.I	N.I

Values are means of triplicate zones of Inhibition± Standard error, N.I =No Inhibition. In each row, means followed by different letters are significantly different (P > 0.05) according to Duncan's multiple range test.

The water extract produced the highest inhibitory effect on *S. cerevisiae* only at concentrations of 50 and 100 mg/ml. Generally, the inhibitory effect of the ethanolic extracts of the plant on *Pseudomonas aeruginosa* was low compared with the other organisms. The highest effect was shown on *Saccharomyces cerevisiae* with mean values ranging from 30. 0 to 35. 0 mm at 50 to 200 mg/ml of the extract (Table 2).

Table 2: Sensitivity of the Pathogens to Ethanolic extract of *Distemonanthus benthamianus*

Organisms	Concentration (mg/ml).			
	50	100	150	200
<i>S. aureus</i> ATCC 8043	23.0±1.0 ^c	28.0±1.0 ^b	31.0±2.0 ^b	35.0±2.6 ^a
<i>P. aeruginosa</i> ATCC 27853	18.0±1.0 ^d	21.0±1.7 ^c	29.0±1.0 ^b	34.0±2.0 ^a
<i>S. cerevisiae</i> ATCC 2601	30.0±1.0 ^b	33.0±0 ^{ab}	33.0±2.0 ^{ab}	35.0±3.0 ^a
Control	N.I	N.I	N.I	N.I

Values are means of triplicate zones of Inhibition ± Standard error, N.I =No Inhibition

In each row, means followed by different letters are significantly different (P > 0.05) according to Duncan's multiple range test.

The highest effects of ethanol and water extracts were observed at the highest concentration of 200 mg/ml while the least effect was seen in the smallest concentration of 50 mg/ml. The Minimum Inhibitory Concentration (MIC) of all the extracts used is shown in Table 3.

Table 3: Minimum Inhibitory concentration of aqueous extract of *Distemonanthus benthamianus* on the organisms.

Organisms	MIC of <i>D. Benthamianus</i>	
	Water (mg/ml)	Ethanol (mg/ml)
<i>S. aureus</i> ATCC 2601	6.3	50.0
<i>P. aeruginosa</i> ATCC 27853	50.0	50.0
<i>S. cerevisiae</i> ATCC 2601	6.3	25.0

The lowest MIC was produced against *Staphylococcus aureus* and *Saccharomyces cerevisiae* with a concentration of 6.3mg/ml by using the water extract of *Distemonanthus benthamianus*. For the ethanolic extracts, the highest MIC of *Distemonanthus benthamianus* was exhibited against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

3.2 Phytochemical properties of extract

The phytochemical properties of *Distemonanthus benthamianus* vary with alkaloids and saponin having the highest mean values of 3.142 mg/100g and 2.731 mg/100 respectively. The lowest value was produced by glycoside with an average of 0.113mg/100g, however, this value was not statistically different from those of phenol, phytate, oxalate, steroids and tannin. There was no significant difference between flavonoids and trypsin inhibitors (Table 4).

Table 4: Phytochemical analysis of *D. benthamianus*.

Active principles Determined	Concentration (mg/ 100g)
Tannin	0.152± 0.0021 ^d
Phenol	0.140± 0.0000 ^d
Saponin	2.731± 0.0014 ^a
Alkaloids	3.142± 0.0028 ^a
Phytate	1.142± 0.0014 ^{bc}
Oxalate	0.373± 0.0035 ^d
Trypsin Inhibitors	1.462± 0.0021 ^b
Glycoside	0.113± 0.0004 ^d
Flavonoids	1.692± 0.0021 ^b
Steroids	0.551± 0.0007 ^d
Hemagultin	1.046± 0.0028 ^{bc}

Mean value of duplicate ± Standard deviation

4.0 Discussion

The inhibitory effect of *D. benthamianus* extracts on some oral pathogens investigated *in-vitro* revealed the antimicrobial potential of these extracts especially the ethanol extract. This is not surprising as the antimicrobial nature of many edible plant extracts have been demonstrated (Aboaba *et al.*, 2005). These results confirmed and showed that *D. benthamianus* has consistent inhibitory effect on all the isolates tested. A few earlier reports, however, demonstrated the antimicrobial properties of *D. benthamianus* (Adeniyi *et al.*, 2011; Ngulefack *et al.*, 2005). 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.

This finding also agrees with previous work of Obi and Onuoha (2000) who reported ethanol to be the best solvent for the extraction of most plant active principles of medicinal importance. The high potency of the ethanol extract may be attributed to the dissolving power of alcohol over water (Majorie, 1999). The observed antibacterial effects on the isolates is believed to be due to the presence of alkaloids, tannins, and flavonoids which have been shown to possess antibacterial properties (Cowan, 1999; Draughon, 2004). Antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infections (Aboaba *et al.*, 2005). 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. It is believed that the tannin present in African chewing stick is responsible for the antibacterial effect. Other preformed compounds like saponins also have antifungal properties. Many plants contain non toxic glycosides which can get hydrolyzed to release phenolics which are toxic to microbial pathogen (Aboaba and Efuwape, 2001). African chewing sticks have antimicrobial properties and so their use will help to promote oral hygiene. The results obtained support the fact that more work needs to be done on the purification, identification and quantification of the active components with the view of their use for *in-vivo* studies.

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