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Antifungal Activities of Ethanol and Aqueous Crude Extracts of Four Nigerian Chewing Sticks

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ABSTRACT

The antifungal activities of the ethanol and aqueous crude extracts of four Nigerian chewing sticks were investigated. Also a preliminary phytochemical analysis of the plants was done. The chewing sticks include *Anogeissus schimperi*, *Distemonanthus benthmianus*, *Vernonia amagdalina* and *Xanthoxylum zanthoxyloides*. All the plants tested, except *Anogeissus Schimperi*, displayed antifungal activities, zone of inhibition above the 10mm standard mark. The ethanol crude extracts of the chewing sticks had a greater zone of inhibition in comparison with the aqueous extract. Among the individual plant extracts, *D. benthmianus* had the highest antifungal activity against *Candida albicans*, *Aspergillus flavus* and *Microsporium gypseum* and *Trichophyton metagrophytes*. The chewing sticks contain antifungal agents, though the concentration and composition of the bioactive substances may differ amongst the plants. *Distemonath benthmianus* exhibited a better antifungal activity and thus made it more suitable for better dental care.

Flavonoid was present in all the plant extracts. Tannin was present in all the plant extract, except that of *Anogeissus schimperi*. Alkaloids were absent in all the plant extract. The ethanolic extracts had more phytochemical compounds than the aqueous extracts.

KEYWORDS: Chewing Sticks; antifungal activity; Ethanol and aqueous extract; flavonoid; Tannin

INTRODUCTION

In many African houses, teeth are cleaned in the morning by chewing the root or skin stem of certain plants, until they acquire brush like ends (EL-Said *et al*., 1971). The fibrous end is then cleaned and it brushes the teeth thoroughly. In certain parts of West Africa, e.g. Ghana, Nigeria, Senegal, Chewing sticks are used frequently during the day. These chewing sticks impact varying taste sensation; a tingling peppery taste, a bitter taste and numbness is provided (Buada and Boakye-Yiadom, 1973).

Investigations carried out on some of these chewing sticks, shows that they all posses antimicrobial activity against oral microbial flora, but to varying degrees when tested by cup-plate

agar method (Enwonwu, 1997). This could indicate therefore that the chewing sticks, in addition to providing mechanical stimulation of the gums, also destroy microbes, presenting the mouth a feature, which is absent in the common toothpaste and brush method (Enwonwu, 1997). The advantage of the chewing sticks over the conventional toothpaste and brush could explain why many Africans have strong teeth (Ugoji *et al.*, 2000).

Some of the plants employed as chewing sticks for dental care include *Anogeissus schiemperi* (Hochst); *Distemonathus benthmianus* (Baill); *vernonian amygdalina* (Del.) and *Xanthoxyllum zanthoxyloides* (Lam.) "formerly *Fagara zanthoxyloides"* (Burkill,1997). *A. schimperi* is in the family combretaceae found in southern Nigeria, Senegal, Ivory Coast and Togo, and commonly called "Pako dudu" in Yoruba. *Distemonanthus benthmianus* known as "Ayan" in Yoruba is in the family caesalpinaceae. *Xanthoxyllum zanthozyloides* "orin ata" is in the family Rutaceae while *Vernonia amagdalina* commonly called bitter leaf is in the family compositae. Ugoji *et al*., (2000) determined the antibacterial activities of the aqueous extracts of these chewing sticks, except *Distemonanthus benthmianus,* on oral pathogens and found that they displayed broad spectrum activity on aerobic and anaerobic bacteria. The antifungal activities of these chewing sticks were not reported, although some of them have documented reports of the antifungal properties of their leaves or bark Sofowora (1982).

The aim of this paper is to report the antifungal activities of ethanol and aqueous extracts of *Anogeissus schimperi, Distemonanthus benthmianus, Xanthoxyllum Zanthoxyloides* and *Vernonia amagdalina* on oral pathogens and some dermatophytes using the paper disc method. Also the preliminary phytochemistry of the extracts will be examined.

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Source of plant materials:

The stem or root of the plant used as chewing stick were purchased from Ketu and Tejuosho markets of Lagos State, Nigeria. The plant species parts were authenticated by a botanist at the Department of Botany and microbiology University of Lagos as well as the Forests research Institute, Ibadan, Nigeria.

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Aspergillus flavus, Candida albicans, Microsporum gypseum, Trichophyton metagrophytes were colleted from patients at the Department medical microbiology, college of medicine, Idi-Araba, University of Lagos, Nigeria. The fungi were storted on Sabouraud dextrose agar (SDA) slants in the refrigerator, 4^0C, prior to use.

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The ethanol and aqueous extracts were carried out using modified methods of Saxena and Mathela (1996). The stem or roots of the different plants (chewing sticks) were chopped into tiny bits. They were then pounded with local pistil and mortar before blending with an electric blender.

Twenty grammes of the ground plant part was soaked separately in 100ml, 70% aqueous ethanol, and another twenty grammes in sterilised distilled water (100ml) for 24 hours. The fluids were then filtered using Whatman No. 1 filter paper. The ethanol and water extract were concentrated using a Rotatory evaporator at 40^0C. The concentrated extracts were the ethanol and aqueous extract of the plants. They were kept in the fridge prior to use.

Antifungal tests:

The paper disc diffusion method if Irobi and Daramola (1994) was modified here. Spore of conidia suspension of 10^5 – 10^7 cells, counted with haemocytometre were made. About 10ml Sabouraud dextrose agar, (SDA) water poured into Petri dishes and allowed to solidify. A micropipette was used to introduce 0.1ml of the spore or conidia suspensions on to the agar plate, and spread with glass spreading rod under sterile conditions. Sterilised disc (6mm, Whatman No AA2017006) were soaked in each of the extracts (100mg/ml) being assayed for 6 hours. Four of these soaked discs were spread on a fungal spore or conidia seeded plate with the help of sterile forceps. There was a control which contains the SDA and fungal inoculums but the discs were soaked in sterilised distilled water only without extracts. Three replicates were produced for each fungus. All the plates containing the discs were then incubated at 28 – 31^0C. Zone of inhibition was measured after 48 – 72 hours. Results were statistically analysed using standard deviation (S.D), analysis of variance (ANOVA, F – Test) and Duncan multiple range test (Parker, 1979).

Preliminary Phytochemical Studies:

Supporting phytochemical studies were carried out using methods described by Fadeyi *et al*., (1987) and Harbone, (1998). Basic phytochemical screening were performed on the chewing stick extracts. They were screened for the presence of anthocyanin, anthraquinone, alkaloids, flavonoids, phlobatarnnin, saponin, steroid and tannin.

RESULTS

The ethanolic, aquesous extract of each of the chewing sticks as well as the missed extract inhibited the growth of all the fungi tested, except the extract of *Anogeissus schimperi* which did not inhibit the growth of *C. albicans* (Tables 1 – 2). Only the extracts of *A. schimperi* had zone of the inhibition less than 10mm (Tables 1 and 2). The ethanol crude extracts of the chewing sticks had a greater zone of inhibition in comparison with the aqueous extract. Amongst the plant extracts *D. benthmianus* had the highest antifungal activity against *C. albicans* and *A. flavus*. The mixed extracts had the highest zone of inhibition on *M. gypseum* and *T. mentagrophytes*. There were significant differences between the zones of inhibition among the extracts of some of the plant part tested. The control, which was with sterilised distilled water, yielded no zone of inhibition.

Table 3 shows the phychemical compounds present in the aqueous and ethanol crude extracts of the four plants. Flavonoids was present in all four plants extracts. Tannin was present in all the extract except in the aqueous and ethanol extract of *Anogeissus schimperi*. Alkaloid was not present

in any of the plant extract. The ethanolic extracts had more phytochemical compounds than the aqueous extracts. The mixed extracts (ethanolic) and ethanolic extracts of *Vernonia amagdalina* had the highest range of phytochemical compounds (Table 3).

DISCUSSION

The result presented here shows the presence of antifungal substances in all the ethanol chewing stick extracts. The aqueous extracts had lower fungal inhibition which might be because the bioactive components were more soluble in the ethanol. This is in agreement with findings of Barnabas and Nagarajan (1988) on other plants. Of all the plant extract tested, *D. benthmianus* was the most active against the fungi tested, since it possessed the highest antifungal inhibition. *A. schimperi* extracts do not contain much phytochemical compound, especially tannin, which probably might be attributed to its low activity. Burapadaja and Bunchoo (1995) reported the presence of tannins in *Terminalia citrina* extracts and explained that the tannins inhibited cell wall formation in fungi leading to the death of the micro-organism. *A. schimperi* extracts might not contain potent antifungal substances even though Ugoji et al., (2000) reported that the aqueous extract contain antibacterial substances. This observation supported Valenciennes *et al.*, (1999), who reported that antibacterial agents might not necessarily be antifungal. It is of significance to note the high sensitivity of some of the fungi to the *D. benthmianus* extracts, because it is claimed locally that the use *D. benthmianus* might reduce or completely stop mouth odour. The mixed extracts (ethanol or aqueous) of all the chewing sticks proved to inhibit the fungi better than the extracts of most be due to the presence of more phytochemical compounds or bioactive components in the mixed extracts as observed in the result of the phytochemical study.

Conclusively, this study has shown the presence of antifungal substances in the chewing sticks. The concentration and composition of the bioactive substances may differ from one plant to another as indicated in the degree of potency and presence of phytochemical compounds. The regular use of the Nigerian chewing sticks may decrease the incidence of dental disease caused by microbes. The chewing stick of *Distemonathus benthmianus* because of its better antifungal properties might be recommended for good oral health.

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Table 1. Antifungal Activity of the Ethanol Crude Extracts of Four Nigerian Chewing Sticks.

	Zone of inhibition (mean ± S.D, mm			
Sample (100mg/ml) extracts	***Aspergillus flavus***	**Candida albicans**	**Microsporum gyseumm**	**Trichophyton mentagrophyte**
Control	0.00 ± 0.00a*	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Anogeissus schimperi	9.40 ± 0.21c	0.00 ± 0.00a	2.50 ± 0.20a	4.60 ± 0.22d
Distemonathus benthmianus	13.40 ± 0.14e	13.70 ± 0.15e	11.40 ± 0.18f	13.40 ± 0.17e
Vernonia amagdalina	12.30 ± 0.12f	12.16 ± 0.13f	11.60 ± 0.12f	11.50 ± 0.12f
Xanthoxyllum zanthoxyloides	10.90 ± 0.10g	11.20±0.11f,g	12.40 ± 0.14f	11.50 ± 0.12f
Mixed extracts	11.50 ± 0.13f	13.40 ± 0.18e	13.00 ± 0.17e	14.80 ± 0.14k

* Zone of inhibition with similar alphabets shows no significant difference p = 0.05

* Zone of inhibition with different alphabets shows significant difference p = 0.05

Table 2. Antifungal Activity of the Aqueous Crude Extracts of Four Nigerian Chewing Sticks.

	Zone of inhibition (mean ± S.D, mm			
Sample (100mg/ml) extracts	***Aspergillus flavus***	**Candida albicans**	**Microsporum gyseum**	**Trichophyton mentagrophyte**
Control	0.00 ± 0.00a*	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Anogeissus schimperi	8.30 ± 0.45c	0.00 ± 0.00a	2.20 ± 0.39a	3.50 ± 0.57d
Distemonathus benthmianus	12.00 ± 0.19f	12.62 ± 0.74f	10.60 ± 0.30g	12.60 ± 0.49f
Vernonia amagdalina	11.90 ± 0.92f	11.84 ± 0.16f	11.40 ± 0.20f	10.80 ± 0.40g
Xanthoxyllum zanthoxyloides	10.50 ± 0.05g	11.00 ±0.10f,g	11.90 ± 0.36f	11.30 ± 0.12f,g
Mixed extracts	11.30±0.18f,g	13.10 ± 0.90e	12.60 ± 0.17f	13.70 ± 0.47e

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Table 3. Phytochemical Compounds in the Crude Extracts (Aqueous and 70% Aqueous Ethanol) of Four Nigerian Chewing Sticks.

	Phytochemical Compound

Sample (100mg /ml) extracts	*Alkaloid*	*Antraquinone*	*Anthocy-anin*	*Flavo-noids*	*Phloba Tannin*	*Sapo-nin*	*Steroid*	*Tannin*
Anogeissus schimperi	–	–	–	+	+	–	+	–
Aqueous	–	+	+	+	+	+	+	–
Ethanol								
Distemonanthus benthmianus								
Aqueous	–	–	+	+	–	+	–	+
Ethanol	–	+	+	+	–	+	+	+
Vernonia amagdalina	–	–	–	+	+	–	–	+
Aqueous	–	+	+	+	+	+	+	+
Ethanol								
Xanthoxylum zanthoxyloides								
Aqueous	–	–	–	+	+	+	–	+
Ethanol	–	–	+	+	+	+	+	+
Mixed extracts								
Aqueous	–	–	+	+	+	+	+	+
Ethanol	–	+	+	+	+	+	+	+

(+) Presence of the phytochemical compound
(–) Absence of the phytochemical compound

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Table 2. Antifungal Activity of the Aqueous Crude Extracts of Four Nigerian Chewing Sticks.

	Zone of inhibition (mean ± S.D, mm			
Sample (100mg/ml) extracts	***Aspergillus flavus***	**Candida albicans**	**Microsporum gyseum**	**Trichophyton mentagrophyte**
Control	0.00 ± 0.00a*	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Anogeissus schimperi	8.30 ± 0.45c	0.00 ± 0.00a	2.20 ± 0.39a	3.50 ± 0.57d
Distemonathus benthmianus	12.00 ± 0.19f	12.62 ± 0.74f	10.60 ± 0.30g	12.60 ± 0.49f
Vernonia amagdalina	11.90 ± 0.92f	11.84 ± 0.16f	11.40 ± 0.20f	10.80 ± 0.40g
Xanthoxyllum zanthoxyloides	10.50 ± 0.05g	11.00 ±0.10f,g	11.90 ± 0.36f	11.30 ± 0.12f,g
Mixed extracts	11.30±0.18f,g	13.10 ± 0.90e	12.60 ± 0.17f	13.70 ± 0.47e

* Zone of inhibition with similar alphabets shows no significant difference p = 0.05

* Zone of inhibition with different alphabets shows significant difference p = 0.05

Table 3. Phytochemical Compounds in the Crude Extracts (Aqueous and 70% Aqueous Ethanol) of Four Nigerian Chewing Sticks.

	Phytochemical Compound							
Sample (100mg /ml) extracts	*Alkaloid*	*Antraquinone*	*Anthocy-anin*	*Flavo-noids*	*Phloba Tannin*	*Sapo-nin*	*Steroid*	*Tannin*
Anogeissus schimperi								
Aqueous	–	–	–	+	+	–	+	–
Ethanol	–	+	+	+	+	+	+	–
Distemonanthus benthmianus								
Aqueous	–	–	+	+	–	+	–	+
Ethanol	–	+	+	+	–	+	+	+
Vernonia amagdalina								
Aqueous	–	–	–	+	+	–	–	+
Ethanol	–	+	+	+	+	+	+	+

Xanthoxylum zanthoxyloides								
Aqueous	–	–	–	+	+	+	–	+
Ethanol	–	–	+	+	+	+	+	+
Mixed extracts								
Aqueous	–	–	+	+	+	+	+	+
Ethanol	–	+	+	+	+	+	+	+

(+) Presence of the phytochemical compound

(–) Absence of the phytochemical compound