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Antifungal activities of crude extracts of some Nigerian chewing sticks

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Preliminary screening of both methanol and aqueous extracts of the stem and root of *Distemonanthus benthmianus*, *Treculia africana, Garcinia kola* and *Anogessus leiocarpus* was carried out to test for antifungal activity. This was investigated using disc diffusion technique by measuring the zone of inhibition of the fungi after infusion with the plant extract. This produced definite antifungal activity against the dermatophytes used which were *Trichophyton mentagrophytes, Candida albicans* and *Aspergillus fumigatus. Distemonanthus benthmianus* and *Anogessus. leiocarpus* extracts exhibited antifungal activity at 0.97mg/ml concentration against *Candida albicans* and *Aspergillus fumigatus*. There was significant difference (P<0.05) in fungicidal activity in the methanolic and aqueous extracts. However, between the methanolic and aqueous extracts, there is no significant difference.

Key words: Antifungal, dermatophytes, crude extract, fungicidal activity.

INTRODUCTION

The use of chewing sticks is very popular in Africa, indeed in Nigeria; it is a common practice to see people in local communities using pieces of wood to clean their mouth early in the morning (Adekunle and Odukoya, 2006). This practice has been from time immemorial. It was stated that chewing sticks were once used by the Babylonians and later by the Egyptians, Greeks and Romans. The same report claimed that "toothbrushes" were also common in pre-Islamic Arabia, and that the use of the chewing stick fell out of favor with the advent of Europeans about 300 years ago, yet it is still popular in many parts of Africa, Asia, and the Middle East (William, 2003; Osho and Adelani 2012).

Despite the widespread use of toothbrushes and toothpaste, natural methods of tooth cleaning, using chewing sticks selected and prepared from the twigs, stems, or roots from a variety of plant species have been practiced for many years in Asia, Africa, the Middle East and the Americans (Wu et al., 2001). Darout et al. (2002) reported that selected clinical status have shown that chewing sticks, when properly used, can be as efficient as toothbrushes in removing dental plaque due to the combined effect of mechanical cleaning and enhanced salivation. It has also been suggested that antimicrobial substances that naturally protect plants against various invading microorganisms or other parasites may leach

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out into the oral cavity, and that these compounds may protect the mouth against carcinogenic aniodontopathic bacteria.

Today, chewing sticks are still used in many developing countries because of religion and/or tradition; and because of their availability, low cost and simplicity. The World Health Organization (WHO, 1987) also encourages their use. The year 2000 consensus report on oral hygiene states that chewing sticks may have a role to play in the promotion or oral hygiene, and that evaluation of their effectiveness warrants further research (Axelsson et al., 2002).

In the local communities around the southern part of Nigeria, the use of chewing sticks is very popular, for the cleaning of mouth as part of oral hygiene and also for medicinal purposes especially the use of these plants against oral cavity infections. Some plants used for such purpose include *Distemonanthus benthmianus, Treculia africana, Garcinia kola* and *Anogeissus leiocarpus*.

D. benthomianus is in the family Leguminosae, locally known as "Ayan" (Yoruba) in South Western Nigeria. The twig is used locally or medicinally to stop mouth odour, in cabinetry and decorative veneers (Christine 2003). *T. africana* belongs to the family Moraceae, traditionally called "Ifon" by the Yorubas in Western part of Nigeria where the plant is widely cultivated (Ajayi et al., 2013). *G. kola*, a multi-purpose fruit tree, produces fruit, seeds, roots and stem which are extensively used in Nigeria, Ghana and other West Africa countries for dental care (Emmanuel and Roy, 2001). *G. kola* is a member of the family Sterculiaceae, known locally by Yoruba as “Orogbo” and commonly found in the middle belt areas of Nigeria. *A. leiocarpus*, locally called "Ayin" in Yoruba belongs to the family Combretaceae.

The oral cavity is possible reservoir for microorganisms, both communal and acquired (Hilal and Nizar, 2012). The mouth consists of multiple habitats offering ecological niches to a variety of organisms. Bacteria have been given a notable attention. Fungi have very scanty report despite the documented reports of their association with tooth root infection. Prominent among these reports include Cannon et al. (1995), Waltimo et al. (1997) and Baumgartner et al. (2000). Common trend that runs through the reports is the fact that *Candida albicans* is associated with root canal infection and a constant inhabitant of oral cavity. Another oral infection, thrush is a fungal infection caused by *C. albicans* which forms a white film that irritates the gum and coats the tongue and corners of the mouth and leaves a bleeding surface. Although fungi related infections may not be as common as bacterial infections, they are more difficult to treat especially in patients whose immunity has been compromised. This is one of the reasons the exploration and development of natural products with potent antifungal activity is important (Brooks and Etim, 2004).

The aim of this research was to investigate the antifungal activities of crude extract of *D. benthmianus, G. kola, T. africana,* and *A. leiocarpus* which are commonly used as chewing sticks.

MATERIALS AND METHODS

Source of materials

The stem or root part used as chewing stick were purchased from the local markets and were authenticated at the Department of Pharmacognosy, College of Medicine, Lagos University Teaching Hospital (LUTH) Idi-Araba, Lagos. Information collected from traditional healers and local hawkers from Oyo, Osun, Ogun Ondo and Lagos States of Nigeria included parts of plants used and the preparation and dosage. Local names were also collected. The use of questionnaires and interviews were employed. *Aspergillus fumigatus, C. albicans*, and *Trichophvton mentagrophytes* with accession numbers (014, 003, 016) respectively were all collected from the Department of Medical Microbiology, Lagos University Teaching Hospital (LUTH) Idi-Araba, Lagos. The fungi were stored in Sabouraud Dextrose Agar (SDA) slants at 4°C prior to use, the fungi are clinical samples.

Preparation of discs

Whatman filter paper number one was punched into 200 pieces, 0.6 cm diameter circular discs. This was done using a perforator, the discs were then sterilized. Method of Okigbo et al. (2015) as modified was employed.

Extract preparation

The stem or root of the different plants used as chewing sticks were rinsed under running tap water after which they were dried and chopped into tiny bits. They were then pounded into powdery form with local pistil and mortar. Two hundred (200) grams of the ground plant sample where split into 100 grams for each plant and soaked separately in 70% methanol. For aqueous extract, the plant samples were soaked in water for 5 days (120 h) at room temperature to allow full extraction of all active ingredients (Akande and Ajao, 2011; Osho and Adelani, 2012). The plant materials were placed in a sterilized clinical tube. The fluid were filtered using Whatman filter paper number one (Whatman International Limited, England), after the fifth day.

The extracts were concentrated through evaporation to dryness, under pressure at 50°C using an improvised rotary evaporator (Bankole et al., 2012). The concentrated extract was later kept in the refrigerator for further use.

Culture media

Sabouraud Dextrose Agar (SDA) was used for the culturing of fungi. It was prepared according to the manufacturer’s instruction.

Screening for antifungal activity

Liquid inoculum of test fungi was prepared. This was obtained by pouring sterile distilled water into actively growing fungal plates. The fungal inoculum in suspension was carefully dropped onto the already prepared agar plates using sterilized hockey sticks. The pre-sterilized discs were soaked separately in 100 ml of sterile distilled water as control and in both the aqueous and methanol

Table 1. Information obtained from local hawkers

Sample plant	Locality collected	Part of plant	Preparation	Dosage (Tea cup)
Distemonanthus benthmianus	Tejuosho Market	Stem	Wash under running water and chew both the bark and the wood itself	Two times daily (morning and night)
Treculia africana	Ketu Market	Stem	Wash before usage	Two times daily until no more pain
Garcinia kola	Ketu Market	Root	Rinse thoroughly before chewing	Two times daily
Anogessus leiocarpus	Ojo market	Root	Rinse thoroughly before chewing	Two times daily (morning and night)

Table 2. Antifungal activity of methanol extract {zone of inhibition mean ± s.d. (mm)}.

Extract	Organism used	Concentration (mg/ml)						
		0.97	0.49	0.24	0.12	0.06	0.03	0.015
T. Africana	*T. mentagrophyte*	12.00±0.01^{a}	10.00±0.60^{a}	8.00±0.50^{a}	7.00±0.10^{a}	5.25±0.05^{a}	2.00±0.50^{a}	0.01±0.01^{a}
	C. albicans	10.00±0.01^{b}	9.25±0.20^{a}	7.00±0.40^{ac}	5.25±0.10^{b}	4.00±0.20^{b}	2.00±0.20^{a}	0.01±0.01^{a}
	A. fumigates	10.50±0.50^{b}	8.00±0.70^{a}	6.25±0.05^{bc}	4.00±0.10^{c}	3.00±0.10^{c}	0.01±0.01^{b}	0.01±0.01^{a}
G. kola	*T. mentagrophyte*	11.25±0.10^{a}	8.00±0.10^{a}	6.25±0.05^{a}	5.25±0.15^{a}	0.02±0.03^{a}	0.02±0.01^{a}	0.01±0.01^{a}
	C. albicans	9.25±0.10^{b}	7.00±0.20^{b}	6.25±0.10^{a}	4.00±0.10^{b}	2.00±0.20^{b}	0.03±0.02^{a}	0.01±0.01^{a}
	A. fumigates	9.25±0.10^{b}	7.00±0.20^{b}	5.25±0.15^{b}	5.25±0.15^{a}	3.00±0.10^{c}	2.00±0.20^{a}	0.01±0.01^{a}
A. leiocarpus	*T. mentagrophyte*	10.00±0.10^{a}	6.25±0.05^{a}	5.25±0.05^{a}	4.00±0.10^{a}	1.00±0.10^{a}	0.03±0.01^{a}	0.01±0.01^{a}
	C. albicans	11.25±0.10^{b}	9.25±0.01^{b}	6.25±0.05^{b}	3.00±0.20^{b}	2.00±0.20^{b}	0.02±0.01^{a}	0.01±0.01^{a}
	A. fumigates	10.00±0.10^{a}	7.00±0.10^{c}	5.25±0.05^{a}	4.00±0.10^{a}	2.00±0.20^{b}	2.00±0.20^{b}	0.01±0.01^{a}
D. *benthmianus*	*T. mentagrophyte*	10.00±0.10^{a}	8.00±0.10^{a}	6.25±0.10^{a}	2.00±0.20^{a}	2.00±0.20^{a}	2.00±0.20^{a}	0.01±0.01^{a}
	C. albicans	12.30±0.05^{b}	10.00±0.10^{b}	8.00±0.10^{b}	4.00±0.10^{b}	4.00±0.10^{b}	2.00±0.20^{a}	0.01±0.01^{a}
	A. fumigates	11.25±0.10^{c}	7.00±0.10^{c}	5.25±0.05^{c}	3.00±0.20^{c}	3.00±0.20^{c}	2.00±0.20^{a}	1.00±0.10^{a}
Control	*T. mentagrophyte*	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	C. albicans	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	A, fumigatus	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Means with the same superscript alphabets and in the same column are not significantly different (p>0.05).

extracts of all the four chewing sticks under study. The discs were soaked for 24 h, after which they were removed using sterile forceps. For each extract, 4 discs were placed separately in the marked Petri-dishes. Seven plates were used for each fungus. Observation was taken at every 24 h to monitor any zone of inhibition on the plates, and measurements were taken as zone of inhibition showed.

Statistical analysis

Data were expressed as mean ± standard deviation. Data were subjected to one-way analysis of variance (ANOVA) (SPSS for Windowns version 17.0) and where there is significant variation, Fisher's Least Significant Difference (LSD) was applied at α = 0.05.

RESULTS AND DISCUSSION

The information collected from traditional healers and local hawkers shows that the parts of the plants used as chewing sticks includes the stem and the root (Table 1). The fungicidal activity of the plant extracts on the test organisms is shown in (Tables 2 and 3). Both the methanol and aqueous extracts of each of the chewing sticks inhibited the growth of all the fungi. This is in line with previous studies by Bankole et al. (2012) and Osho and Adelani (2012) where both aqueous and ethanolic extracts exhibited growth inhibition against all tested organisms.

Only the extract of *G. kola* had the zone of inhibition less than 10 mm for all the fungi tested at 0.97 mg/ml on both methanol and aqueous extracts and a Minimum Inhibitory Concentration (MIC) of 0.06 mg/ml on methanol and 0.12 mg/ml in aqueous extracts for *G. kola* against *T. mentagrophytes*.

Among the plant extracts, *D. benthmianus* and *A. leiocarpus* had the highest antifungal activity against *C. albicans* on aqueous extract with mean range of 13.0±0.10 mm at 0.97 mg/ml, with a minimum inhibitory

Table 3. Antifungal activity of aqueous extract {zone of inhibition mean ± s. d. (mm)}.

Extract	Organism used	Concentration (mg/ml)						
		0.97	0.49	0.24	0.12	0.06	0.03	0.015
T. Africana	*T. mentagrophyte*	11.25±0.10^{a}	9.25±0.20^{a}	8.00±0.10^{a}	0.01±0.01^{a}	0.01±0.01^{a}	0.02±0.01^{a}	0.01±0.01^{a}
	C. albicans	10.00±0.10^{b}	9.25±0.20^{a}	7.00±0.10^{b}	5.25±0.15^{b}	4.00±0.10^{b}	2.00±0.20^{b}	0.01±0.01^{a}
	A. fumigatus	10.00±0.10^{b}	8.00±0.10^{b}	6.25±0.05^{c}	4.00±0.10^{c}	3.00±0.20^{c}	0.03±0.02^{a}	0.01±0.01^{a}
G. kola	*T. mentagrophyte*	11.15±0.10^{a}	8.00±0.10^{a}	8.00±0.20^{a}	4.00±0.10^{a}	0.01±0.01^{a}	0.01±0.01^{a}	0.01±0.01^{a}
	C. albicans	9.25±0.20^{b}	7.00±0.10^{b}	6.25±0.05^{b}	4.00±0.10^{a}	0.01±0.01^{a}	0.01±0.01^{a}	0.01±0.01^{a}
	A, fumigatus	9.25±0.20^{b}	9.25±0.20^{c}	8.00±0.20^{a}	4.00±0.10^{a}	0.01±0.01^{a}	0.01±0.01^{a}	0.01±0.01^{a}
A. leiocarpus	*T. mentagrophyte*	10.00±0.10^{a}	7.00±0.10^{a}	6.25±0.05^{a}	3.00±0.20^{a}	2.00±0.20^{a}	0.01±0.01^{a}	0.01±0.01^{a}
	C. albicans	13.00±0.10^{b}	10.00±0.10^{b}	7.00±0.10^{b}	4.00±0.10^{b}	2.00±0.20^{a}	0.01±0.01^{a}	0.01±0.01^{a}
	A. fumigatus	11.25±0.10^{c}	8.00±0.10^{c}	6.25±0.05^{a}	4.00±0.10^{b}	2.00±0.20^{a}	0.01±0.01^{a}	0.01±0.01^{a}
D. benthmianus	*T. mentagrophyte*	10.00±0.10^{a}	8.00±0.10^{a}	6.25±0.05^{a}	3.00±0.20^{a}	2.00±0.20^{a}	2.00±0.20^{a}	0.01±0.01^{a}
	C. albicans	13.00±0.10^{b}	10.00±0.10^{b}	8.00±0.10^{b}	6.25±0.05^{b}	4.00±0.10^{b}	2.00±0.20^{a}	0.01±0.01^{a}
	A. fumigatus	11.25±0.10^{c}	9.25±0.20^{c}	7.00±0.10^{c}	5.25±0.15^{c}	3.00±0.10^{c}	2.00±0.20^{a}	1.00±0.10^{b}
Control	*T. mentagrophyte*	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	C. albicans	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	A. fumigatus	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Means with the same superscript alphabets and in the same column are not significantly different (p>0.05).

concentration of 0.03 mg/ml in *A. leiocarpus* and no MIC in *D. benthmianus.* In the methanol extract, the highest antifungal activity was observed with *D. benthmianus* and *T. africana* against *C. albicans* and *T. mentagrophytes* respectively, at 0.97 mg/ml for both samples.

For both methanol and aqueous extract, observation shows that the aqueous extract of *D. benthmianus* and *A. leiocarpus* were the most effective of all four chewing sticks investigated for potential treatment of fungal infections in the oral cavity. Ogundiya et al. (2006) also reported that *A. leiocarpus* showed a higher antimicrobial activity among two other chewing sticks used in that research. The control which was sterile distilled water gave no zone of inhibition against any of the dermatophytes used.

The result presented here showed the presence of antifungal substances in the methanol and aqueous extracts of the chewing sticks used, *D. benthmianus* and *A. leoicarpus* possessed the highest antifungal activity on the test fungi, while *G. kola* showed the least antifungal activity, which properly might be attributed to its low activity. A similar observation was made by Ogundiya et al. (2006) where the extracts of the three chewing sticks used in the study had no activity on *C. albicans.* Ikenebomeh and Methitiri (1988) also reported that extract of *Casia alata* at various dilutions did not inhibit the growth of *C albicans*, indicating that it might be resistant to some antimycotic agents. However, the result from this study agrees with that of Adejumobi et al. (2008) and Osho and Adelani (2012) that *A. leiocarpus* is active against *C. albicans.*

For both methanolic and aqueous extracts, at concentrations of 0.03 and 0.015 mg/ml, generally there is no significant difference (p>0.05) between the activity of the plant extracts against the test

organisms. However, for *T. africanum* against *A. fumigatus* and *A. leiocarpus* against *A, fumigatus* at 0.03 mg/ml, there is significant difference ($p<0.05$) in the activity of the methanolic extracts. Also for aqueous extracts, *T. africanum* against *C. albicans* and *D. benthmianus* against *A. fumigatus* at 0.03 and 0.015mg/ml respectively showed significant difference.

Conclusion

The regular use of the African chewing sticks may decrease the incidence of gingivitis and dental carriers by controlling plague formation. Although the report of William (2003) demonstrated that the dental caries is an infection, and so extracts of some sticks have been shown to have antibacterial and antifungal properties to act against these infections in the oral cavity. From this study, it can be deduced that these chewing sticks have the potential of controlling infections caused by these fungi.

Conflict of Interests

The authors have not declared any conflict of interests.

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