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In Vitro Antimicrobial Activity and Phytochemical Composition of *Dichrostachys cinerea*

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ABSTRACT

Studies on phytochemical composition and antimicrobial activity of aqueous and ethanol extracts of roots and stems of *Dichrostachys cinerea* against clinical isolates of *Candida albicans*, *Streptococcus mutans* and *Staphylococcus saprophyticus* were carried out. The main phytochemicals present in the stem and roots included alkaloids, saponins and tannins, with roots containing the greater share. Steroids and cyanoglycoside were present in the stem. Both ethanol and aqueous extracts of the tested chewing stick inhibited the growth of all three tested microorganisms. There was no significant difference ($P>0.05$) between the inhibitory effect of the aqueous and ethanolic extracts of the roots of *D. cinerea* on *C. albicans*. However, the ethanolic extract of the stem exhibited a significantly higher ($P<0.05$) bioactivity than that exhibited by the ethanolic extract of the root. The pattern of inhibition of *S. mutans* and *S. saprophyticus* by the extracts were similar. Solvent used in extraction did not produce any significant effect ($P>0.05$), but the stem extracts exhibited a significant inhibition ($P<0.05$) compared to the root extract. Our results clearly show that *D. cinerea* is a potential candidate plant that could be used in the development of a dentifrice.

Keywords: *Candida albicans*, chewing stick, dentifrice, oral pathogen, *Staphylococcus saprophyticus*, *Streptococcus mutans*

INTRODUCTION

The use of chewing sticks is deeply rooted in many cultures. The Babylonians recorded the use of chewing sticks in 7000 BC and its use ultimately spread throughout the Greek and Roman empires. Chewing sticks were also used by Egyptians, Jews and in the Islamic Empires (Almas and Al Lafi 1995). Lewis and Elvin-Lewis (1977) stated that the use of chewing sticks persists today among many African and southern Asian communities as well as in isolated areas of tropical America and southern United States. It is believed that the counterpart of the modern day toothbrush was unknown in Europe until about 300 years ago (Almas and Al Lafi 1995). However, a more recent report has indicated that a chewing stick is more effective than toothbrushing for reducing plaque and gingivitis, especially when preceded by professional instruction as to its correct application (Al-Otaibi *et al.* 2003).

There are various plants which are used as chewing sticks in West Africa. Lime tree (*Citrus aurantafolia*) and orange tree (*Citrus sinensis*) sometimes serve as chewing sticks. The roots of senna (*Cassia vinnea*) were used by African Americans and those of African laburn (*Cassia sieberianba*) are used in Sierra Leone. Neem (*Azadirachta indica*) is widely used to provide chewing sticks in the Indian sub-continent (Almas 1993). In Nigeria, the major plants that serve as source of chewing sticks include *Terminalia glaucescens*, *Anogeissus leiocarpus* and *Pseudocedrella kotschyi*. However, the use of *Dichrostachys cinerea* as a chewing stick is common in some parts of south-western Nigeria.

D. cinerea is a semi-deciduous tree up to 7 m tall with an open crown. Its native range includes Cameroon, Ethiopia, Ghana, Nigeria, Swaziland, Uganda and Zambia, among others. *D. cinerea* is widely use as a source of food, fuel, fibre and timber. In medicine, its bark is use to treat dysentery, headaches, toothaches, and elephantiasis. Root infusions are taken for leprosy, syphilis, coughs, as an antihelmintic, purgative and strong diuretic (World Agroforestry 2008).

Reports on the antimicrobial activity of the major chewing sticks in Nigeria against oral pathogens have been documented (Rotimi *et al.* 1988; Akande and Hayashi 1998; Adekunle and Odukoya 2006; Ogundiya *et al.* 2006) with virtually no documentation on the antimicrobial activity of *D. cinerea* on oral microbes. The aim of the present paper was to provide information on the phytochemical composition and antimicrobial activity of aqueous and ethanol extracts of *D. cinerea* on some common oral pathogens such as *Candida albicans*, *Streptococcus mutans* and *Staphylococcus saprophyticus*.

MATERIALS AND METHODS

Plant collection and pre-extraction preparation

Different plant parts such as leaves, stem, root and fruit of *D. cinerea* were collected from Oke-Ogun axis of south Western Nigeria (a woody savannah vegetation). The plant was identified by a plant Taxonomist at the Forestry Research Institute of Nigeria, Ibadan, Nigeria. All the plant parts were collected for identification purpose, only the stem and root were analysed. The stem and roots of a single plant was sun-dried for seven days then grinded using a pestle and wooden mortar.

Extraction procedure

The ethanol extract preparation was done as previously described by Ogundiya *et al*. (2006). However, for water extraction, the procedure was basically the same except that soaking was done for 48 h and the filtrate was evaporated to dryness. The crude extracts were reconstituted into an aqueous solution using sterile distilled water to obtain extract concentrations of 0.4 and 0.2 g/ml.

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Short Communication

Microorganisms

Pure cultures of *Candida albicans*, *Streptococcus mutans and Staphylococcus saprophyticus* isolated from patients with dental diseases were obtained from the Medical Microbiology Department of the University College Hospital (UCH) Ibadan, Nigeria. Bacterial cultures were maintained on Nutrient agar slants and the fungus on Potato dextrose agar slants, both at 6-8°C (Acheampong *et al.* 1988).

Phytochemical studies

Both qualitative and quantitative analyses of the phytochemicals present were carried out using methods described by Fadeyi *et al.* (1987) and Harbone (1998).

Antimicrobial assay

The antimicrobial activity of different concentrations of both ethanolic and aqueous extracts was determined by a modified agar-well diffusion method of Perez *et al.* (1990) as described by Popoola *et al.* (2007). The bacterial plates were incubated at 37°C (fungal plates at 28°C) and the zone of inhibition measured in mm after 24, 48 and 72 h of growth. A control experiment was set up by using an equal amount of sterile distilled water in place of different extract concentrations.

Statistical analysis of data

Data were expressed as mean ± standard deviation. The statistical significance of differences was assessed using analysis of variance. A two-tailed P value of less than 0.05 was considered to be statistically significant. Values that were significantly different were separated using Duncan's Multiple Range test using SPSS for Windows ver. 11.0 statistical package.

RESULTS

Table 1 shows the result of phytochemical analysis of the stem and root of *D. cinerea.* The prominent phytochemicals present in the stem and root of the tested plant included alkaloids, saponins and tannins, with the root contained a significantly higher amount (P<0.05) of these phytochemicals. Steroids were present in appreciable amounts in the stem while the root contained only trace amounts. In a similar trend, cyanoglycosides were present in the stem but not in the root.

The results of the antimicrobial assay of both the root

Table 1 Results of the quantitative estimation of the phytochemicals (mg/100 g) present in the ethanol extracts of *Dichrostachys cinerea*.

	Alkaloids	Steroids	Phenols	Tannins	Cyanoglycosides	Saponins
Stem	105.5 ± 1.5 b	11.5 ± 2.5	Trace	49.2 ± 3.5 b	1.5 ± 0.1	108.3 ± 0.7 b
Roots	127.4 ± 0.9 a	Trace	Trace	80.0 ± 1.9 a	ND	117.6 ± 0.4 a

Values are mean of triplicate determinations. ND implies not detected.
Within column values with different letters are statistically significant (P<0.05)
Means were separated using T- test.

Table 2 Inhibition of *Candida albicans* by aqueous and ethanol extract of *Dichrostachys cinerea*.

Plant part	Incubation period (h)	Aqueous extract		Ethanolic extract	
		Extract concentration (g/ml)			
		0.4	0.2	0.4	0.2
Roots	24	22.5 ± 0.5 c	17.5 ± 2.5 c	24.5 ± 2.5 c	18.5 ± 3.0 d
	48	32.0 ± 2.0 a	32.0 ± 1.0 a	19.5 ± 2.0 d	18.0 ± 1.0 d
	72	20.5 ± 0.5 c	17.5 ± 0.5 c	18.5 ± 0.5 d	18.0 ± 3.0 d
Stem	24	22.5 ± 0.5 c	17.5 ± 2.5 c	35.0 ± 0.0 a	35.0 ± 0.0 a
	48	29.5 ± 3.5 a	31.0 ± 4.0 a	35.0 ± 0.0 a	30.5 ± 3.5 b
	72	26.0 ± 1.6 b	23.0 ± 2.7 b	29.5 ± 4.5 b	27.0 ± 0.0 bc

Values are mean ± standard deviation (n=3)
Within column values with different letters are statistically significant (P<0.05)
Means were separated using Duncan's Multiple Range test.

Table 3 Inhibition of *Streptococcus mutans* by aqueous and ethanol extract of *Dichrostachys cinerea*

Plant part	Incubation period (h)	Aqueous extract		Ethanolic extract	
		Extract concentration (g/ml)			
		0.4	0.2	0.4	0.2
Roots	24	39.0 ± 0.0 a	28.5 ± 1.5 a	35.0 ± 0.0 a	32.5 ± 2.5 a
	48	32.5 ± 1.5 bc	28.0 ± 1.5 a	20.5 ± 0.0 c	20.5 ± 0.0 b
	72	16.5 ± 1.5 d	14.0 ± 1.0 b	18.5 ± 5.5 c	13.5 ± 0.5 c
Stem	24	39.0 ± 0.0 a	28.5 ± 1.5 a	30.0 ± 0.0 b	30.0 ± 0.0 a
	48	35.0 ± 4.0 b	28.0 ± 0.0 a	35.0 ± 5.0 a	30.5 ± 6.5 a
	72	31.0 ± 2.0 bc	27.1 ± 0.3 a	31.0 ± 7.0 b	30.5 ± 5.0 a

Values are mean ± standard deviation (n=3)
Within column values with different letters are statistically significant (P<0.05)
Means were separated using Duncan's Multiple Range test.

Table 4 Inhibition of *Staphylococcus saprophyticus* by aqueous and ethanol extract of *Dichrostachys cinerea*

Plant part	Incubation period (h)	Aqueous extract		Ethanolic extract	
		Extract concentration (g/ml)			
		0.4	0.2	0.4	0.2
Roots	24	35.0 ± 5.0 a	25.5 ± 2.5 b	27.0 ± 0.0 bc	26.5 ± 0.5 bc
	48	28.5 ± 2.5 b	26.0 ± 0.0 b	26.0 ± 1.0 c	26.5 ± 1.5 bc
	72	21.0 ± 1.0 c	15.0 ± 0.0 c	26.0 ± 1.5 c	25.0 ± 0.0 c
Stem	24	35.0 ± 5.0 a	25.5 ± 2.5 b	39.0 ± 1.0 a	37.6 ± 2.5 a
	48	35.5 ± 0.0 a	31.0 ± 1.0 a	29.5 ± 0.5 b	28.0 ± 1.0 b
	72	26.0 ± 0.1 b	24.0 ± 3.0 b	28.5 ± 1.5 b	23.5 ± 0.0 d

Values are mean ± standard deviation (n=3)
Within column values with different letters are statistically significant (P<0.05)
Means were separated using Duncan's Multiple Range test.

and stem extracts of *D. cinerea* are presented in **Tables 2-4**. At the tested concentrations, results obtained indicated that both ethanolic and aqueous extracts of the tested chewing sticks had an inhibitory effect on the growth of the three tested microorganisms. There was no significant difference ($P>0.05$) between the inhibitory effect exhibited by aqueous and ethanolic extracts of the roots of *D. cinerea* on *Candida albicans*. However, the ethanolic extract of the stem exhibited a significantly higher ($P<0.05$) bioactivity than that exhibited by the ethanolic extract of the root.

The result of antimicrobial assay of the different extracts of *D. cinerea* on *Streptococcus mutans* is shown in **Table 3**. ANOVA test of the data depicted that the solvent used in the extraction process did not produce any significant effect ($P>0.05$) on the bioactivity of the extract, although the stem extracts produced a significant effect ($P<0.05$) compared to the bioactivity exhibited by the root extract. The sensitivity of *Staphylococcus saprophyticus* to the aqueous and ethanolic extracts of the root and stem of the tested plant is presented in **Table 4**. Statistical analysis of the data obtained depicted that the patterns of sensitivity of *S. saprophyticus* to both aqueous and ethanolic extracts was similar to those observed for *C. albicans* and *Strep. mutans*.

DISCUSSION

Well known examples of constitutive plant compounds with antimicrobial properties include phenols, unsaturated lactones, saponins, cynanogenic glycosides, glucosinates, alkaloids, tannins, and linoleic and stearic acids (Ingham 1973; Osbourn 1996; Darout *et al*. 2000; Abd El Rahman *et al.* 2003). The presence of some of these phytochemicals in an appreciable amount in the investigated plant parts may have contributed to the observed anti-oral pathogenic activities of the stem and root extracts of *D. cinerea.* The alkaloid, saponin and tannin contents of the stem extract were lesser than those of the root extract, although the former had a significantly higher antimicrobial activity. It seems that the additional bioactivity contributed by steroids and cyanoglycosides might have been responsible for this. This becomes more plausible as cyanoglycosides and steroids have been reported to have antimicrobial activity (Ingham 1973; Osbourn 1996).

Many studies have demonstrated antimicrobial, anticaries, anti-periopathic and antifungal properties of both aqueous and ethanol extracts of various chewing sticks (Buada and Boak-Yiadom 1973; Rotimi *et al.* 1988; Akande and Hayashi 1998; Ugoji *et al*. 2000; Adekunle and Odukoya 2006). Prominent examples of such chewing sticks include *Anogeissus leiocarpus*, *Terminalia glaucescens*, and *Pseudocedrela kotschyi.* Results from the present study have placed *D. cinerea* in the same group of those other chewing sticks.

The African continent is a continent endowed with some of the richest biodiversity in the World, with many food plants used as herbs, health foods and for therapeutic purposes. In addition, many species of this biodiversity have been found to be useful in traditional medicine for prophylaxis and cure of diseases (Iwu 1993). Kasilo (2001) stated that increase in the patronage of herbal medicine is likely to continue because of global economic downturn and as bodies like the World Health organization African Region continue to advocate for its promotion and integration in the National Health Systems. In a related development, Killoy (1998) submitted that a recent trend is to employ local antimicrobial delivery in the treatment of periodontitis. Alkaloid extract of *Sanguinaria canadensis* incorporated into various dentifrices and oral rinses have been shown to possess broad-spectrum in vitro activity against a wide variety of microorganism (Dzink and Socransky 1985). Similarly, fagaronine, a compound extracted from *Fagara zanthoxyloides* (a Nigerian chewing stick) has been shown to provide beneficial effects for the oral hygiene of some rural natives (Odebiyi and Sofowora 1979). Results from this study have clearly shown that *D. cinerea* is a potential candidate plant that could be used in the development of dentifrice.

REFERENCES

Abd El Rahman HF, Skaug N, Whyatt A (2003) Volatile compounds in crude *Salvadorapersica* extracs. *Pharmaceutical Biology* **41**, 392-404

Acheampong YB, El-Mahmood AM, Olurinola PF (1988) The antibacterial properties of the liquid antiseptic TCP. *Indian Journal of Pharmaceutical Science* **3**, 183-186

Adekunle AA, Odukoya KA (2006) Antifungal activities of ethanol and aqueous crude extracts of four Nigerian chewing sticks. *EthnoBotanical Leaflet.* Available online: http://www.siu/~ebl/leaflets

Akande JA, Hayashi Y (1998) Potency of extract contents from selected tropical chewing sticks against *Staphylococcus aureus* and *Staphylococcus auricularis*. *World Journal of Microbiology and Biotechnology* **14**, 235-238

Almas K (1993) Miswak (Chewing stick) and its role in oral health. *Postgrad Dentist Middle East* **3**, 214-218

Almas K, Al Lafi TR (1995) The natural toothbrush. *World Health Forum* **16**, 206-210

Al-Otaibi M, Al-Harthy M, Soder B, Gustafsson A, Angmar-Mansson B (2003) Comparative effect of chewing sticks and toothbrushing on plaque removal and gingival health. *Oral Health and Preventive Dentistry* **1(4)**, 301-307

Buada CV, Boakye-Yiadom K (1973) The antibacterial activity of some Ghanian chewing sticks. *Ghana Pharmaceutical Journal* **1**, 150-151

Darout I, Christy A, Skaug N (2000) Identification and quantification of some potentially antimicrobial anionic components in Miswak extract. *Indian Journal of Pharmacology* **32**, 11-14

Dzin JL, Socransky SS (1985) Comparative *in vitro* activity of sanguinarine against oral microbial isolates. *Antimicrobial Agents Chemotherapy* **27**, 663-665

Fadeyi MO, Adeoye AO, Olowokudejo ID (1987) Epidermal and phytochemical Studies in the genus *Bohervia* (Nyctaginaceae) in Nigeria. *International Journal of Crude Drug Research* **27**, 178-184

Harbone JB (1998) *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (3rd Edn), Chapman and Hill, London, 279 pp

Ingham JL (1973) Disease resistance in higher plants. The concept of pre-infectional and post-infectional resistance. *Phytopathology Journal* **78**, 314-335

Iwu MM (1993) *Handbook of African Medicinal Plants*, CRC Press, Inc., FLA, p 1

Kasilo O (2001) Integration of traditional medicine in national health systems. *World Health Organisation Regional Meeting on Traditional Medicine*, Harare. 26-29 November 2001

Killoy W (1998) Chemical treatment of periodontitis: local delivery of antimicrobials. *International Dental Journal* **48 (Suppl 1)**, 305-315

Lewis WH, Elvin-Lewis MPF (1977) *Medical Botany: Plants Affecting Man's Health*, John Wiley and Sons, New York, pp 226-270

Odebiyi OO, Sofowora EA (1979) Antimicrobial alkaloids from Nigerian chewing stick (*Fagara zanthoxyloides*). *Planta Medicine* **36**, 204-207

Ogundiya MO, Okunade MB, Kolapo AL (2006) Antimicrobial activities of some Nigerian chewing sticks. *EthnoBotanical Leaflet.* Available online: http://www.siu/~ebl/leaflets

Osbourn AE (1996) Preformed antimicrobial compounds and plant defense against fungal attack. *The Plant Cell* **8**, 1821-1831

Perez C, Pauli A, Bazerque P (1990) An antibiotic assay by agar-well diffusion method. *Acta Biologiae et Medicine Experimentalist* **15**, 113-115

Popoola TOS, Yangomodu OD, Akintokun AK (2007) Antimicrobial activity of cassava seed oil on skin pathogenic microorganisms. *Research Journal of Medicinal Plant* **1(2)**, 60-64

Rotimi VO, Laughon BE, Bartlett JG, Mosadomi HA (1988) Activities of Nigerian chewing sticks extracts against bacteriodes-gingivalis and bacteriodes-melaninogenicus. *Antimicrobiological Agents and Chemotherapy* **32**, 598-600

Ugoji E, Egwari LO, Obisesan B (2000) Antibacterial activities of aqueous extracts of ten African chewing sticks on oral pathogens. *Nigerian Journal of Internal Medicine* **3(1)**, 7-11

World Agroforestry Centre (2008) Agroforestree Database. http://www.worldagroforestrycentre.org/sites/treedbs/aft/speciesPrinterFriendly.asp?Id=675