Original Article

Antimicrobial potential of *Dialium guineense* (Wild.) stem bark on some clinical isolates in Nigeria

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Abstract

Context: The persistent increase in the number of antibiotic-resistant strains of microorganisms has led to the development of more potent but also more expensive antibiotics. In most developing countries of the world these antibiotics are not readily affordable, thus making compliance difficult. This calls for research into alternative sources of antimicrobials. *Dialium guineense* is a shrub of the family Leguminosae. Its stem bark is used for the treatment of cough, toothache, and bronchitis. **Aims**: Despite the acclaimed efficacy of *D guineense*, there is no scientific evidence in its support. This work was carried out to assess the antimicrobial activity of *D guineense in vitro* against some clinical isolates. **Materials and Methods**: *D guineense* stem bark was collected and 50 gm of air-dried and powdered stem bark of the plant was soaked for 72 hours in 1 l of each of the six solvents used in this study. Each mixture was refluxed, agitated at 200 rpm for 1 hour, filtered using Whatman No. 1 filter paper and, finally, freeze dried. The extracts were then tested for antimicrobial activity using the agar diffusion method. **Results**: The highest percentage yield of 23.2% was obtained with ethanol. Phytochemical screening showed that *D guineense* contains *anthraquinone*, alkaloids, flavonoids, tannins, and saponins. The antimicrobial activity of the extracts revealed a broad spectrum of activity, with *Salmonella typhi* and Staphylococcus aureusa showing the greatest zones of inhibition (18.0 mm). Only Candida *albicans* among the fungi tested was inhibited by the extract. The greatest zone of inhibition among the fractions was 16.0 mm. *D guineense* exhibited bactericidal activity at the 7th and 9th hours against *Streptococcus pneumoniae* and *S. aureus* 25923 while the 10th hour against *S. typhi* and *C. albicans*. The greatest activity was noted against *S pneumoniae*, where there was reduced viable cell count after 6 hours of exposure. **Conclusion**: Stem bark extract of *D guineense* (Wild.) has the potential to be developed into an antimicrobial agent

Key words: *Dialium guineense*, antimicrobial activity, broad spectrum, clinical isolates

Introduction

The oldest form of health care known to mankind involves the use of plants and their parts for therapeutic purposes, a practice that has been embraced by all cultures throughout the world.[1] The World Health Organization estimates that about 80% of the world's population uses herbal medicine for some aspect of their health care.[2] Despite the popularity of modern medicine and the variety of drugs available for various ailments, it has been observed that 85% of patients combine herbal therapy with the medicines prescribed at hospitals or clinics.[3] This shows the level of confidence patients have in herbal recipes. The situation in the United States is not any different, and it has been noticed that 25% of prescription drugs dispensed in the US contain at least one active ingredient derived from plant material.[4]

The persistent increase in antibiotic-resistant strain of microorganisms has led to the development of more potent but also more expensive antibiotics, such as the third-generation fluoroquinolones and the cephalosporins.[5–7] In most developing countries of the world, these antibiotics are not readily affordable, which makes compliance difficult. This calls for research into alternative sources of antimicrobials. *Dialium guineense* is a shrub of the family Leguminosae. It has a straight, grayish and smooth stem. The stem bark is

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used for the treatment of cough, toothache, and bronchitis.[8] Despite its acclaimed efficacy, there is as yet no scientific in its support. This work was carried out to assess its *in vitro* antimicrobial activity against some clinical isolates.

Materials and Methods

Plant collection and authentication

D guineense stem bark was collected with the assistance of a herbal practitioner at Ode-Lemo, Ogun State of Nigeria. A sample of the plant was taken to the Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria, for further confirmation and a voucher specimen was deposited there [Forest Herbarium, Ibadan (FHI) No. 108009].

Extraction procedure

A 50-g portion of air-dried, powdered, stem bark of *D guineense* was soaked in 1 l of each of the six solvents used [i.e., sterile distilled water, absolute ethanol, ethanol 50% (v/v), chloroform, petroleum ether, and acetone] for 72 hours according to the method of Rojas *et al*.[9] Each mixture was refluxed, agitated at 200 rpm for 1 hour, and filtered using Whatman No. 1 filter paper. Following this, the aqueous filtrates were freeze dried, while the alcohol filtrates were placed in a vacuum oven at 40°C and dried for 3 days to obtain the dry extracts. The percentage yield and the pH of the extracts in the different solvents were determined. All crude extracts were stored at 4°C until they were needed for use.

Phytochemical analysis

Qualitative chemical analysis of the plant was carried out using the standard methods described by Treas and Evans.[10]

Fractionation of the crude extract

Forty grams (40 g) of the crude extract (coded CEDG), was adsorbed onto silica and allowed to dry before it was packed on the Buchner funnel column of Kieselgel 60 (ASTM 70–230 mesh). The elution was effected with *n*-hexane, dichloromethane, ethyl acetate, acetone, and ethanol successively. The extracts were in turn concentrated to dryness in a vacuum oven. The resultant fractions were then tested for antimicrobial activity using the agar diffusion method.

Thin-layer chromatography

TLC was done on bioactive fractions using analytical silica gel 60 $GF_{254+366}$ precoated aluminum-backed plates (Merck; 0.25 mm thick). One milligram of the sample was dissolved in 2 ml of methanol and spotted on a silica gel sheet and developed using an ethyl acetate: acetone (9:1) solvent system. The resulting spots on the chromatography paper were visualized under ultraviolet (UV) light (254 nm) and detected using 10% H_2SO_4 in methanol spray reagent. Fractions having the same TLC patterns were bulked together and concentrated *in vacuo*, resulting in four pure fractions. These were further subjected to antimicrobial activity testing.

Assay of antimicrobial activity

The microorganisms used for the assessment of the antimicrobial activity of the plant extracts were procured from the Nigerian Institute of Medical Research and Olabisi Onabanjo University Teaching Hospital. Different strains were also used [Table 1].

The method of McFarland was modified for the preparation of the inoculum. The optical densities of the 0.5 McFarland turbidity standard and the organism suspensions were compared and further adjusted on a UNICO® 2100 spectrophotometer at 520 nm wavelength.

The agar well diffusion method described by Shahidi and Rashidi[11] was adopted for antibacterial activity assessment.

Müller-Hinton (MH) agar (Fluka Bichemika, Lot 1198898, Spain) and heated blood agar (HBA) were used for the bacterial isolates, while Sabouraud dextrose agar (Lab M Batch No. 053741, UK) was used for the fungal isolates. Agar plates were seeded with 2.0 ml of the bacteria preparation. Any excess suspension was drained off using a pad of filter paper. Wells of 6-mm diameter were bored in the MH and HBA culture media with a sterile cork borer. A concentration of 100 mg/ml of the extract was prepared in the appropriate solvent and 0.2 ml of

Table 1: Characteristics of microorganisms used to assess the antimicrobial activity of the plant extracts

Organism	Relevant property	Source
Staphylococcus aureus[a]	R: CRO, TE	OOUTH
Staphylococcus aureus[b]	R: Gen	OOUTH
Staphylococcus aureus[c]	S: to all	OOUTH
Escherichia coli[a]	R: COT, Amp	OOUTH
Escherichia coli[b]	R: Amp, TE	OOUTH
Proteus mirabilis	R: Gen, COT	OOUTH
Pseudomonas aeruginosa[a]	S: only to COL	OOUTH
Pseudomonas aeruginosa[b]	S: CAZ, COL.	OOUTH
Klebsiella pneumonia	R: CRO COT	OOUTH
Streptococcus pneumonia	R: Gen, COT	OOUTH
Enterohemorrhagic E coli		NIMR
Salmonella typhi		NIMR
Shigella flexneri		NIMR
Bacillus subtilis		NIMR
Candida albicans		OOUTH
Aspergillus niger		OOUTH
Penicillium notatum		OOUTH
Epidemophyton fluccosum		OOUTH
Staphylococcus aureus	ATCC 25923	NIMR
Escherichia coli	ATCC25922	NIMR

R: Resistant; S: Sensitive; CRO: Ceftriaxone; TE: Tetracycline; Gen: Gentamycin; COT: Cotrimoxazole; Amp: Ampicillin; COL: Colistin sulfate; CAZ: Ceftazidine; NIMR: Nigerian Institute of Medical Research; OOUTH: Olabisi Onabanjo University Teaching Hospital; a, b, c: different strains of test organisms

the extract was used to fill each well. The plates were incubated at 37°C for 24 hours. The diameters of zones of inhibition were measured in millimeters. All samples were tested in duplicate and the average was recorded as the mean inhibition zone. Gentamycin at 1 µg/ml and the various extractants served as positive and negative controls, respectively.

For screening the antifungal activity of the extract, the method described by Awoderu *et al*.[12] was adopted. Incubation was done at 30°C for 48 hours, and griseofulvin and the extractants served as positive and negative controls, respectively.

Determination of minimum inhibitory concentration and minimum bactericidal concentration of the extract

Starting from the initial concentration of 100 mg/ml, the plant extract was serially diluted with molten Müller-Hinton agar to achieve final concentrations of between 10 mg/ml and 0.63 mg/ml and poured aseptically into sterile petri dishes as described by Musa *et al*.[13] These agar–plant extract mixtures were allowed to set. Sterile paper discs (Whatman No. 1 filter paper, 5 mm in diameter) were firmly placed on the agar surface. Twenty microliters (20 µl) of freshly standardized bacteria cultures were then dispensed on the paper disc. The plates were incubated at 37°C for 24 hours. The lowest extract concentration that inhibited the growth of the test organisms was taken as the MIC. The paper discs in the MIC determination that showed no visible growth around their edges were placed in 5 ml glucose broth and incubated for 24 hours and then subcultured on freshly prepared 10% blood agar (BA). The BA plates were incubated at 37°C for 24 hours. The lowest concentration of the plant extract at which no growth was observed on the cultured plates was taken as the MBC of the extract.

Kinetics of bactericidal activity of the extract

Twenty milliliter (20 ml) of the MBC of plant extract was prepared with sterile normal saline using a modification of the method described by Olonitola *et al*.[14] The extract was then inoculated with 20 µl of 1.5×10^8 cfu/ml of some selected isolates that had exhibited sensitivity to the extract, namely, *Staphylococcus aureus* 25923, *Streptococcus pneumoniae*, *Salmonella typhi*, and *Candida albicans*. At different time intervals (0, 1, 2, 3, …12 h), 20 µl from decimal dilutions of this reaction mixture was placed on five different spots of a freshly prepared plate count agar and Sabouraud dextrose agar (SDA), which were then incubated at 37°C and 28°C, respectively, for 24 hours. The average of the five plate counts was recorded to the nearest whole number.

Results

The colors of the crude extracts were between light brown in isopropanol to brownish-black in chloroform. The highest percentage yield of 23.2% was obtained with ethanol, while the lowest yield of 0.2% was obtained with petroleum ether [Table 2].

The phytochemical screening showed that *D guineense* contains anthraquinone, alkaloids, flavonoids, tannins, and saponins. The antimicrobial activity of the extracts was revealed by a broad spectrum of action, with *S typhi* and *S aureus*[a] showing the highest zone of inhibition (18.0 mm). Only *C albicans*, among the fungi tested, was inhibited by the extract. The highest zone of inhibition with the fractions was 16.0 mm [Table 3].

The MIC of *D guineense* crude extract against *Escherichia coli* 25922, *Bacillus subtilis*, and *S typhi* was 0.63 mg/ml, while the MBC was 5.0 mg/ml against *S typhi* and *C albicans* [Table 4].

D guineense exhibited bactericidal activity at the 7th and 9th hours against *Streptococcus pneumoniae* and *S. aureus* 25923 while the 10th hour against *S. typhi* and *C. albicans*. The greatest activity was noted against *S pneumonia*, with drastically reduced viable cell count after 6 hours of exposure.

Fractionation by TLC produced four fractions, which were further tested for antimicrobial activity. The DGEF1 fraction of the ethanolic extract showed the maximum activity [Table 5].

Discussion

The ethanolic extract of *D guineense* was the most active against the microorganisms studied. This finding agrees with that of some other studies[9,15] and shows that preparation of this herb in tinctures is the best method for extracting and concentrating the active components of the plant. The use of alcohol as an extractant will also enhance the preservation of the extracts and its assimilation by the body.

Anthraquinone which has been known to form an irreversible complex with nucleophilic amino acids, leading to their inactivation and loss of function, was found in *D guineense* extract and might be responsible for its broad-spectrum antimicrobial activity.[16] The crude extract of *D guineense* was able to inhibit multiple drug-resistant strains of *Pseudomonas aeruginosa*[a]. This agrees with the work of Nascimento *et al*., who demonstrated that *P aeruginosa* that was resistant to many

Table 2: Characteristics of crude extracts of *D guineense*

Solvent	Relative polarity	Color	pH	% yield
Petroleum ether	0.117	Reddish-brown	6.9	0.2
Chloroform	0.259	Brownish-black	-	8.2
Acetone	0.355	Reddish-brown	6.8	8.8
Isopropanol	0.617	Light brown	6.5	12.3
Absolute ethanol	0.654	Reddish-brown	6.1	23.2
Ethanol (50%)	-	Reddish-brown	6.3	17.1
Water	1.000	Light brown	5.8	14.6

Table 3: Antimicrobial activities of *D guineense* crude extracts expressed as inhibition zones (in millimeters)

Organisms	Abs Eth	Eth (50%)	Chlor	Water	Pet eth	Acet	Gen 10 µg/ml	Gri 10 µg/ml
Staphylococcus aureus[a]	16.0	12.0	0.0	0.0	0.0	12.0	22.0	NT
Staphylococcus aureus[b]	14.0	10.0	0.0	0.0	0.0	0.0	0.0	NT
Staphylococcus aureus[c]	18.0	14.0	0.0	0.0	0.0	0.0	23.0	NT
Escherichia coli[a]	13.0	10.0	0.0	0.0	0.0	8.0	18.0	NT
Escherichia coli[b]	13.0	13.0	0.0	0.0	0.0	0.0	16.0	NT
Proteus mirabilis	13.0	10.0	0.0	0.0	0.0	8.0	0.0	NT
Pseudomonas aeruginosa[a]	14.0	12.0	0.0	0.0	0.0	10.0	0.0	NT
Pseudomonas aeruginosa[b]	11.0	10.0	0.0	0.0	0.0	0.0	0.0	NT
Klebsiella pneumoniae	13.0	14.0	0.0	0.0	0.0	8.0	12.0	NT
Streptococcus pneumoniae	12.0	10.0	0.0	0.0	0.0	0.0	0.0	NT
Enterohemorrhagic E coli	13.0	10.0	0.0	0.0	0.0	10.0	18.0	NT
Salmonella typhi	18.0	14.0	0.0	0.0	0.0	10.0	21.0	NT
Shigella flexneri	14.0	14.0	0.0	0.0	0.0	16.0	24.0	NT
Bacillus subtilis	16.0	16.0	0.0	0.0	0.0	0.0	20.0	NT
Candida albicans	16.0	10.0	0.0	0.0	0.0	12.0	NT	27.0
Aspergillus niger	0.0	0.0	0.0	0.0	0.0	0.0	NT	16.0
Penicillium notatum	0.0	0.0	0.0	0.0	0.0	0.0	NT	18.0
Epidermophyton flucossum	0.0	0.0	0.0	0.0	0.0	0.0	NT	18.0
Staphylococcus aureus ATCC 25923	18.0	16.0	0.0	0.0	0.0	0.0	22.0	NT
*Escherichia coli ATCC*25922	16.0	16.0	0.0	0.0	0.0	16.0	20.0	NT

Abs: Absolute; Eth: Ether; Chlor: Chloroform; Pet: Petroleum; Acet: Acetone; Gen: Gentamycin; Gri: Griseofulvin; NT: Not tested

Table 4: Minimum inhibitory concentrations and minimum bactericidal concentrations of the ethanolic extract of *D guineense* (in mg/ml) and standard drugs (in µg/ml) on sensitive bacteria isolates

Organism	MIC and MBC* values		
	D guineense	Gen	Gri
Staphylococcus aureus[a]	2.5 (>10.0)	0.63 (0.63)	NT
Staphylococcus aureus[b]	2.5 (>10.0)	- -	NT
Staphylococcus aureus[c]	1.25 (>10.0)	0.63 (0.63)	NT
Escherichia coli[a]	1.25 (>10.0)	0.63 (0.63)	NT
Escherichia coli[b]	1.25 (>10.0)	1.25 (1.25)	NT
Proteus mirabilis	2.5 (>10.0)	–	NT
Pseudomonas aeruginosa[a]	5.0 (>10.0)	–	NT
Pseudomonas aeruginosa[b]	5.00 (>10.0)	–	NT
Klebsiella pneumoniae.	5.0 (>10.0)	0.63 (1.25)	NT
Streptococcus pneumoniae	0.63 (10.0)	–	NT
Enterohemorrhagic E coli	5.0 (>10.0)	0.63 (0.63)	NT
Salmonella typhi	0.63 (5.0)	0.63 (0.63)	NT
Shigella flexneri	2.5 (>10.0)	0.63 (0.63)	NT
Bacillus subtilis	0.63 (10.0)	0.63 (2.5)	NT
Candida albicans	5.0 (5.0)	NT	1.25 (5.0)
Staphylococcus aureus 25923	1.25 (10.0)	0.63 (0.63)	NT
Escherichia coli 25922	0.63 (10.0)	0.63 (0.63)	NT

*Figures in bracket are the MBC values, Gen: Gentamycin; Gri: Griseofulvin: NT: Not tested

Table 5: Activity of pure fractions from *D guineense* extracts on some organisms, expressed as zone of inhibition (in millimeters)

Pure fractions	*S aureusc*	*S aureus ATCC*	*E coli*[b]	*B subtilis*	*C albicans*
DGEF1	14.0	15.0	15.0	14.0	8.0
DGEF2	0.0	0.0	0.0	0.0	0.0
DGEF3	0.0	0.0	0.0	0.0	0.0
DGEF4	0.0	0.0	0.0	0.0	16.0

DGEF 1–4: *D guineense* fractions 1–4

antibiotics was inhibited by extracts from clove, jambolan, and thyme.[17] This puts *D guineense* in the ranks of the potential novel antimicrobials that may help solve the current problem of bacterial drug resistance.

The effectiveness of an antibacterial agent is measured by its ability to inhibit or kill bacteria.[18] The MIC and the MBC of *D guineense* were 0.63 mg/ml and 5 mg/ml, respectively; though these values are higher than that of the control antibiotic gentamycin, they can still be considered reasonably good. Our findings are in agreement with the work of Akinpelu and Onakoya.[19] *D guineense* demonstrated bactericidal activity against *S aureus*[a], *S pneumoniae*, *S typhi,* and *C albicans*, which justifies its use in the treatment of enteric and respiratory infections.[1,8] The reduction in antimicrobial activity of the fractions versus the crude plant extract (as revealed by the smaller inhibition zones) might be an indication that other components in the plant, though not antimicrobial in nature, might contribute to the overall performance of the whole plant.

Studies are ongoing to ascertain the mode of antimicrobial activity of this plant, which has the potential for being developed into a novel antimicrobial drug.

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