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Antimicrobial Potentials of *Diospyros soubraeana* (Ebenaceae)

B.A. Adeniyi* and H.A. Rasaq

Department of Pharmaceutical Microbiology and Clinical Pharmacy,
College of Medicine, University of Ibadan, Ibadan, Nigeria.

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ABSTRACT: The petroleum ether, chloroform and methanol extracts of the leaf, stem bark and root of *Diospyros soubraeana* were studied for their antimicrobial activities. The crude extracts showed broad spectrum antimicrobial activity against six (6) Gram – positive bacterial, six (6) Gram – negative bacterial and two (2) fungal strains. Comparative antimicrobial activity of extracts from the three extracting solvents revealed that petroleum ether extract of the root had the greatest activity on the organisms followed by methanol and then chloroform. Some tetracyclines and ampicillin resistant strains of *Staphylococcus aureus* and gentamicin resistant strains of *Pseudomonas aeruginosa* were sensitive to some of the extract tested. Preliminary phytochemical screening revealed the presence of the anthraquinones, tannin, steroidal nucleus, saponin glycoside, cardenolides, sugar while volatile oils and alkaloids were absent except traces of alkaloids in only the leaf.
The antimicrobial activities observed are discussed in relation to the preliminary phytochemical screening and chemical constituents reportedly isolated from several species of this plant and their traditional uses.

Key Words: Medicinal plants; Antimicrobial activities; *Diospyros soubraeana.*

Introduction

Diospyros is one of the four genera that made up the Ebenaceae family. It is the only genus in the family represented in Nigeria and in fact the largest genus which contains not less than 500 species of tropical trees and shrubs (1), whose heartwood are sometimes black and yield ebony of commerce (2). The genus has an economic importance as a source of heartwood and edible fruits. The wood of most species are hard, strong and elastic. The smaller stems of many species are used to make spear, shafts, walking sticks, implement handles and in house building (2), *Diospyros* species are generally valuable as chewing sticks. Several ethno pharmacological claims has been made in respect of *Diospyros* sp. The infusion of the inner bark and leaves of *D. gabunensis* are used in Liberia as antiseptic wash for sores and wounds. The boiled leaves is applied as a poultice (3). The root bark of *D. usamberences* has a reputation in Malawi as a cure for schistosomiasis. Fresh leaves of *D. soubraeana* are well known for their haemostatic properties (4).

*To whom correspondence should be addressed.

Information on *in-vitro* antimicrobial activity of species of Nigeria *Diospyros* sp. is of recent. Adeniyi, *et al*., (5) reported that the crude extract of *D. mespiliformis* showed broad spectrum antimicrobial activity, while the chloroform extracts of the root exhibited the highest antimicrobial activity. Lajubutu *et al* (6) reported that the minimum inhibitory concentration (MICs) of diosquinone from the roots of *D. mespiliformis* against *Staphylococcus aureus* ranged from 3-30µg/ml, while those against *Escherichia coli* and *Pseudomonas aeruginosa* ranged from 15-60µg/ml. However, there is no information on the biological properties of *D. soubraeana*.

Previous research on the phytochemistry of the bark or wood of 17 African species of *Diospyros* has revealed the presence of triterpene and naphthoquinone (7). A naphthoquinone epoxide, diosquinone and plumbagin along with a-amyrin, b-sitosterol and betulinic acid has been reported from *D. mespiliformis* (6). There is however no information about the chemical constituents of *D. soubraeana*. The present study is designed to investigate its antimicrobial potential and justify, scientifically, its antimicrobial relevance in traditional medicine.

Materials and Methods

The stem and root bark of *D. soubraeana* were collected from Olokemeji in Ogun State of Nigeria during the dry season and authenticated by Mr. Gabriel of the Forest Research Institute of Nigeria (FRIN). Voucher specimens were deposited at FRIN, which were pressed, poisoned and annotated with reference No. FHI 31498. The samples used in the following analysis were air dried and milled with a Hammer mill. Each of the plant parts was successively extracted using soxhlet with redistilled petroleum ether 60-80°C, chloroform and methanol in succession for 12 hours each. Each extract was filtered and concentrated *in-vacuo*. Extracts were stored in the refrigerator until needed for analysis.

Organisms

The microorganisms used for the study consisted of 6 Gram-positive and 6 Gram-negative bacterial and 2 fungal (Table 1).

Media

Nutrient broth No. 2 pH 7.4, nutrient agar pH 7.4, malt extract broth pH 5.6 and Sabourand dextrose agar pH 5.4, all products of Oxoid Laboratories, England were used in this study.

Antimicrobial agents

The following chemotherapeutic agents were included in the test as positive controls. Gentamicin sulphate, 2mg/ml. (Nicholas Laboratories Limited, England), Ampicillin 5mg/ml. (Laboratory Oftalmiso, Spain) and Tioconazole 1% w/v (Pfizer Inc., New York).

Phytochemical screening:

The qualitative chemical analysis of the powders was carried out for the presence of anthraquinones, volatile oils, tannins, saponins, steroids, cyanogenic glycosides, cardiac glycosides, flavonoids and alkaloids using the method adopted in similar surveys [5,6].

Determination of antimicrobial activity

The antimicrobial activity of the extracts were determined using agar well diffusion technique [6]. Nutrient agar plates were each seeded with 0.1ml of a 1:100 dilution of an overnight culture of each bacterial isolate, while the Sabourand dextrose agar plates were each similarly seeded with each fungal strain. The seeded plates were allowed to dry in the incubator at 37°C for 20 minutes. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar, into which was added 60 µl of each extract re-suspended in 50% methanol at a concentration of 20 mg/ml. Nutrient agar plates with bacterial isolates were incubated at 37°C for 24 hours and Sabourand dextrose agar plates seeded with

fungal strains were incubated at 25°C for 72 hours after which diameters of zones of inhibition were measured. Since each of the petroleum ether, chloroform, and methanol extracts was re-suspended in 50% methanol before being tested, 50% methanol was included in each plate as a solvent control besides the chemotherapeutic agents included as positive control.

Results

The colour and percentage yield of the petroleum ether, chloroform and methanolic extracts of the leaf, stem bark and root bark of *D. soubraeana* are presented in Table 2. Phytochemical screening (Table 3) showed that the constituents found in the plant are saponin glycoside, tannins, steroidal nucleus, anthraquinones glycosides, flavonois while alkaloids were absent except in trace form in the leaf. The result of antimicrobial activity is presented in Table 4. All extracts were found to possess varying degrees of antimicrobial activities. The zones of inhibition obtained in respect of the extracts moderately compared favourably with those obtained from ampicillin and gentamicin. The petroleum ether extracts of the roots generally showed greater antimicrobial activities than the others. This is followed by the water extract of the leave, which surprisingly has broad antibacterial activity than the chloroform extract of the root. It worth noting that some clinical strains of *Staphylococcus aureus* that were resistant to both tetracycline and ampicillin and *Pseudomonas aeruginosa* that were resistant to gentamicin were found to be sensitive to some extracts under test. The water extracts of the leave has pronounced anti-pseudomonas property. The petroleum extract of the root has pronounced activity on *Aspergillus flavus* while both the chloroform and petroleum extract of the leaf has moderate activity against both *Candida albicans* and *Aspergillus flavus*. The aqueous extract of the stem is ineffective against most organisms.

Table 1: List of microorganisms used to assess antimicrobial activity of *Diospyros soubraeana*.

Test Microorganisms	Relevant Properties	Source
Bacillus aereus		Laboratory stock
Staphylococcus aureus NCTC 6571		Dept. of Pharmacy, Univ. of Strathclyde, Glasgow
S. aureus UCH 246	Resistant to Te.	Clinical Strain
S. aureus UCH 565	Resistant to Te and CT	Clinical Strain
S. aureus UCH 573	Resistant to Te, CT & Am.	Clinical Strain
S. aureus UCH 579	Resistant to Am. And P.	Clinical Strain
Escherichia coli NCTC 9001		Dept. of Pharmacy, Univ. of Strathclyde, Glasgow
E. coli UCH 379	Resistant to Am.	Clinical Strain
E. coli UCH 459	Resistant to Am. & CT.	Clinical Strain
Pseudomonas aeruginosa NCTC 6750		Dept. of Pharmacy, Univ. of Strathclyde, Glasgow
P. aeruginosa UCH 359	Resistant to Am, CIP and Caz.	Clinical Strain
P. aeruginosa UCH 479	Resistant to Am, CIP.	Clinical Strain
Candida albicans		Dept. of Vet. Microbiology Univ. of Ibadan, Ibadan.
Aspergillus flavus		

Te, Tetracycline, CT, Cotrimoxazole; Am, Ampicillin; P, Penicillin, CIP, Ciprofloxacin; Caz, Cerftzidin; NCTC: National Collection Type Cultures; UCH: University College Hospital Collection, Ibadan.

Table 2: Characteristics of crude extracts of *Diospyros soubraeana*

Extracting solvent	Morphological part	Colour	Percentage (%) yield
Petroleum ether	Leaf	Deep green	5.0
	Stem	Greenish brown	2.5
	Root	Light orange	2.0
Chloroform	Leaf	Grennish black	5.5
	Stem	Dark brown	2.0
	Root	Light yellow	1.8
Methanol	Leaf	Shinning dark brown	7.5
	Stem	Brown	6.1
	Root	Dark brown	5.9
Water	Leaf	Brown mass	3.5
	Stem	Light brown sticky mass	1.8
	Root	Brown mass	2.0

Table 3: Phytochemical screening of *Diospyros soubraeana*

Phytochemical grouping	Morphological Parts		
	Leaves	Stem	Root
Anthraquinones	+	+ +	+ +
Tannins	+ + +[g]	+ + +[b]	+ + +[b]
Saponins	+ +	+ +	+ + +
Steriodal nucleus	+ + +	+ + +	+ +
Volatile oils	-	-	-
Sugars	+ +	+ +	+ +
Alkaloids	+	-	-
Cardenolides	+ +	-	+ +
Flavonoids	+ +	+	+ +

Discussion

All the secondary metabolites present in the different morphological parts of *D. soubraeana* studied except traces of alkaloids in the leaf have been reported in some of the *Diospyros* sp. [2,5]. Phytochemical screening showed that tannin is present in highest concentration in the root bark and least in the stem bark. This might be attributed to the antibacterial and haemostatic activities on fresh wound [10]. The relative difference in the antibacterial and haemostatic activities of *Diospyros* species is attributed to the type of certain tannin present. The hydrolysable tannin have been reported to have some astringent properties due to their ability to bind to the protein of exposed and traumatized tissue, thereby precipitating the proteins to form a mildly antiseptic protective coat on the wound [10].

Table 4: Antimicrobial activity of crude extracts of *Diospyros soubraeana*

Test Organisms	Diameter of zone of inhibition (mm)															
	Petroleum ether			Chloroform			Methanol			Water			Controls			
	L	S	R	L	S	R	L	S	R	L	S	R	Am	Gn	T	M
Bacillus aerus	-	12±0.2	17±0.2	90±0.1	11±0.2	13±0.1	11±0.2	NT	NT	12±0.3	-	12±0.2	9±0.2	22±0.3	NT	-
Staphylococcus aureus NCTC 6571	-	-	16±0.2	10±0.3	-	12±0.2	9±0.3	NT	NT	15±0.2	-	12±0.3	9±0.3	17±0.2	NT	-
S. aureus UCH 246	10±0.1	14±0.2	20±0.3	13±0.2	15±0.2	12±0.3	10±0.3	NT	NT	20±0.3	10±0.1	11±0.3	9±0.3	22±0.1	NT	9±0.2
S. aureus UCH 565	11±0.2	14±0.3	20±0.1	10±0.3	16±0.1	14±0.2	9±0.1	NT	NT	19±0.2	11±0.1	12±0.2	-	20±0.3	NT	9±0.1
S. aureus UCH 573	10±0.2	-	12±0.2	10±0.2	11±0.3	9±0.3	9±0.1	NT	NT	11±0.3	-	9±0.3	-	16±0.3	NT	-
S. aureus UCH 579	10±0.2	-	12±0.3	10±0.3	9±0.2	10±0.2	10±0.1	NT	NT	13±0.2	-	9±0.2		17±0.3	NT	-
Escherichia coli NCTC 9001	9±0.1	-	11±0.1	9±0.2	9±0.3	10±0.1	-	NT	NT	9±0.3	-	9±0.2		19±0.2	NT	-
E. coli UCH 379	10±0.1	10±0.3	12±0.4	10±0.3	-	19±0.3	11±0.2	NT	NT	12±0.3	9±0.1	-		20±0.3	NT	-
E. coliI UCH 459	10±0.3	10±0.4	14±0.3	10±0.2	10±0.2	9±0.2	9±0.3	NT	NT	11±0.3	9±0.2	9±0.3	9±0.3	15±0.2	NT	-
Pseudomonas aeruginosa NCTC 6750	10±0.3	10±0.3	13±0.2	11±0.2	11±0.3	9±0.3	10±0.2	NT	NT	13±0.2	-	11±0.1	-	18	NT	-
P. aeruginosa UCH 359	9±0.1	-	14±0.3	9±0.3	-	-	10±0.3	NT	NT	13±0.1	-	12	-	14	NT	-
P. aeruginosa UCH 479	9±0.3	11±0.2	14±0.3	10±0.2	10±0.3	10±0.2	11±0.2	NT	NT	13±0.3	-	11	-	14	NT	-
Candida albicans	11±0.3	9±0.3	9±0.1	10±0.3	-	-	-	NT	NT	10±0.2	-	10	NT	NT	14	-
Aspergillus flavus	10±0.3	-	13±0.2	10±0.2	10±0.3	-	-	NT	NT	-	-	-	NT	NT	12	-

NT – Not Tested; L – Leave; S – Stembark; R – Root; Am – Ampicillin; Gn – Gentamicin; T – Tioconazole; M – 50% Methanol

The antibacterial activity of some of the extracts: petroleum ether root, water extract root and water extract leaf appeared to be broad spectrum because both Gram – positive, Gram – negative bacteria and fungi were sensitive to the extracts. The antimicrobial properties demonstrated by these plant parts could be traced also to the possession of flavonoids and quinones. *D. usamberensis* has been reported to contain antifungal naphthoquinones like 7-methyljuglone at 0.025 µg/ml, both 2-methoxyl-7-methyljuglone and 3-methoxyljuglone at 10µg/ml [11]. The antibacterial activities of isoflavonoids and flavonoids have been reported [12]. Naphthoquinones such as diospyrin and isodiospyrin, which have been identified in many species of *Diospyros*, have been shown to be antimicrobial [6,13].

The majority of the bacteria used in this study has been implicated in diseases such as diarrhoea, dental and oral infections, sepsis, respiratory tract infections, wound sepsis and dysentery while *C. albicans* has been implicated in serious infections of mucous membrane and skin thrush, vaginal thrush, acute atrophic candidiasis, and chronic atrophic candidiasis [14,15]. The antimicrobial activities shown by the crude extracts of this plant may, therefore, justify some of the ethno pharmacological claims about this plant in the treatment of diseases such as cough, dysentery, leprosy, sepsis and wound. The susceptibility of some ampicillin and tetracycline resistant strains of *S. aureus* and gentamicin resistant of *P. aeruginosa* to some of the extracts shows that this plant possesses a great potential as a substitute for these well known antimicrobial agents. Phytochemical studies are in progress to isolate, characterize and identify the specific active compounds in this plant responsible for the antimicrobial activity.

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