

# The African Journal of Medicine and Medical Sciences

Editors: T.A. Junaid  
O. Bademosi and D.D.O. Oyebola

## Editorial Board:

A.K. Addae  
S.A. Adebajo  
O.O. Adekunle  
A. Adeloye  
B. Adelusi  
A.F. Aderounmu  
C.O. Adesanya  
A. Adetugbo  
A.A. Adeyokunnu  
A. Agboola  
O.O.O. Ajayi  
E.O. Akande  
O.O. Akinkugbe  
O.O. Akinyemi  
T. Atinmo  
O. Ayeni  
E.A. Ayoola  
E.A. Bababunmi  
E.A. Badoe  
T.O. Cole  
O.A. Dada  
A.B.O. Desalu

L. Ekpechi  
R.A. Elegbe  
G. Emerole  
J.G.F. Esan  
E.M. Essien  
G.O. Ezeilo  
A. Fabiyi  
A.O. Falase  
J.B. Familusi  
D. Femi-Pearse  
K.A. Harrison  
P.A. Ibeziako  
A.C. Ikeme  
A.O. Iyun  
F. Jaiyesimi  
A.O.K. Johnson  
T.O. Johnson  
T.M. Kolawole  
O.A. Ladipo  
S.B. Lagundoye  
D.G. Montefiore  
E.O. Nkposong

N.C. Nwokolo  
H.O. Obianwu  
S.A. Oduntan  
E.O. Ogunba  
O. Ogunbode  
M.O. Olatawura  
D.A. Olatunbosun  
E.O. Olurin  
Oyin Olurin  
A. Omololu  
B.O. Onadeko  
G. Onuaguluchi  
A.O. Osoba  
B.O. Osotimhin  
B.O. Osunkoya  
B.O. Osuntokun  
A.B.O.O. Oyediran  
L.A. Salako  
T.F. Solanke  
O. Tomori  
F.A.O. Udekwu  
A.O. Uwaifo

Volume 17  
1988

BLACKWELL SCIENTIFIC PUBLICATIONS  
Oxford London Edinburgh Boston Palo Alto Melbourne

## Preliminary investigation of in-vitro antimicrobial activity of two Nigerian *Diospyros* species (Ebenaceae)

H. A. ODELOLA\* AND V. I. OKOROSOBO

Department of Pharmaceutical Microbiology and Clinical Pharmacy, University of Ibadan, Ibadan, Nigeria

### Summary

The aqueous and alcoholic extracts from the leaves of *Diospyros bati* and *D. monbutensis* were studied for their antimicrobial activities. The alcoholic extracts of the two *Diospyros* spp. showed strong antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria, while only the aqueous extract of *D. bati* showed antibacterial activity against all the bacteria tested. The two fungi included in the study *Aspergillus niger* and *Candida albicans* were found to be resistant to the extracts of both *Diospyros* spp.

### Résumé

Les extraits alcooliques et aqueux des feuilles de *Diospyros bati* et *D. monbutensis* étaient étudiés à propos de leurs activités antimicrobiennes. Les extraits alcooliques des deux espèces de *Diospyros* spp. ont montré une forte activité antibactérienne contre un ample assortiment de bactéries gram positives et gram negatives. Par contre seul l'extrait aqueux de *D. bati* a montré une activité antibactérienne contre toutes les bactéries testées. Les deux fongis inclus dans l'étude, *Aspergillus niger* et *Candida albicans* étaient résistants aux extraits des deux *Diospyros* spp.

### Introduction

*Diospyros* spp. are forest trees [1] that are commonly used in many Nigerian communities as chewing sticks. Certain *Diospyros* spp. have also been used as herbal medicines in the treatment of leprosy. In some communities, infu-

sions of bark and leaves of *Diospyros* spp. are used as antiseptic washes for sores and wounds, while boiled leaves are applied as poultices.

Several workers from developed countries have reported in-vitro activity of *Diospyros* spp. The antibacterial activity of extracts from leaves and seeds of *D. montana* was studied by Goutam and Purohit [2] who observed that the petroleum-ether extracts were active against *Bacillus subtilis* and *Corynebacterium pyogenes*. Paris and Prista [3] also found that a 10% infusion of the bark of *Canaliculata* was bacteriostatic against *Staphylococcus* spp.

Information on in-vitro antimicrobial activity of species of Nigerian *Diospyros* is very scanty; hence this study was designed to identify their antimicrobial potentials.

### Materials and methods

#### Plant extract

Authenticated samples of leaves of *D. bati* and *D. monbutensis*, which were collected from the Botanical Garden of the University of Ibadan during the rainy season (April to July), were dried and pulverized before the extraction was carried out in a soxhlet apparatus using water or methanol. The strength of each extract varied between 10% and 12% (w/v) depending on the weight of the dry powder and volume of extraction solution. The extracts were dispensed into sterile bottles and stored at 4°C until used.

#### Organisms

The micro-organisms employed in this study consisted of Gram-positive and Gram-negative bacteria and two fungi (Table 1).

\*To whom correspondence should be addressed.

Table 1. List of micro-organisms used to assess the antimicrobial activity of the plant extracts

Organism		Source
<i>Staph. aureus</i>	NCTC 6572	Department of Pharmacy, University of Glasgow
<i>Bacillus subtilis</i>		Laboratory stock
<i>Bacillus cereus</i>		Laboratory stock
<i>Strep. pyogenes</i>		Clinical isolate
<i>Strep. faecalis</i>		Clinical isolate
<i>Escherichia coli</i>	Enteroreference	Department of Pharmacy, University of Glasgow
<i>Klebsiella spp.</i>		Clinical isolate
<i>Proteus mirabilis</i>		Laboratory stock
<i>Proteus rettgeri</i>		Laboratory stock
<i>Salmonella typhi</i>		Clinical isolate
<i>Pseudomonas aeruginosa</i>	NCTC 6750	Department of Pharmacy, University of Glasgow
<i>Pseudomonas aeruginosa</i>		Clinical isolate
<i>Pseudomonas aeruginosa</i>		Clinical isolate
<i>Pseudomonas aeruginosa</i>	UCH 17739	Clinical isolate
<i>Pseudomonas aeruginosa</i>	UCH 17567	Clinical isolate
<i>Pseudomonas aeruginosa</i>	UCH 19286	Clinical isolate
<i>Pseudomonas aeruginosa</i>	UCH 19475	Clinical isolate
<i>Pseudomonas aeruginosa</i>	UCH 17586	Clinical isolate
<i>Pseudomonas aeruginosa</i>	UCH 17818	Clinical isolate
<i>Aspergillus niger</i>		Laboratory stock
<i>Candida albicans</i>		Clinical isolate

### Media

Nutrient broth No. 2 (Oxoid) pH 7.4, nutrient agar (Mast Laboratories) pH 7.4 and Sabouraud dextrose agar (Oxoid) pH 5.4 were employed in this study.

### Antimicrobial agents

The following chemotherapeutic agents were included in the test as positive controls: Gentamicin sulphate 1 µg/ml (Eupharma Laboratory, Bombay) Ampicillin 2.5 µg/ml (Lab. Oftalmiso, Spain) and Tioconazole 1% (w/w) (Pfizer Inc. New York).

### Determination of antimicrobial activity

The minimum inhibitory concentration (MIC) of each antibiotic included in the test was determined using the broth dilution method as reported by Reeves *et al.* [4]. Nutrient agar plates

were seeded with bacterial isolates, while sabouraud dextrose agar plates were seeded with the fungi. A standard cork borer was used to cut uniform and equidistant wells on the surface of the agar into which known dilutions of the extracts and the antimicrobial agents were added. The bacteria seeded plates were incubated at 37°C for 24 h and the fungi seeded plates were incubated at 25°C for 3 days, after which zones of inhibition were measured.

### Phytochemical tests

The aqueous extracts were tested for the presence of sugar, protein, glycoside, volatile oil, loid, saponin and tannin using standard methods as reported by Sofowora [5].

### Results

The water extract of *D. bateri* had an intense wine colour with a pH value of 4.7, while the



water extract of *D. monbutensis* was dark brown in colour and of pH 5.3. The methanol extracts of *D. bateri* and *D. monbutensis* had pH values of 5.9 and 6.1, respectively. The antibacterial activity of each extract was not pH dependent. This was detected when the antibacterial activity of each extract was determined at varying pH values using citric acid/phosphate buffered nutrient agar pH 5.0–8.5.

The water extract of *D. monbutensis* was found to have no activity against all test organisms. The antibacterial activities of the extracts were compared with those of standard antibacterial agents shown in Table 2. The zones of inhibition obtained in respect of the extracts compared favourably with those obtained with gentamicin and ampicillin. The results showed water extract of *D. bateri* to be active against some clinical isolates of *Pseudomonas aeruginosa*, even when such isolates were resistant to either gentamicin or ampicillin. Generally, the

extracts of *D. bateri* were found to be more potent in antibacterial activity than those of *D. monbutensis*. The extracts of the two *Diospyros* spp. studied did not possess antifungal activity against *Candida albicans* and *Aspergillus niger*. The extracts of the two species contained saponin and tannin, but the tannin in *D. monbutensis* was condensed. The extracts of both species obtained during the dry season of the year did not show any appreciable antibacterial activity.

### Discussion

The data obtained from this preliminary study have shown interesting features which could point to the wide spectrum of antibacterial activity of extracts of Nigerian *Diospyros* spp. The antibacterial activity exhibited by the aqueous extract of *D. bateri* against clinical isolates of *Pseudomonas aeruginosa* was quite sig-

Table 2. Zones of inhibition (mm) of micro-organisms by *Diospyros* extracts

Organism	<i>D. bateri</i> extracts		<i>D. monbutensis</i> extract		Gentamicin	Ampicillin	Tioconazole
	Water	Methanol	Water	Methanol			
<i>Staph. aureus</i> NCTC 6572	12	15	R	9	17	11	NT
<i>Bacillus subtilis</i>	13	18	R	11	18	12	NT
<i>Bacillus cereus</i>	16	19	R	12	18	14	NT
<i>Strep. pyogenes</i>	13	16	R	9	16	13	NT
<i>Strep. faecalis</i>	14	18	R	13	17	12	NT
<i>Escherichia coli</i>	20	23	R	15	23	16	NT
<i>Klebsiella</i> spp.	18	19	R	11	20	13	NT
<i>Proteus mirabilis</i>	18	23	R	16	22	24	NT
<i>Proteus rettgeri</i>	30	29	R	25	35	40	NT
<i>Salmonella Typhi</i>	26	29	R	25	20	22	NT
<i>Pseudomonas aeruginosa</i> NCTC 6750	11	15	R	9	18	9	NT
<i>Pseudomonas aeruginosa</i>	14	NT	NT	NT	R	17	NT
<i>Pseudomonas aeruginosa</i> UCH 17739	22	NT	NT	NT	20	16	NT
<i>Pseudomonas aeruginosa</i> UCH 17567	13	NT	NT	NT	20	R	NT
<i>Pseudomonas aeruginosa</i> UCH 19286	22	NT	NT	NT	28	16	NT
<i>Pseudomonas aeruginosa</i> UCH 19475	12	NT	NT	NT	18	9	NT
<i>Pseudomonas aeruginosa</i> UCH 17586	19	NT	NT	NT	13	17	NT
<i>Pseudomonas aeruginosa</i> UCH 17818	19	NT	NT	NT	14	17	NT
<i>Aspergillus niger</i>	R	NT	NT	NT	NT	NT	18
<i>Candida albicans</i>	R	NT	NT	NT	NT	NT	14

NT = Not tested;

R = Resistant.

nificant, especially when it was observed that two of the isolates were resistant to 1 µg/ml gentamicin and 2.5 µg/ml ampicillin, respectively.

There is no doubt that the extracts of *D. bateri* were more potent than those of *D. monbuttensis*. Irvine [6] reported the use of fruit of *D. monbuttensis* in the treatment of ailments in Southern parts of Nigeria. He also reported the use of decoction of the bark and leaf tips of *D. monbuttensis* with those of *Cassia occidentalis* and *Lippia odoensis* in bathing as a remedy for leprosy on the Ivory Coast. On the other hand, *D. bateri*, *D. elliotii* and *D. tricolor* are highly valued in many Nigerian and other West African communities for their efficacy as chewing sticks, probably due to the presence of high fluoride content in each of them [7]. It was significant to observe the presence of saponin and tannin in the extracts of the Nigerian *Diospyros* species studied. However, more importantly the tannin in *D. bateri* was hydrolysable while that in *D. monbuttensis* was condensed that is, in trace. The presence of hydrolysable form of tannin in *D. bateri* could probably be responsible for the stronger antibacterial activity observed in the extracts of this *Diospyros* spp. since tannin is a phenolic substance.

It was observed that the antibacterial activity of the extracts of the two Nigerian *Diospyros* spp. is seasonal, as extracts obtained during the dry season (unpubl. obs.) had no appreciable antibacterial activity as was also observed by Obaseiki-Ebor [8] on the antibacterial activity of *Bryophyllum pinnatum* leaf juice. This preliminary investigation has revealed the antibacterial potentials of the two *Dio-*

*spyros* spp. studied, but there is need for further work to explore the antimicrobial potentials of the remaining 24 out of the 26 known *Diospyros* spp. in Nigeria [1].

#### Acknowledgments

The efforts of Mr Bankole Ige, a final year pharmacy student in 1984/85 session, are acknowledged. The authors also wish to thank Mr B. U. Ikhile of the Department of Pharmacognosy for his technical assistance and Mr J. I. Akpomie for his secretarial assistance.

#### References

1. Keay RW, Onochie J, Standfield DP. Nigerian Trees Vol. II. FRIN Ibadan, 1964.
2. Goutam MP, Purohit RM. Antibacterial activity of leaves and seeds of *Diospyros montana* Roxb. Indian J Pharm 1973;35:93-4.
3. Paris RA, Prista C. Sur les quinones du *Diospyros tricolor* Hiern, Ebénacée africaine anti-lépreuse. Ann Pharm (Franc) 1954;12:375-80.
4. Reeves DS, Philips I, Williams JD, Wise R. Laboratory Methods in Antimicrobial Chemotherapy. London: Churchill Livingstone, 1978: 8-49.
5. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Chichester: John Wiley & Sons, 1982:74-88.
6. Irvine FR. Woody Plants of Ghana. London: Oxford University Press, 1961:18-25.
7. Lewis WH, Elvin L, Memory LPF. Medical Botany. Plants Affecting Man's Health. New York: John Wiley & Sons, 1977:45-57.
8. Obaseiki-Ebor EE. Preliminary report on the in vitro antibacterial activity of *Bryophyllum* leaf juice. Afr J Med med Sci 1985;14:199-202.

(Accepted 18 June 1987)