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*SHORT COMMUNICATION*

# Antimicrobial Spectrum of *Alchornea cordifolia* Leaf Extract

**Iruka N. Okeke,[1] Abiodun O. Ogundaini,[2] Francis O. Ogungbamila[2] (Late) and Adebayo Lamikanra[1]***

[1]Department of Pharmaceutics, Obafemi Awolowo University, Ile-Ife, Nigeria
[2]Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

**The 50% aqueous ethanol extract of *Alchornea cordifolia* (Schum and Thonn) Muell. Arg. leaf was screened for activity against 74 microbial strains representing aerobic, facultative and anaerobic bacteria as well as fungi. The panel of test strains included organisms from culture collections as well as clinical and environmental isolates. A concentration of 5 mg/mL of extract inhibited 36.5% of the isolates and 95.9% were inhibited by a concentration of 20 mg/mL. Only three strains, all filamentous fungi, were not susceptible to 40 mg/mL of the extract, the highest concentration tested. The extract showed the best activity against gram-positive bacteria and yeasts with inhibitory concentrations against these organisms being under 5 mg/mL. The results demonstrate that the *A. cordifolia* extract has a very broad spectrum of activity and suggests that it may be useful in the treatment of various microbial infections.** © **1999 John Wiley & Sons, Ltd.**

*Keywords: Alchornea cordifolia*; antimicrobial activity.

## INTRODUCTION

Various preparations of *Alchornea cordifolia* (Schum and Thonn) Muell. Arg. leaf are used traditionally in the management of infectious disease. Ogunlana and Ramstad (1975) reported that a methanol extract of this plant showed appreciable activity against *Staphylococcus* spp. and *Escherichia coli*. Lamikanra *et al.* (1990) demonstrated that the extract also had activity against type cultures of *Bacillus subtilis* and *Pseudomonas aeruginosa* and proposed that this activity was caused by several constituents including phenolic acids and triisopentenyl guanidine.

In this report the activity of a 50% aqueous ethanol extract against a broad range of microorganisms obtained mainly from clinical sources and including aerobic and anaerobic bacteria as well as fungi, was evaluated to establish its spectrum of activity with the ultimate aim of examining the possibility of employing it for chemotherapeutic purposes.

## MATERIALS AND METHODS

**Collection and extraction of plant material.** *Alchornea cordifolia* (Schum and Thonn) Muell. Arg. Leaves were collected in August 1993 at the Obafemi Awolowo University (O.A.U.) Campus, Ile-Ife, Nigeria. The plant was authenticated by Mr Adesakin of the Herbarium Section, Faculty of Pharmacy, O.A.U. where a voucher specimen was deposited. The leaves were air dried at room temperature and ground into powder. The leaf powder (1 kg) was extracted with aqueous ethanol (50%). The extract obtained was filtered, concentrated *in vacuo* and freeze-dried to give a powder (125 g) which was used in subsequent experiments.

**Test organisms.** Reference strains were from stocks of culture collections maintained in our laboratory. Clinical isolates were obtained from specimens from human infection (including diarrhoea, abscesses, necrotizing fascitis, osteomyelitis and oral and mucosal infections) and identified by conventional biochemical tests (Murray *et al.*, 1995). Fungi were obtained from the department of Microbiology O.A.U. Bacteria were maintained by cryopreservation according to the method of Gibson and Khoury (1986) and fungi were maintained on Sabaraud dextrose agar slants at 4°C and sub-cultured monthly.

**Determination of Minimum Inhibitory Concentrations (MICs) from the freeze-dried extract.** MICs were determined by the agar dilution method on solid media. A stock suspension of 400 mg/mL of extract in methanol was prepared and from this, plate concentrations containing 40, 20, 10, 5 and 2.5 mg/mL of *Alchornea cordifolia* extract (AC) in test medium were made. Plates containing chlorocresol (BDH) 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.015625 mg/mL were similarly prepared and employed as controls.

Aerobic bacteria were grown overnight in nutrient broth (Oxoid). The cultures were diluted to a final density

* Correspondence to: A. Lamikanra, Department of Pharmaceutics, Obafemi Awolowo University, Ile-Ife, Nigeria.
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**Table 1. MICs of different organisms**

| Species | Source | No of strains tested | MIC μg/mL Chlorocresol | MIC mg/mL AC extract |
|---|---|---|---|---|
| **Gram-negative aerobic/facultative bacteria** | | | | |
| *Escherichia coli* | NCTC 10418 | 1 | 31.25 | 20 |
| *Escherichia coli* K-12 (C600) | Reference strain | 1 | 31.25 | 20 |
| *Escherichia coli* B | Reference strain | 1 | 31.25 | 20 |
| *Pseudomonas aeruginosa* | ATCC 10145 | 1 | 250 | 20 |
| *Escherichia coli*, normal flora | Faecal | 3 | 31.25 | 20 |
| *E. coli*, alpha-haemolytic | Clinical | 3 | 31.25 | 20 (2); 10 (1) |
| *E. coli*, enterovirulent (enteroinvasive, enteroaggregative, enterohaemorrhagic) | Clinical | 6 | 31.25 | 20 |
| *Enterobacter cloacae* | Clinical | 3 | 31.25 | 20(2); 10(1) |
| *Citrobacter freundii* | Clinical | 3 | 31.25 | 20(2); 10(1) |
| *Klebsiella pneumoniae* | Clinical | 3 | 31.25 | 20(2); 10(1) |
| *Hafnia alvei* | Clinical | 1 | 31.25 | 20 |
| *Shigella flexneri* 2a | Clinical | 1 | 31.25 | 20 |
| *Salmonella paratyphi* A | Clinical | 1 | 31.25 | 20 |
| *Pseudomonas* spp. | Clinical | 1 | 31.25 | 20 |
| *Acinetobacter baumanii* | Clinical | 2 | 31.25 | 20 |
| *Serratia marcescens* | Environmental | 1 | 31.25 | 20 |
| **Gram-positive aerobic/facultative bacteria** | | | | |
| *Staphylococcus aureus* | NCTC 6571 | 1 | ≤15.63 | ≤2.5 |
| *Bacillus subtilis* | NCTC 8236 | 1 | ≤15.63 | ≤2.5 |
| *Staphylococcus aureus* | Clinical | 5 | 31.25(2); ≤15.63(3) | ≤2.5 |
| *Staphylococcus epidermis* | Clinical | 4 | ≤15.63 | ≤2.5 |
| *Streptococcus* sp. | Clinical | 6 | ≤15.63 | 5(2); ≤2.5(4) |
| *Bacillus* spp. | Environmental | 2 | ≤15.63 | ≤2.5 |
| *Micrococcus luteus* | Environmental | 1 | ≤15.63 | 5 |
| **Obligate anaerobic bacteria** | | | | |
| *Prevotella* spp. | Clinical | 8 | 31.25 | 20(3); 10(5) |
| *Porphyromonas* spp. | Clinical | 1 | 31.25 | 10 |
| *Peptostreptococcus* spp. | Clinical | 2 | 31.25 | ≤2.5 |
| *Staphylococcus assacharolyticus* | Clinical | 1 | 31.25 | ≤2.5 |
| **Fungi** | | | | |
| *Candida pseudotropicalis* | NCYC 6 | 1 | 250 | ≤2.5 |
| *Cladosporum cucumerium* | Reference strain | 1 | 31.25 | >40 |
| *Candida albicans* | Clinical | 2 | 250 | ≤2.5 |
| *Trichophyton rubidum* | Clinical | 1 | 125 | ≤2.5 |
| *Fusarium solanii* | Clinical | 1 | 125 | >40 |
| *Aspergillus flavus* | Clinical | 1 | 125 | >40 |
| *Cochlibolus lunatus* | Clinical | 1 | ≤15.63 | ≤2.5 |
| *Trichoderma* spp. | Clinical | 1 | 125 | ≤2.5 |
| *Epidemophyton* spp. | Clinical | 1 | 125 | ≤2.5 |

of $2 \times 10^5$ cfu/mL in normal saline and applied to the surface of nutrient agar (Oxoid) plates containing dilutions of AC, chlorocresol or solvent alone employing a multi-point inoculator. Plates were incubated at 37°C for 48 h.

Anaerobic bacteria were grown in cooked meat medium (Oxoid) for 72 h and dilutions were prepared in peptone water containing 0.1% sodium thioglycollate. The test was carried out on Fastidious anaerobe agar (Techlab, USA) and plates were incubated at 37°C in an atmosphere containing less than 1% $O_2$ and 8% $CO_2$ generated with commercial gas-generating kits (BBL, Cockleysville, USA) in an anaerobic jar. Plates were incubated for 72 h.

Yeasts were grown on Sabouraud dextrose agar (SDA) slants for 3 days. The slants were washed with 2 mL normal saline and diluted to an inoculum density of 0.5–$2.5 \times 10^3$ cfu/mL. Filamentous fungi were grown on SDA slants for 7 days. Growth was washed with 5 mL normal saline and allowed to settle for 5 min. The upper part of the washings (consisting mainly of conidia) was diluted to a concentration of approximately $1 \times 10^4$ cfu/mL, which was employed as inoculum. Test plates for fungal determinations were prepared with SDA, the organisms were surface inoculated with a multi-point inoculator and the plates were incubated for 5 days at 25°C.

All plates were observed for growth and the minimum dilution completely inhibiting the growth of each organism was taken as the MIC.

## RESULTS AND DISCUSSION

The minimum inhibitory concentrations of *A. cordifolia* leaf extract for the test organisms are shown in Table 1. Sixty-five (87.8%) of the test strains were inhibited by 10 mg/mL of the extract when incorporated into agar. The least sensitive strains were three of the filamentous fungi, which were not inhibited by 40 mg/mL of extract under test conditions and these were followed by the gram-negative organisms, 29 (90.6%) of which were only inhibited by concentrations greater than 10 mg/mL of the

 

extract. By contrast, gram-positive bacteria were highly susceptible to the inhibitory activity of the extract. Only 3 (15%) of the test strains demonstrated viability in the presence of 2.5 mg/mL and all were inhibited by concentrations of 5 mg/mL of the extract. The more significant activity against gram-positive organisms is to be expected since gram-negative organisms possess a more sophisticated cell envelope and are usually less sensitive to the activity of antibacterial agents.

The antifungal spectrum of the extract was impressive enough to warrant further investigation, especially as the need for antifungal agents, which has always been pressing is made even more so by the rising number of immunocompromized patients as a result of the spread of AIDS and increased number of malignancies, as well as by the acquisition of resistance by the agents of human mycoses. This is the first report describing the antifungal activity and spectrum of this extract. Similarly, there have been no previous reports of the activity of the extract against obligate anaerobes such are those that are usually encountered in oral, deep wound and bone infections. The results of this study demonstrate that their susceptibility to the extract is similar to what was seen with facultative aerobic bacteria with the gram-positive anaerobic cocci being more susceptible to the extract at all test concentrations and the gram-negative *Prevotella* and *Porphyromonas* spp., less susceptible.

It should be pointed out that the extract was very active against the whole spectrum of organisms used in this study, with the activity against gram-negative organisms including the notoriously resistant *Pseudomonas* spp. being especially noteworthy. These organisms, because of their low intrinsic susceptibility to antimicrobial agents and the ease with which they acquire resistances horizontally, are proving to be especially troublesome within the hospital environment. That these organisms are uniformly susceptible to the extract of *A. cordifolia*, *in vitro*, suggests the possibility of using the plant as a source of active antimicrobial principles against infections caused by susceptible organisms. The infrequent occurrence of variations in MICs within species and between related organisms suggests that resistance to *A. cordifolia* extract, when it occurs, is due to intrinsic properties of the species involved rather than acquired characters. For this reason, it would be useful if the extract or its active principles can be exploited for development into antimicrobial chemotherapeutic agents. This is in line with the current search for such substances to augment or replace the antibiotics in current clinical use which because of the spread of the spread of resistant organisms are less useful than before (Iwata, 1992; Chopra *et al.*, 1997).

Although bioactivity guided fractionation of *A. cordifolia* leaf extract has revealed several active principles, none of these can fully account for the high level of activity and broad spectrum associated with the unfractionated extract (Lamikanra *et al.*, 1990). It is not unlikely therefore that this activity is as a result of a combination of effects generated by the different constituents. In this way, it desirable to employ the total extract as an antimicrobial agent since there may be an appreciable degree of potentiation and synergism between various constituents of the plant. This does not, however, preclude further attempts to isolate and characterize the principles responsible for the impressive antimicrobial activity of *A. cordifolia* leaf extract.

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