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Antibacterial activity of the roots, stems and leaves of *Alchornea floribunda*

X. Siwe Noundou [a], R.W.M. Krause [b,*], S.F. van Vuuren [c], D. Tantoh Ndinteh [b], D.K. Olivier [d]

[a] Department of Applied Chemistry, University of Johannesburg, PO Box 17011, Doornfontein, Johannesburg 2028, South Africa
[b] Department of Chemistry, Rhodes University, Grahamstown 6140, South Africa
[c] Department of Pharmacy and Pharmacology, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa
[d] School of Mining, Metallurgy and Chemical Engineering, University of Johannesburg, PO Box 17011, Doornfontein, Johannesburg 2028, South Africa

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ABSTRACT

Ethnopharmacological relevance: *Alchornea floribunda* Müll. Arg. is used in traditional medicine across Africa for the treatment of bacterial, fungal, parasitic and inflammatory disorders.
Aim of the study: To evaluate the antibacterial activity of the crude extracts of different plant parts in order to provide a scientific rationale for the proposed broad efficacy of *Alchornea floribunda* in the treatment of bacterial infections.
Materials and methods: Extracts of roots, stems and leaves were prepared using solvents of various polarities in order to extract a wide range of phytochemicals. The antibacterial activity of these crude extracts was evaluated by micro-dilution assay, against Gram-positive (i.e. *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus saprophyticus*) as well as Gram-negative (i.e. *Escherichia coli*, *Klebsiella pneumoniae*, *Moraxella catarrhalis* and *Proteus mirabilis*) bacteria.
Results: Generally, the ethanol (EtOH), methanol (MeOH), ethyl acetate (EtOAc) and chloroform ($CHCl_3$) extracts demonstrated the best activities, with the leaves exhibiting the highest average activity for six of the eight pathogens. Of these, the ethanolic leaf extract was the most active against *Staphylococcus aureus* with an MIC value of 50 μg/mL. Some other notable activity was observed for the ethyl acetate and chloroform root extracts against *Staphylococcus aureus* (50 μg/mL), and for selected stem extracts against *Staphylococcus aureus* (50 μg/mL), *Klebsiella pneumoniae* (63 μg/mL) and *Staphylococcus saprophyticus* (63 μg/mL).
Conclusion: This study demonstrates the promising antibacterial activity of *Alchornea floribunda* against both Gram-positive and Gram-negative bacteria responsible for gastrointestinal, skin, respiratory and urinary ailments, and validates its use in the ethnopharmacology of the region.

1. Introduction

Species of the genus *Alchornea* (from the Euphorbiaceae family) have been described in traditional pharmacopoeias to treat a range of microbial infections, especially in Cameroon (Adjanohoun et al., 1996; Jiofack et al., 2009). *Alchornea floribunda* Müll. Arg., in particular, is a shrub commonly found throughout central, western, eastern and southern Africa. Roots, bark, leaves or fruits are used in various ways to treat urinary, respiratory and intestinal problems (Mosango, 2007). In Cameroon, the decoction of its leaves and twigs is a well-known treatment for bacterial and stomach parasitical infections (Adjanohoun et al., 1996). In the upper Nyong Valley, Centre Province of Cameroon, where the practice of traditional medicine plays a very important role in basic healthcare, people use the leaves of *Alchornea floribunda* to treat painful micturition (urination) in children (Jiofack et al., 2009). In the Democratic Republic of Congo, *Alchornea floribunda* is commonly known as *ngombo* in the Bolongo area, Bandundu province, where the aqueous extract (leaves and root bark) is used traditionally for the treatment of various parasitic diseases, and have been investigated for its potency against human African trypanosomiasis (Musuyu Muganza et al., 2012). Leaf macerations may also be taken orally against "pains in the heart", while decoctions of young leaves are taken to treat diarrhoea. Ovarian problems, stomach ailments and intestinal disorders are treated by taking leaf decoctions orally or including the leaves in the diet as a vegetable (Mosango, 2007). A root bark decoction in palm wine is

Abbreviations: ATCC, American type culture collection; CFU/ml, colony forming units/ml; $CHCl_3$, chloroform extract; DMSO, dimethyl sulfoxide; EtOH, ethanol extract; EtOAc, ethyl acetate extract; HNC, Herbier National du Cameroun; MeOH, methanol extract; MIC, minimum inhibitory concentration; INT, iodonitrotetrazolium chloride; w/v, weight by volume; IZD, inhibition zone diameter

* Corresponding author. Tel.: +27 46 603 7030; fax: +27 46 622 5109.
E-mail addresses: r.krause@ru.ac.za, xaviersiwe@yahoo.fr (R.W.M. Krause).

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used as a remedy for urinary, respiratory and intestinal disorders (Mesia et al., 2008; Musuyu Muganza et al., 2012).

Alchornea floribunda reportedly also exhibits anti-inflammatory and analgesic properties when applied topically and is commonly used for the treatment of arthritis, muscle pain and other inflammatory disorders (Duke et al., 2002). The crushed leaves are usually rubbed on painful joints or heated leaves are applied topically as a bandage for the treatment of sprains or fractures (Musuyu Muganza et al., 2012). Leaf or root sap (even leaf pulp) is applied to wounds, including painful stingray wounds and circumcision wounds, as well as areas affected by ringworm and eczema. The root sap may also be applied as eye drops to treat ophthalmic problems such as conjunctivitis. Furthermore, the ash of burnt roots mixed with palm oil is applied to scarifications made to treat chest pain and headache (Mosango, 2007).

Apart from its proposed healing properties, the use of *Alchornea floribunda* as an aphrodisiac with stimulant and intoxicating abilities, under the names *niando* (Raymond-Hamet, 1952a, 1952b) or *ononn*, has a long history in central Africa (De Wildeman, 1920; Raymond-Hamet, 1952a; Mesia et al., 2008; Musuyu Muganza et al., 2012). In this instance, De Smet (1996) reported its hallucinogenic potential, while Fernandez (1972) elaborated on its importance in initiation ceremonies (i.e. the roots are sometimes mixed with or used as a substitute for those of *Tabernanthe iboga* Baill., extracted with water and taken orally; also see Mosango, 2007). Powdered dried roots or root bark scrapings may also be mixed with food or macerated for several days in palm wine, banana beer or other local beers and consumed as a tonic to provide energy during festivals and formerly during warfare. It is reported to provide a state of intense excitement followed by a deep, sometimes fatal, depression depending on dosage and habit (Mosango, 2007). The use of root scrapings or dried leaves as a substitute for tobacco (Mosango, 2007), or the mixing of root bark powder with tobacco to be smoked (Musuyu Muganza et al., 2012) are also well-known practices.

Other miscellaneous uses of *Alchornea floribunda* include consumption of leaves as a vegetable with meat or fish as an antidote to poison, or one tea spoon of root bark powder may be eaten daily to cure impotence. The use of root and leafy stem extracts in the treatment of hepatitis has also shown promising results in clinical experiments (Mosango, 2007).

The antibacterial activities of crude extracts prepared by using solvents with a large range of polarities (hexane, chloroform, ethyl acetate, methanol, ethanol and water) from different plant parts (roots, stems and leaves) against pathogens related to stomach disorders (*Bacillus cereus*, *Enterococcus faecalis* and *Escherichia coli*), skin ailments (*Staphylococcus aureus*), respiratory ailments (*Klebsiella pneumoniae* and *Moraxella catarrhalis*) and urinary ailments (*Proteus mirabilis* and *Staphylococcus saprophyticus*) are studied here for the first time in order to validate the use of *Alchornea floribunda* in such traditional treatments.

2. Materials and methods

2.1. Plant material

The plant material was collected in bulk from an uncultivated farmland of the Elounden Mount, Yaoundé central region of Cameroon in January 2010. Identification was made by Mr. Victor Nana, a botanist from the National Herbarium of Cameroon in Yaoundé, where a voucher specimen (No. 4595/HNC) has been deposited.

2.2. Extraction of plant materials

The plant material was dried, finely ground into powder and extracted for 72 h at room temperature (10 g plant material/150 mL solvent). Six different solvents were used for sequential extraction of the same plant sample, i.e. hexane, chloroform, ethyl acetate, ethanol, methanol and water (in that order). The extracts, with the exception of water, were concentrated using a rotary evaporator and dried in a fume hood. The water extracts were freeze-dried.

2.3. Antibacterial activity

The antibacterial activity of the crude extracts was evaluated by the micro-dilution assay (Eloff, 1998) against four Gram-positive bacterias, i.e. *Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus saprophyticus* ATCC 15305, as well as four Gram-negative bacterial strains, i.e. *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Moraxella catarrhalis* ATCC 23246 and *Proteus mirabilis* ATCC 43071. All strains were confirmed purity stock cultures, maintained in the pharmaceutical microbiology laboratories at the University of Witwatersrand. Stock solutions of all the extracts (32 mg/mL) were prepared in acetone, or 25% dimethylsulphoxide (DMSO)/water (where selected extracts were insoluble in acetone) or water, and were serially diluted with sterile water in a 96-well microtitre plate. Equal volumes (100 μL) of bacterial suspension yielding approximately an inoculum size of 1×10^6 colony forming units (CFU)/mL was added to all the wells. The plates were sealed and incubated for 24 h. Thereafter, 0.04% (w/v) INT (*p*-iodonitrotetrazolium) was added to each well and the plate was left at room temperature for 6 h before the results were recorded. The assay was performed at least in triplicate. Sterile broth containing bacterial suspension was used to monitor the viability of the test organism, while ciprofloxacin (0.01 μg/mL) was used as the positive antibacterial control. The final concentration of acetone or DMSO in the well had no effect on bacterial growth.

3. Results and discussion

The antimicrobial activity of *Alchornea floribunda*, as exhibited here, shows that the crude extracts of all three plant parts (leaves, stems and roots) of *Alchornea floribunda* displayed mostly noteworthy inhibitory effects (leaves and roots with MIC values as low as 50 μg/mL, and stems from 63 μg/mL) on eight selected pathogens (Table 1). In all the three cases, the activities exhibited by the medium polar extracts (ethanol, methanol, ethyl acetate, and chloroform) were higher (MIC values between 50 and 1000 mg/mL) than those exhibited for the aqueous and non-polar extracts (MIC values from 1000 or 130 μg/mL respectively, mostly $>$ 8000 μg/mL), and agrees with the means of preparation where traditional infusions and decoctions using palm wine are used. Traditionally, leaves or roots may be used on their own, and sometimes also in conjunction to treat infections. Leaves do, however, seem to be preferentially used for medicinal purposes while the roots tend to be used for their hallucinogenic properties. The results obtained here, i.e. the leaves showing highest activity in general, provide a scientific rationale for this notion.

The classes of compounds previously identified from *Alchornea floribunda* leaves include terpenes, sterols, saponins, flavonoids, tannins, carbohydrates and glycosides as well as alkaloids (Okoye and Ebi, 2007). Preliminary disc diffusion assays against *Pseudomonas aeruginosa*, *Salmonella keitambii* and *Bacillus subtilis* revealed weak activity for the terpene fractions only, with IZD 42 mm, 34 mm and 32 mm respectively (Okoye and Ebi, 2007). Isolation studies revealed the identities of the major stigmastane-type steroids [i.e. stigmastane-22-ene-3,6-dione and 3-hydroxystigmastane-22-ene