



Short communication

Extracts of the leaves and twigs of the threatened tree *Curtisia dentata* (Cornaceae) are more active against *Candida albicans* and other microorganisms than the stem bark extract

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Abstract

Curtisia dentata is a popular medicinal plant in South Africa that has become vulnerable and scarce owing to over-exploitation by collection of bark. The aim of this study was to compare the antifungal activities of extracts of the leaves, twigs and bark of *C. dentata* to identify possible substitution of leaf material for bark for medicinal purposes. The methods used were thin layer chromatography (TLC) to compare the chemical composition, bioautography to ascertain the number of active compounds and microdilution assay to determine the minimal inhibitory concentrations of the extracts. TLC revealed differences in chemical compositions of the extracts of the leaves, twigs and bark of *C. dentata*. These differences were largely centred on the quantities of the various components in the extracts. The acetone extracts of the leaves had four bands active against *Candida albicans* and two in extracts of the stem bark on bioautograms. The R_f values of these bands indicated that they are also present in the leaves. The leaves were more active with an MIC of 0.11 mg/ml while the stem bark had an MIC of 0.61 mg/ml against *C. albicans*. The total activity values confirmed that the leaves (1072 ml/g) were five-fold more active than the bark (190 ml/g).

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1. Introduction

In the continent of Africa, plant-derived preparations are an important component of primary health care for approximately 80% of the population (Farnsworth, 1988; Balick et al., 1994). This widespread usage of plant-based remedies in South Africa involves an estimated 147 plant families, and the most prominent in Zulu, Sotho and Xhosa ethnomedicine are the Fabaceae, Asteraceae, Euphorbiaceae, Rubiaceae and Orchidaceae families (Hutchings et al., 1996). Medicinal tree bark is the most preferred source of herbal remedies in South Africa,

constituting approximately 27% of market produce traded annually in KwaZulu–Natal (Mander, 1998).

Curtisia dentata is traditionally used for stomach ailments, diarrhoea, as a blood strengthener and as an aphrodisiac (Hutchings et al., 1996). It is also used in treatment of heartwater in cattle in the Eastern Cape (Dold and Cocks, 2001) and treatment of pimples (Grierson and Afolayan, 1999). The harvesting of bark endangers the survival of the plant. *Curtisia dentata* is classified as vulnerable, declining, conservation-dependent and protected in KwaZulu–Natal (Cunningham, 1988; Scott-Shaw, 1999). Approximately 24 t of *C. dentata* bark are harvested and traded annually in KwaZulu–Natal (Cunningham, 1988; Mander, 1998). The traditional medical practitioners use this species in special mixtures because it is scarce (Cunningham, 1988).

Grace et al. (2002) and Zschocke et al. (2000a) suggested replacing non-sustainable stem bark, roots and bulbs with aerial parts such as leaves and twigs for medical applications as this

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practice inflicts much less damage on the plants. Depending on the plant species, phytochemical constituents of the bark and leaves may be similar and show identical biological activity (Zschocke et al., 2000a,b). In other words, traditional healers and other users may realise the same efficacy using the bark, twigs or the leaves. In support of this, Lewu et al. (2006) reported that the leaves of *Pelargonium sidoides* may substitute for its roots to treat bacterial infections, as similar bioactivity of extracts of the different plant parts was proven against several test organisms.

After a random screening of leaves of more than 400 southern African trees against a range of bacterial and fungal pathogens in the Phytomedicine Programme (www.up.ac.za/phyto) several species with very good activity against *Candida albicans* were identified and examined in greater detail (Shai et al., 2008). Reports concerning isolation of compounds from *C. dentata* are scanty. Leaf extracts had good activity against *C. albicans*. The antifungal pentacyclic triterpenoids lupeol, betulinic acid, ursolic acid and colosolic acid were isolated from the leaves of *C. dentata* by bioactivity guided fractionation (Shai, 2007).

The aim of the study was to compare the activity of extracts of bark, twigs and leaves of *C. dentata* against fungal and bacterial pathogens. If the activity of the extracts of the leaves is comparable to those of the bark, the harvesting of *C. dentata* leaves for medicinal purposes by local South Africans may be encouraged. This may, in turn, promote the conservation of *C. dentata*.

2. Materials and methods

2.1. Plant collection

Leaves, twigs and stem bark of *C. dentata* were harvested from a mature tree growing in the University of Pretoria Botanical Gardens in March 2005, dried at room temperature and ground to powder using a Macsalab mill (Model 200 Lab). A voucher specimen (Shai 003) has been deposited in the medicinal plant herbarium of the Phytomedicine Programme at the University of Pretoria. The leaf, twig and stem bark powders were stored separately in sealed glass containers at room temperature in the dark until use.

2.2. Extraction

For preparation of extracts from different plant parts, the plant material (1 g) was extracted with acetone (10 ml) (Eloff, 1998a). Acetone was chosen as a solvent in this comparative study because it extracts a range of polar and non-polar compounds (Eloff, 1998a). The mixture was vigorously agitated on a linear shaker and the filtrate collected through Whatman No. 1 filter paper. The filtrate was dried and the residue reconstituted to 10 mg/ml in acetone.

2.3. TLC fingerprints

The plant extracts (stem bark, twigs and leaves) were analyzed by separation on aluminium-backed Merck F_{254} thin layer chromatography (TLC) plates. TLC plates were loaded with 100 μ g of acetone extracts of stem bark and leaves and

developed in chloroform/ethyl acetate/formic acid (CEF) (5:4:1), or dichloromethane/ethyl acetate (DE) (4:1). The plates were visualized under UV light at 254 and 365 nm before being sprayed with vanillin-sulphuric acid and heated at 110 °C for a few minutes until optimal colour development. Replicate plates not sprayed with vanillin-sulphuric acid reagent were used for bioautography analysis.

2.4. Antimicrobial activity

2.4.1. Fungal and bacterial cultures

Candida albicans, isolated from a Goldian finch, was obtained from the fungal culture collection in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science at the University of Pretoria. *Candida albicans* was cultured at 30 °C in universal bottles as slants in Sabouraud dextrose agar and Sabouraud dextrose broth prior to bioactivity assay procedures. The density of *C. albicans* before antimicrobial activity testing was 2.5×10^6 cells/ml.

Bacterial test organisms used in the screening tests were *Staphylococcus aureus* (Gram-positive, American Type Culture Collection [ATCC] number 29213), *Enterococcus faecalis* (Gram-positive, ATCC 29212), *Pseudomonas aeruginosa* (Gram-negative, ATCC 27853) and *Escherichia coli* (Gram-negative, ATCC 25922). These species are considered the most important nosocomial pathogens and these specific strains are used to compare antibiotics in different laboratories (NCCLS, 1992). Bacterial cells were inoculated into fresh Müller–Hilton (MH) broth (Fluka, Switzerland) and incubated at 37 °C for 14 h prior to the screening procedures. Densities of bacterial cultures after incubation overnight were as follows: *S. aureus*, 2.6×10^{12} cells/ml; *E. faecalis*, 1.5×10^{10} cells/ml; *P. aeruginosa*, 5.2×10^{13} cells/ml; *E. coli*, 3.0×10^{11} cells/ml.

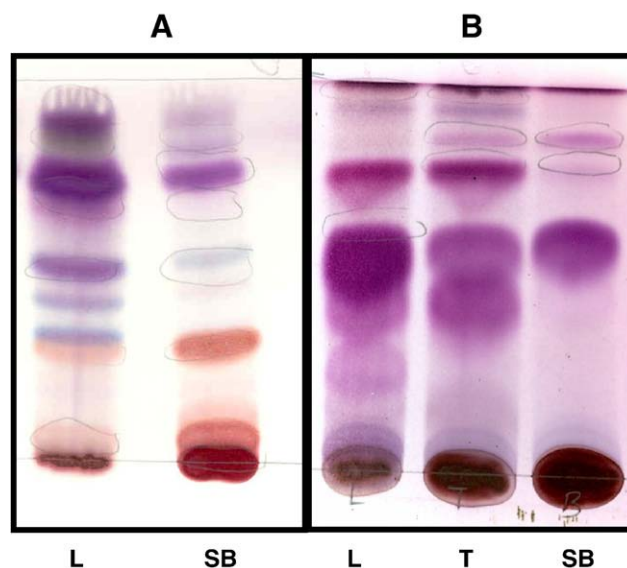


Fig. 1. Comparison of the chemical components present in leaves (L), twigs (T), and stem bark (SB) of *Curtisia dentata*. TLC plates were developed in CEF (A) and DE (4:1) (B) and sprayed with vanillin-sulphuric acid.

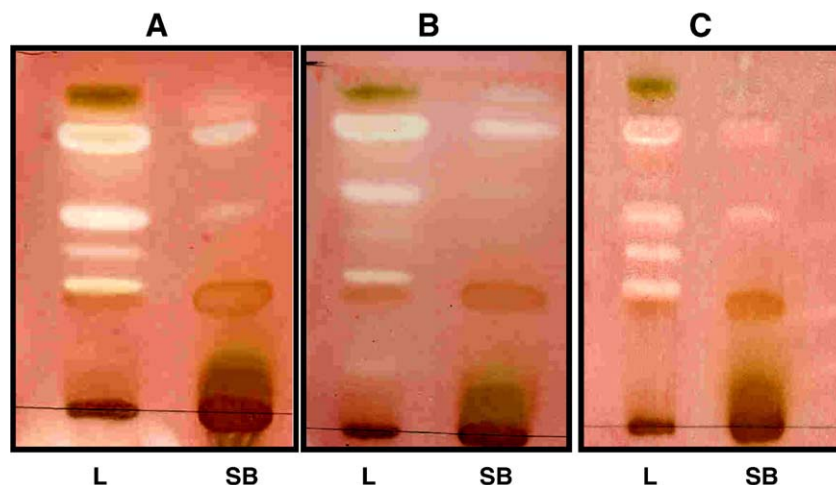


Fig. 2. Comparison of the antibacterial chemical components present in leaves (L) and stem bark (SB) of *Curtisia dentata*. Acetone extracts of leaves and bark were analysed on TLC plates using CEF as mobile phase, and then sprayed with *E. coli* (A), *S. aureus* (B) or *C. albicans* (C). Clear lines indicates inhibition of growth of the microorganism.

2.4.2. Bioautography

Thin-layer chromatography plates were loaded with extracts, developed and used for bioautography using the method developed in our laboratory (Masoko et al., 2005) using *Candida albicans* as a fungal test organism. *Escherichia coli*, *S. aureus*, *E. faecalis* and *P. aeruginosa* were used as bacterial test organisms. The bioautography plates were compared with the reference TLC plates prepared as described in Section 2.3.

2.4.3. Microdilution method

To determine the minimal inhibitory concentration (MIC), the microplate dilution method developed by Eloff (1998b), with slight modifications for *C. albicans* by Masoko et al. (2005) was used. MIC values were regarded as the lowest concentrations of extracts that inhibited growth of test organisms. The total activity of the extracts was calculated as the total mass (mg) of the extract prepared from 1 g of powdered plant material divided by the MIC value (mg/ml). The total activity value indicates the volume to which 1 g of the extract can be diluted and still inhibit the growth of microbial cells (Eloff, 2004).

Table 1
MIC values (mg/ml) of *C. dentata* leaf and stem bark acetone extracts against *C. albicans*

Plant part	MIC values (mg/ml)	Mass of extract (mg/g)	Total activity (ml/g)
Stem bark	0.61±0.27	116	190
Leaves	0.11±0.04	118	1072
Amp B	0.2 µ/ml	–	–

Key: Amp B, amphotericin B.

The results represent means of three independent triplicate experiments. Total activity was calculated using average MIC's.

3. Results and discussion

Plant leaves, twigs and stem bark extracted with acetone were analysed using TLC to compare their chemical compositions. There were slight differences in chemical composition, mostly concerning the levels of specific chemical components as indicated by the intensity of spots on TLC plates. Extracts of the twigs and leaves contained similar components at approximately equal quantities on TLC plates. Some components that were observed in the extracts of the leaves and twigs were undetectable in the bark extract. In the bark extract there was a high concentration of a band that stained orange-red when sprayed with vanillin, while in the leaves there was an accumulation of three components that stained dark blue-purple (Fig. 1).

Table 2
MIC values (mg/ml) of the acetone extracts of leaves, twigs and stem bark of *Curtisia dentata* against bacterial test organisms

Bacterial species	Plant extracts		
	Leaves	Twigs	Stem bark
<i>MIC values (mg/ml)</i>			
<i>E. coli</i>	0.56±0.11	0.32±0.15	0.60±0.17
<i>S. aureus</i>	0.21±0.08	0.12±0.03	0.30±0.12
<i>P. aeruginosa</i>	0.12±0.04	0.11±0.02	0.15±0.06
<i>E. faecalis</i>	0.09±0.03	0.10±0.05	0.15±0.05
Average MIC values	0.25	0.16	0.30
<i>Total activity (ml/g)</i>			
<i>E. coli</i>	210	359	387
<i>S. aureus</i>	562	958	387
<i>P. aeruginosa</i>	983	1045	773
<i>E. faecalis</i>	1311	1150	773
Average total activity	767	878	580

The results represent means of two independent triplicate experiments. Total activity was calculated using average MIC's.

Since extracts of the twigs and leaves of *C. dentata* contained similar compounds on TLC plates, only the extracts of the leaves were used for antibacterial and antifungal activities using bioautography. Four compounds in the leaf extract were active against *C. albicans* compared with two in the bark extract. The number of compounds separated by TLC active against *C. albicans* was determined by bioautography. The leaf extract contained four antibacterial and anti-*Candida* bands compared to two in the bark extract (Fig. 2). Similar observations were recorded with bacterial species, where extracts of the leaves had more active compounds inhibiting the growth of these micro-organisms.

The leaf acetone extract was five to six times more active than the bark extract (0.11 vs 0.61 mg/ml). The total activity of the leaf extract was more than five-fold higher than that of the bark extract, at 1072 and 190 ml/g respectively (Table 1). This means that the extract from one gram of dried leaves can be diluted to more than one litre and it will still kill the fungus (Eloff, 2000). The TLC fingerprints of the extracts of the leaves and twigs were comparable, hence only leaf extracts were used. The MIC of the leaves and bark against bacteria were comparable (Table 2). The average MIC values for the extracts were: 0.25 mg/ml for extracts of the leaves, 0.16 mg/ml for extracts of the twigs and 0.30 mg/ml for bark extracts. The lowest MIC value (0.09 mg/ml) was obtained with leaf extracts against *E. faecalis*. However, the calculated average total activity values of the twigs (878 ml/g) and leaves (767 ml/g) were higher than that calculated for the bark extract (580 ml/g).

Some literature reports suggest that the phytochemical constituents of the bark and leaves are similar for certain species (Zschocke et al., 2000a,b). The MIC values reported in this study question the preference of bark over leaves in preparation of traditional medicine cocktails using *C. dentata* for treating infections. The MIC and total activity values of the bark, leaves and twigs against bacteria imply these plant parts can be used interchangeably. Harvesting of the leaves and twigs inflicts much less damage on the plant, and promotion of usage of these aerial parts may help in the conservation of this plant species (Zschocke et al., 2000a). These results suggest that leaves may be used to replace stem bark in *C. dentata* traditional medicine preparations for treatment of bacterial and fungal-related infections as the plant species is threatened (Cunningham, 1988; Scott-Shaw, 1999). In future work, a comparison of activity in the water extracts of the leaves and stem bark or for non-infection related diseases may serve to further encourage harvesting of leaves for traditional medical purposes thereby conserving the vulnerable *C. dentata*.

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