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Crude extracts of, and purified compounds from, *Pterocarpus angolensis*, and the essential oil of *Lippia javanica*: their in-vitro cytotoxicities and activities against selected bacteria and *Entamoeba histolytica*

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In a recent study, various extracts of *Pterocarpus angolensis* were prepared and tested against bacteria. The acetone extract was found to be the most active against all the bacteria investigated, with minimum inhibitory concentrations varying from 0.0156 mg/ml against *Staphylococcus aureus* to 2 mg/ml against *Enterobacter cloacae*. Seven pure compounds were subsequently isolated from the ethanol extract of *P. angolensis*. Using several chromatographic and spectroscopic methods, the structures of five of these compounds — phthalate and four derivatives of epicatechin [(-)-epicatechin, epicatechin-3-*O*-galate, epicatechin (4β–8)-epicatechin (B2), and a hexamer of epicatechin] — were successfully determined. The seven purified compounds were then further tested, *in vitro*, against *Staphylococcus aureus* and *Entamoeba histolytica*, and for their in-vitro cytotoxic activity. Although all seven were active against *S. aureus*, just one of the purified compounds from *P. angolensis* and piperitenone, a pure compound isolated from *Lippia javanica* essential oil, were found to have marked activity against *Entamoeba histolytica*, with median inhibitory concentrations (IC_{50}) of 25 and 100 μg/ml, respectively. The other *P. angolensis* compounds were either weakly active or showed no activity against the amoebae when tested at concentrations up to 400 μg/ml. All seven compounds isolated from *P. angolensis* showed less toxicity against cultures of human (HCT-8) cells than piperitenone, with IC_{50} of 175–375 μg/ml. The presence of epichatechin and derivatives (with strong antibacterial activities but generally weak activities against *Entamoeba histolytica*) in the stem bark of *P. angolensis* has thus been demonstrated. Further investigation of the activities of these compounds and their potential use in the treatment of bacterial diseases appears justified.

Infectious diseases are important health problems, particularly in developing countries, where the number of emerging and opportunistic infections has been increasing as the result of the changing environment and habits and HIV. In the Venda region of southern Africa, for example, *Cryptosporidium*, *Entamoeba histolytica*, *Campylobacter* spp, entero-aggregative *Escherichia coli* and *Clostridium difficile* are all now quite easy to find (Samie *et al*., 2008). Although there are drugs available to treat and control such infections, their wide-spread use is often hampered by high costs and their efficacy may be limited by resistance in the pathogens. In an attempt to develop relatively cheap but effective alternatives to existing antimicrobial drugs, Samie *et al*. (2009) recently studied the antimicrobial and cytotoxic activities of Venda medicinal plants *in*

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vitro. The results of this investigation indicated that crude extracts of *Pterocarpus angolensis* and *Lippia javanica* were active against *Campylobacter* spp. and *Entamoeba histolytica*.

Pterocarpus angolensis, commonly known as bloodwood in English and as *mutondo* in Tshi-Venda, is a deciduous, spreading and slightly flat-crowned tree with a high canopy. It reaches about 15 m in height and has dark bark. The shiny leaves are compound (i.e. divided into leaflets) and characteristically hang downwards (Van Wyk and van Wyk, 1997). The species belongs to the family Fabaceae and sub-family Papilionoideae. It is indigenous to East and southern Africa, growing from sea level, on the Mozambican coast, up to 1650 m above sea level (Stahle *et al.*, 1999). The tree is commercially important, its dense timber having high durability and strength. In countries such as Tanzania, it is widely utilized for furniture, veneer, carving and general-purpose timber (Monela *et al.*, 1993). Extracts of the tree are commonly used by traditional healers, in the Venda region as well as other regions of Africa, for the treatment of malaria, gonorrhoea, headaches, stomach aches, diarrhoea, mouth sores and rashes. In South Africa, the sap is used traditionally for the treatment of ringworm, ulcer, malaria, skin inflammation and urinary schistosomiasis (Watt and Breyer-Brandwijk, 1962; Palgrave, 1981; Ndamba *et al.*, 1994; Nyanzema *et al.*, 1994; Van der Reit *et al.*, 1998). Although the seeds of *Pt. angolensis* are known to contain lectins (Bezuidenhoudt *et al.*, 1980), no compounds from the tree's stem bark — which is commonly used by traditional healers in Africa — appear to have been characterised.

In the present study, crude extracts of *Pt. angolensis* stem bark were prepared using various organic solvents and tested against several bacterial organisms. Several compounds in an ethanol extract were then isolated, characterised and tested, *in vitro*, for their activity against selected bacterial pathogens and *Enta. histolytica* and for their cytotoxicity against a human cell line.

MATERIALS AND METHODS

Plant Collection

Between November 2003 and February 2004, stem bark was collected from several *Pt. angolensis* growing at Mbaye and Makwarela, near the South African town of Thohoyandou.

Preparation of Crude Extracts

The bark was washed with distilled water and air dried in the laboratory for 2 weeks before being ground in a Wiley mill grinder (Thomas Scientific, Swedesboro, NJ) with a 2-mm wire mesh. Samples of the ground bark were soaked in various organic solvents (hexane, dichloromethane, chloroform, ethanol, acetone or methanol, with 50 g bark/500 ml solvent) for at least 72 h, with frequent shaking. Each resultant crude extract was suction-filtered through Whatman No.1 filter paper (Whatman, Maidstone, U.K.) and then the filtrate was evaporated to dryness in a rotary evaporator under reduced pressure, at 40°C. Each residue was dissolved, at 0.2 g/ml, in 12% (v/v) dimethyl sulphoxide (DMSO) in water, to give a stock solution. All the stock solutions (and the essential oil of *L. javanica*; see below) were kept at 4°C in the dark until used.

Fractionation

STEM BARK OF *Pt. angolensis* (FIG. 1)

For the isolation of compounds from an ethanolic extract of the stem bark of *Pt. angolensis*, 1.4 kg of the powdered dried bark was extracted with 5 litres of ethanol, by sonication for 30 min and overnight maceration. As previously, the crude extract was suction-filtered through Whatman No. 1 filter paper and concentrated to dryness under reduced pressure at 40°C, in a rotary evaporator. The mass of the dried

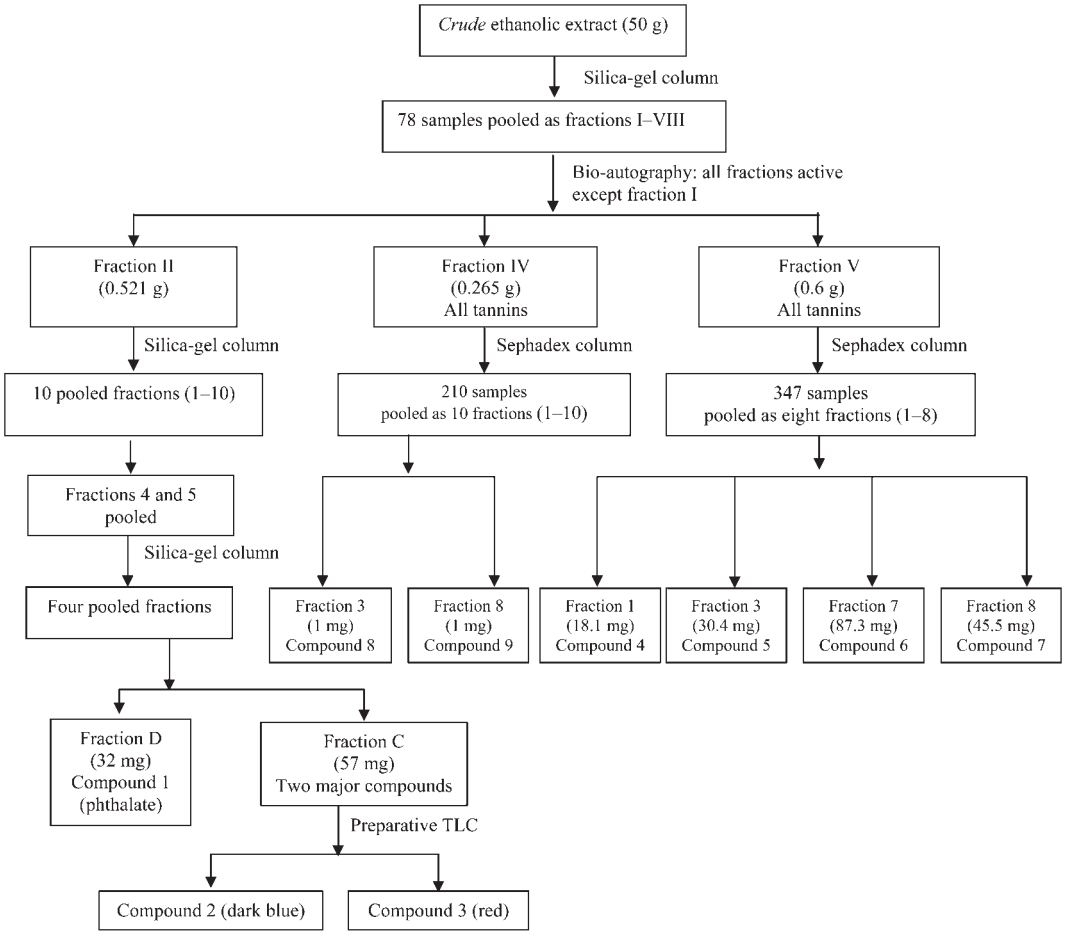

FIG. 1. Schematic representation of the steps taken in the isolation of the major compounds from an ethanolic extract of *Pterocarpus angolensis* stem bark.

extract obtained was 61.595 g, giving a yield of 11.54%.

Most (50 g) of the dried ethanol extract was loaded on a large silica-gel column and eluted with seven different hexane: ethyl-acetate solvent systems (with gradually increasing polarity) to give 78 samples, which were pooled to give eight main fractions (I–VIII). When the antibacterial activity of each of these main fractions was tested against *Staphylococcus aureus* by bio-autography (see below), all except fraction I (which was not investigated further) showed very strong antibacterial activity.

Fraction II (0.521 g) was loaded on a silica-gel column and eluted with hexane:ethyl acetate (9 : 1, by vol.) to give 10 fractions (1–10). Fractions 4 and 5 were pooled and run on another silica-gel column, with 7% ethyl acetate in hexane as the eluent. The 225 samples that were collected were pooled as four fractions (A–D). Fraction D (32 mg) gave a single, blue band on preparative thin-layer chromatography (TLC), whereas fraction C (57 mg) gave two bands, one red and the other bluish; the results of preliminary analysis indicated that fraction D was phthalate.

Columns of Sephadex™ (GE Healthcare, Uppsala, Sweden) were used for the fractionation of fractions IV (0.265 g) and V (0.6 g) from the original

extract, each column being eluted with ethanol and run for 9 days. The chromatography of fraction IV produced 10 pooled fractions, two of which — fractions 3 (1 mg) and 8 (1 mg) — were 'clean'. The chromatography of fraction V gave eight pooled fractions and four of the eight — fractions 1 (18.1 mg), 3 (30.4 mg), 7 (87.3 mg) and 8 (45.5 mg) — were 'clean'.

Although fraction III (0.164g) was run on a silica-gel column, with 10% ethyl acetate in hexane as eluent, each of the 16 fractions so obtained was 'mixed' and not investigated further.

ESSENTIAL OIL FROM *Lippia javanica*

A 300-mg sample of the essential oil of *L. javanica*, previously obtained by hydrodistillation and tested against several bacterial species (Samie *et al.*, 2005), was loaded on a silica-gel column and eluted with 3% ethyl acetate in hexane. Twelve fractions (1–12) were obtained and fraction 5 was further purified on a small silica-gel column. The major compound collected from the small column was identified (see below).

IDENTIFICATION OF THE ISOLATED COMPOUNDS

Attempts were made, using nuclear mass resonance (NMR), gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC) to identify the seven compounds isolated, in apparently pure form, from the stem bark of *Pt. angolensis* and the single compound isolated from the *L. javanica* oil. Both ^{1}H and ^{13}C NMR spectra were recorded.

Bacteria

Each of the six crude extracts of *Pt. angolensis* was tested *in vitro* against five Gram-positive bacteria (*Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, *St. aureus* and *Enterococcus fecalis*) and four Gram-negative bacteria (*Enterobacter cloacae*, *Escherichia coli, Pantoea agglomerans* and *Proteus mirabilis*). All of the bacterial strains, which came from the Departments of Microbiology and Biological Sciences at the University of Venda in Thohoyandou, were maintained on nutrient agar, with subculture every 3 days. For the present study, an inoculum of each bacterial strain was suspended in 5 ml Mueller–Hinton broth and incubated overnight at 37°C. The overnight cultures were then diluted with fresh Mueller–Hinton broth, to give a concentration of bacterial cells that matched a 0.5 McFarland turbidity standard, prior to the assays of antibacterial activity.

Antimicrobial Assays

EFFECTS OF DMSO

As the stock solutions of the crude extracts of *Pt. angolensis* contained DMSO, the activity of DMSO against each of the test strains of bacteria was determined using the disc-diffusion and microdilution methods (Samie *et al.*, 2005) and Mueller–Hinton broth containing 0.1% to 25% (v/v) DMSO.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

The microdilution method was also used to determine the minimum inhibitory concentration (MIC) — the lowest concentration inhibiting all visible growth of each bacterial strain — of each of the crude extracts of *Pt. angolensis*, the seven compounds purified from them, and the single compound isolated from *L. javanica* oil. Gentamicin or kanamycin (each at concentrations varying between 0.25 and 32 μg/ml) was used as the positive control, while 12% (v/v) DMSO in the culture broth was used as the negative control.

ACTIVITY OF ISOLATED COMPOUNDS AGAINST *Enta. histolytica*

Each of the seven compounds isolated from the ethanol extract of *Pt. angolensis* stem bark and the single compound isolated from

L. javanica oil were tested, *in vitro*, for anti-amoebic activity against a standard strain of *Enta. histolytica* (HM-1:IMSS), using another microdilution method (Samie *et al.*, 2009). The concentrations tested varied between 3.8 and 400 μg/ml. Each test included metronidazole, at concentrations varying between 0.01 and 2 μg/ml, as the positive control, diluent (i.e. culture medium with an appropriate concentration of DMSO) as the negative control, and a blank (culture medium without DMSO). The lowest concentration that showed inhibition (the destruction or rounding-up) of half of the cells in the well was treated as an approximate median inhibitory concentration (IC_{50}). More accurate IC_{50} were then determined by Trypan-Blue staining of the amoebae (Samie *et al.*, 2009).

CYTOTOXICITY ASSAY AGAINST HCT-8 CELLS

The isolated compounds were also each tested for cytotoxicity against a cell line (HCT-8; American Type Culture Collection, Rockville, MD) derived from a human intestinal adenocarcinoma. Monolayers of HCT-8 cells were prepared in 96-well microtitre trays by seeding each well with 200 μl 10% Eagle's minimum essential medium (MEM) containing 1×10^5 cells/ml. Doubling dilutions of each test compound, from 400 to 0.8 μg/ml, were prepared in medium before 200 μl of a dilution (or, as a negative control, the same volume of medium) were added to each well. The cells were incubated for 7 days at 37°C. The well contents were inspected daily on an inverted microscope and checked for loss of the monolayer and rounding, shrinking, granulation and vacuolization of the cells. The results were expressed as the concentrations of each compound that, after 7 days, inhibited 50% of the cell growth seen in the negative-control wells.

Bio-autography Assay

The fractions of the crude extracts as well as the isolated compounds from *Pt. angolensis* and *L. javanica* were tested for antibacterial activity by direct bio-autography on TLC. The fractions (of about 5 μl) were applied to plates of silica gel 60 (Merck) and developed in ethyl acetate:hexane (7 : 3, by vol.). The isolated compounds were dissolved in either dichloromethane:methanol (9 : 1, by vol.; *Pt. angolensis*) or ethyl acetate (*L. javanica*). Each TLC plate was observed under ultraviolet light (at 254 and 366 nm) after development, then left overnight, for the solvent to evaporate completely, before being sprayed with a suspension in nutrient broth (matching the 0.5 McFarland turbidity standard) of Gram-positive *St. aureus* (Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria). The TLC plates were then dried for a few minutes until they appeared translucent and incubated overnight at 37°C in humid conditions. The plates were then sprayed with an aqueous solution of p-iodonitrotetrazolium violet (2.0 mg/ml) and re-incubated at 37°C for 4 h. White areas indicated the presence of antibacterial compounds, as bacterial growth converts the indicator tetrazolium salt to a red product.

RESULTS AND DISCUSSION

Both *Pt. angolensis* and *L. javanica* are common in the Venda region, in the far north–east of South Africa, and both are used by traditional healers for the treatment of a wide variety of ailments (Samie *et al.*, 2005, 2009). Extracts and essential oil from *L. javanica* collected from the Venda region have previously been found to have good activity against several species of bacteria (Samie *et al.*, 2005). Whilst many studies have characterised *L. javanica* compounds and tested their activity against various organisms, no study has determined the antimicrobial activity of isolated *Pt. angolensis* compounds. The objectives of the present study were to determine the activity of *Pt. angolensis* bark extracts against several bacterial organisms of clinical interest and to

isolate pure compounds from a bark extract and test their activities against bacteria and *Enta. histolytica* and their cytotoxicities against monolayers of HCT-8 cells. In addition, the major compound from the essential oil of *L. javanica* was isolated and assayed for its in-vitro cytotoxicity and antibacterial and anti-amoebic activities.

Of the five solvents used for the extraction of compounds from *Pt. angolensis* stem bark, the fractions produced using the more polar solvents (i.e. ethanol, methanol and acetone) showed good activities against all of the bacterial organisms tested, the recorded MIC all being <0.5 mg/ml with the exception of those (all of 2 mg/ml) measured against *Enterobacter cloacae* (Table 1). The acetone extract was particularly active, with an MIC of just 15.6 μg/ml against *St. aureus*, although the ethanol extract was the most active against *B. cereus*, with an MIC of 62 μg/ml. The hexane extract was the least active against all the bacterial organisms, giving MIC of >4 mg/ml against all the organisms except *B. pumilus* and *B. subtilis* (Table 1). In a previous study, similarly, *Pt. angolensis* extracts were found to be active against *Aeromonas* spp., with higher activity associated with an acetone extract than with methanol and hexane extracts (Obi *et al.*, 2007). Acetone extracts of other medicinal plants have also been found more active against bacterial isolates than the corresponding methanol or hexane extracts (Aqil and Ahmad, 2007; Sabir *et al.*, 2007). In the present study, however, the ethanol extract was shown to be even more active than the acetone extract against certain bacteria (*B. cereus* and *Enterococcus fecalis*). Ethanol extracts of other plants have recently been found active against *Mycobacterium* spp. (Cruz-Vega *et al.*, 2008; Mativandlela *et al.*, 2008) as well as other bacterial and fungal organisms (Kloucek *et al.*, 2007; Al-Bayati and Al-Mola, 2008).

Disc diffusion is regularly used to test the antibacterial activity of medicinal-plant extracts. Bio-autography provides more information, requires a smaller weight of sample and can be used for the bio-assay-guided isolation of compounds, simplifying the process of the identification and isolation of the active compounds (Rahalison *et al.*, 1991). Bio-autography (based on an agar overlay) is considered one of the most efficient methods for the detection of antimicrobial compounds (Runyoro *et al.*, 2006). In the present study, after the first chromatography of the ethanolic extract of *Pt. angolensis* bark on a silica-gel column, the antibacterial activity of the eight fractions was tested against *St. aureus* using bio-autography on a TLC plate. This revealed that all the fractions except the first were very active against *St. aureus* (Fig. 2).

In their recent study, Samie *et al.* (2009) found stem-bark extracts of *Pt. angolensis* to be active against *Campylobacter* spp. and *Enta. histolytica*. In the present study, the major compounds, including, phthalate, flavonoids and tannins, were isolated from a ethanolic extract of this bark (the ethanolic being investigated rather than the acetone because ethanolic extracts of the bark are sometimes used by Venda traditional healers). Although seven compounds were isolated in pure form (Fig. 3), only five were collected in sufficient quantity to be fully identified, as phthlate and four derivatives of epicatechin [(-)-epicatechin, epicatechin-3-*O*-galate, epicatechin (4β–8)-epicatechin (B2), and a hexamer of epicatechin]. The structures of the epicatechin derivatives are shown in Figure 4. This is the first study to demonstrate the presence of these compounds in *Pt. angolensis* stem bark, although similar compounds have been isolated from the bark of other, related plants. (-)-Epicatechin, for example, has been isolated as an active principle in a water extract of the bark of *Pt. marsupium* (Sheehan *et al.*, 1983). In in-vitro experiments, this compound was found to increase the cyclic-adenosine-monophosphate content of rat islets, a change associated with increased insulin release, the conversion of proinsulin to insulin and cathepsin B activity (Ahmad *et al.*, 1991). Epicatechin derivatives have

TABLE 1. *The minimum inhibitory concentrations (MIC) of various extracts of* Pterocarpus angolensis *stem bark and other samples, measured,* in vitro, *against nine species of bacteria*

Sample	Units for MIC	MIC against: *Bacillus cereus*	*Bacillus pumilus*	*Bacillus subtilis*	*Staphylococcus aureus*	*Enterococcus fecalis*	*Enterobacter cloacae*	*Escherichia coli*	*Pantoea agglomerans*	*Proteus mirabilis*
Hexane extract	µg/ml	>4000	1000	4000	>4000	>4000	>4000	>4000	>4000	>4000
Dichloromethane extract	µg/ml	1000	1000	4000	1000	1000	>4000	4000	4000	>4000
Chloroform extract	µg/ml	1000	4000	2000	1000	1000	>4000	1000	4000	>4000
Ethanol extract	µg/ml	62	500	125	62	125	2000	250	500	500
Acetone extract	µg/ml	125	250	62	15.6	250	2000	125	250	125
Methanol extract	µg/ml	500	250	125	31.2	250	2000	250	250	250
Dimethyl sulphoxide	(%, v/v)	>8	>8	>8	>8	>8	>8	>8	>8	>8
Gentamicin	µg/ml	4	4	4	4	4	4	4	4	4

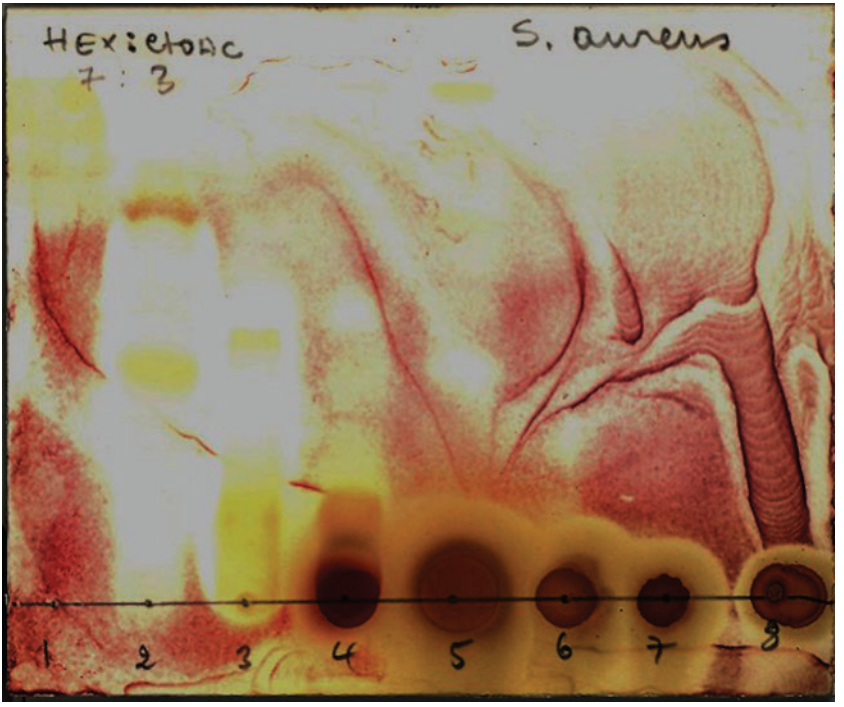

FIG. 2. Bio-autograph of the antibacterial activity of fractions I–VIII (in lanes 1–8, respectively), showing that all the fractions were active against *Staphylococcus aureus* except fraction I.

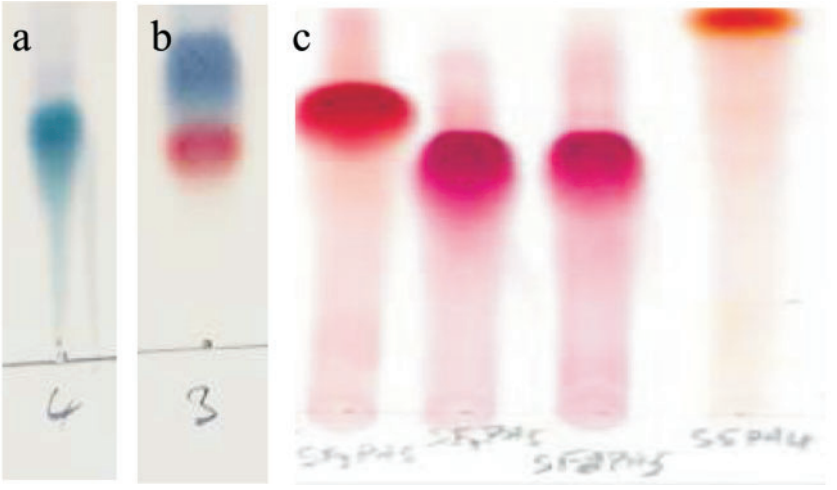

FIG. 3. The results of the thin layer chromatography of six compounds isolated from an ethanolic extract of *Pterocarpus angolensis* stem bark, including compound 1, identified as a phthalate by nuclear magnetic resonance (a), compounds 2 and 3 (b), which were available in insufficient amounts to be identified, and compounds 4–7 (c), identified, respectively, as (-)-epicatechin, epicatechin (4β–8)-epicatechin (B2), a hexamer of epicatechin and epicatechin-3-*O*-galate (see Figure 4).

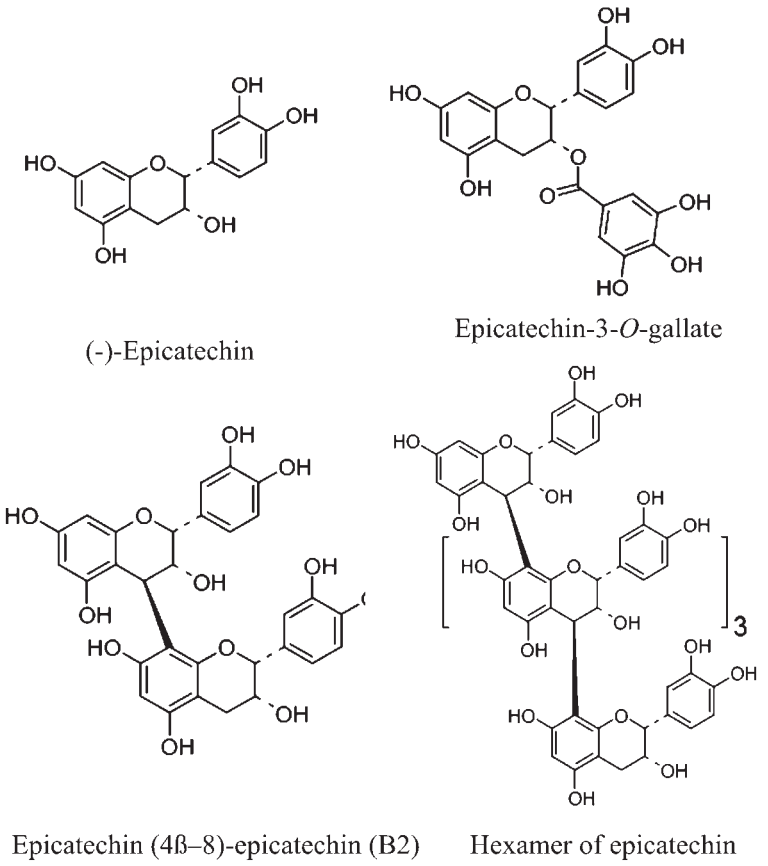

FIG. 4. The structures of the four tannins that were successfully isolated from an ethanolic extract of *Pterocarpus angolensis* stem bark: (-)-epicatechin (=compound 4), epicatechin-3-*O*-galate (=compound 7), epicatechin (4β–8)-epicatechin (B2) (=compound 5), and a hexamer of epicatechin (=compound 6).

also been isolated from green tea. The results of studies by Koparal *et al.* (2004) indicated that the growth of endothelial cells from rat adipose tissue was inhibited by green-tea catechins such as epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin and epigallocatechin gallate (ECGC), the rat cells being more sensitive to these compounds than endothelial cells from human umbilical vein. In humans, a catechin-rich beverage might have several therapeutic uses, including the prevention of obesity, the recovery of insulin-secretory ability, and as a way to maintain low haemoglobin-A (1c) levels in patients with type-2 diabetes who do not yet require insulin therapy (Nagao *et al.*, 2009).

In the present study, all seven pure compounds isolated from the ethanolic extract were found to be very active when tested, by bio-autography, against *St. aureus*. The results of several other studies have indicated that catechins have versatile biological activities, including antimicrobial activity. The incorporation of catechin in an edible *Gelidium corneum* film improved the film's tensile strength, water-vapour permeability and activity against *Eschericha coli* O157:H7 (Ku *et al.*, 2008). Gradisar *et al.* (2007) demonstrated that catechins inhibit bacterial DNA gyrase by binding to the ATP-binding site of the gyrase-B subunit. Of the four catechins tested by these authors, EGCG had the highest such

activity, followed by ECG and EGC. Molecular-docking calculations, which indicated that the benzopyran ring of EGCG penetrates deeply into the active site whereas the galloyl moiety anchors the molecule to the cleft through interactions with its hydroxyl groups, helped explain the relatively high activities of EGCG and ECG. Si *et al.* (2006) isolated catechin-related compounds, including ECG, EGCG, epicatechin and caffeine, from Chinese green tea, and demonstrated that, of these compounds, EGCG gave the greatest inhibition of methicillin-sensitive and methicillin-resistant *St. aureus* (with concentrations of 58 and 37 mg/litre, respectively, inhibiting 90% of bacterial growth). By scanning electron microscopy, Si *et al.* (2006) showed that ECG and EGCG both altered bacterial cell morphology, possibly as the result of disturbed cell division.

Catechin-related compounds are also known to have immunomodulatory effects. In a study by Rogers *et al.* (2005), for example, treatment of murine bone-marrow-derived dendritic cells with EGCG inhibited the cells' production of interleukin-12, in a dose-dependent manner.

Viljoen *et al.* (2005) identified at least five chemotypes of *L. javanica*: myrcenone-rich carvone-rich, piperitenone-rich, ipsenone-rich, and linalool-rich. The isolation of piperitenone as the main compound in the *L. javanica* essential oil investigated in the present study (Fig. 5) indicates that the piperitenone-rich type may be the most common in the Venda region. The piperitenone isolated in the present study was found to have strong activity against various bacteria (Table 2) and *Enta. histolytica* (Table 3). Piperitenone was also found in *L. javanica* from Mozambique and showed good activity against the reverse transcriptase of HIV-1 (Mujovo *et al.*, 2008).

Curiously, although the results of previous tests indicated that some crude extracts of *Pt. angolensis* had significant activity against *Enta. histolytica in vitro* (Samie *et al.*, 2009), none of the pure compounds isolated from *Pt. angolensis* stem bark in the present study possessed strong anti-amoebic activity (Table 3). The anti-amoebic activity observed by Samie *et al.*

TABLE 2. *The minimum inhibitory concentrations (MIC) of piperitenone (from* Lippia javanica*), seven pure compounds isolated from an ethanolic extract of* Pterocarpus angolensis *and the dimethyl sulphoxide and kanamycin used as controls, measured,* in vitro*, against four species of bacteria*

		MIC against:			
	Units for MIC	*Staphylococcus aureus*	*Salmonella typhi*	*Micrococcus kristinae*	*Acinetobacter calcaoceuticus*
PLANT AND COMPOUND					
Lippia javanica					
Piperitenone	µg/ml	12	25	50	50
Pterocarpus angolensis					
Compound 2	µg/ml	500	>1000	500	ND
Compound 3	µg/ml	50	25	50	50
Compound 6	µg/ml	50	25	50	ND
Compound 5	µg/ml	400	>1000	>1000	>1000
Compound 1	µg/ml	1000	>1000	>1000	>1000
Compound 4	ng/ml	500	>1000	500	>1000
Compound 7	µg/ml	50	100	100	50
Dimethyl sulphoxide	%, v/v	>12	>12	>12	>12
Kanamicin	µg/ml	4	8	8	8

ND, Not determined.

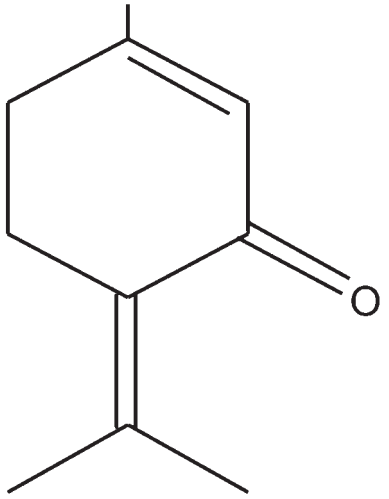

FIG. 5. The major compound isolated, by silica-gel column chromatography, from *Lippia javanica* essential oil, identified as piperitenone. Although three other compounds were isolated by preparative thin-layer chromatography, they were available in insufficient amounts for full characterisation.

(2009) might therefore be the result of synergism between two or more of the compounds present in crude extracts. The isolated compounds also showed low cytotoxicity against intestinal adenocarcinoma cells (i.e. the HCT-8 monolayers; Table 4). When Rogers *et al.,* (2005) treated dendritic cells with EGCG, a significant decrease in cell viability was only observed when the compound was used at 100 μg/ml, and not when it was used at 10 or 50 μg/ml.

Compounds previously isolated from *Pt. angolensis* heartwood include the isoflavonoids prunetin, munin, 7-methyltectoriginin angolensin, (αR)-4-*O*-α-cardinylangolensin, (αR)-4-*O*-*T*-cardinylangolensin, (αS)-4-*O*-methylangolensin (αR)-angolensin and bis-(2-ethylhexyl) phthalate (Bezuidenhoudt *et al.*, 1980). Ndamba *et al.* (1994) indicated that *Pt. angolensis* extracts were active against urinary schistosomiasis. Although Steenkamp *et al.* (2004) tested extracts of *Pt. angolensis* seeds for antibacterial activity, they did not find any (although the extracts gave extremely low yields). Studies by Ho *et al.* (2001) showed that the tannin epicatechin-(4β–8)-epicatechin-(4β–8, 2β–*O*–7)-catechin isolated from *Vaccinium vitis-idaea* had strong antimicrobial activity against periodontal pathogens such as *Porphyromonas gingivalis* and *Po. intermedia*. Other tannins, isolated from *Terminalia citrina* and identified as corilagin, punicalagin, 1,3,6-tri-*O*-galloyl-β-D-glucopyranose, chebulagic acid, and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose, also show antimicrobial action (Burapadaja and Bunchoo, 1995).

TABLE 3. *The median inhibitory concentrations (IC_{50}) of piperitenone (from* Lippia javanica*), seven pure compounds isolated from an ethanolic extract of* Pterocarpus angolensis *and the dimethyl sulphoxide and metronidazole used as controls, measured,* in vitro*, against the HM-1:IMSS strain of* Entamoeba histolytica

Sample	IC_{50}
PLANT AND COMPOUND	
Lippia javanica	
Piperitenone	25 μg/ml
Pterocarpus angolensis	
Compound 2	25 μg/ml
Compound 3	100 μg/ml
Compound 6	400 μg/ml
Compound 5	400 μg/ml
Compound 1	>400 μg/ml
Compound 4	>400 μg/ml
Compound 7	>400 μg/ml
Dimethyl sulphoxide	>5%, v/v
Metronidazole	0.2 μg/ml

TABLE 4. *The median inhibitory concentrations (IC_{50}) of piperitenone (from* Lippia javanica*) and seven pure compounds isolated from an ethanolic extract of* Pterocarpus angolensis*, measured, in vitro, against the HCT-8 cell line that was initially derived from a human intestinal adenocarcinoma*

Sample	Mean IC_{50} and (S.D.) (μg/ml)
PLANT AND COMPOUND	
Lippia javanica	
Piperitenone	265.6 (5.302)
Pterocarpus angolensis	
Compound 2	362.2 (0.789)
Compound 3	>400.0
Compound 6	>400.0
Compound 5	386.2 (2.879)
Compound 1	>400.0
Compound 4	398.4 (4.628)
Compound 7	>400.0

In conclusion, this study identified some major compounds from *Pt. angolensis* stem bark and determined some of their biological activities, including antibacterial activity against *St. aureus, Salmonella typhi, Micrococcus kristinae* and *Acinetobacter calcaoceuticus* as well as antiprotozoal activity against a standard strain of *Enta. histolytica*. Although other compounds have been previously isolated from the heartwood of *Pt. angolensis*, the present study demonstrates the presence of epichatechin and its derivatives in the stem bark of this tree for the first time. The compounds isolated from the bark had generally strong antibacterial activities but weak activities against *Enta. histolytica*. Further studies, to characterise the activities of the isolated compounds in detail and to explore their potential use in the treatment of bacterial and other diseases, appear warranted.

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