Journal of Ethnopharmacology 141 (2012) 1041–1050

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Antioxidant, antimicrobial and antiverotoxic potentials of extracts of *Curtisia dentata*

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ARTICLE INFO

Article history:
Received 31 January 2012
Received in revised form 10 March 2012
Accepted 30 March 2012
Available online 6 April 2012

Keywords:
DPPH radical scavenging activity
Antioxidant
Antiverotoxic
Relative zone diameter of inhibition
Organic compounds

ABSTRACT

Ethnopharmacological relevance: There is an increase in antimicrobial resistance and complexities arising from verotoxic related bacterial infections as well as rise in demand for application of natural antioxidants to combat oxidative damage by free radicals in many oxidative stress-mediated disease conditions such as cancer. Thus the potential of *Curtisia dentata* as antimicrobial, antioxidant and antiverotoxin against environmental isolates of *Escherichia coli* and *Acinetobacter* spp. as well as the presence of phytochemicals and some organic compounds, was determined.
Materials and methods: Phytochemical analysis was carried out using standard methods and antioxidant activity was determined using the DPPH radical scavenging activity. Effect of extracts on bacterial cell wall was also determined.
Results: Extracts contained anthraquinones, alkaloids, essential oils, glycosides, phenols, steroids, saponins, tannins, quinones, anthocyanins, amines and carboxylic acids as phytochemicals. Extracts demonstrated high antimicrobial activity and low minimum inhibitory concentrations as well as inhibitory action against the expression of both Vtx1 and Vtx2 genes in *Escherichia coli*, *Acinetobacter haemolyticus* and *Acinetobacter lwoffii*. Ethanol root bark extracts consistently showed the highest DPPH radical scavenging activity (62.43%), total phenol content (TPH) (57.62 26 mg GAE/g) and reducing power (RP) (41.32%), followed by those of the stem bark and leaf extracts with the respective values of 54.68%, 37.77 mg GAE/g and 21.83%. The extracts induced the leakage of Na^+ and K^+ ions from both test bacteria.
Conclusion: Curtisia dentata is a very effective source of antioxidant and a possible alternative to sourcing antiverotoxic antibiotics with novel mechanism of action.

1. Introduction

Antioxidant supplements are vital to combat oxidative damage by free radicals in many oxidative stress-mediated disease conditions such as cancer, atherosclerosis, diabetes, inflammation and aging. Recently, natural antioxidants are in high demand for application as nutraceuticals and as food additives (Tawaha et al., 2007; Jayasri et al., 2009; Kalim et al., 2010). Exertion of oxidative stress on human cells by free radicals which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells cause protein and DNA damage along with lipid peroxidation resulting in pathological processes (Niki et al., 1994; Maxwell, 1995; Braca et al., 2002; Hazra et al., 2008). While plants serve as rich, natural, and safer sources of antimicrobials, the rapid incidences of increased resistance to available antibiotics worldwide have turned the attention of researchers and the pharmaceutical industries to plants in search of viable alternatives. Recent outbreaks due to verotoxic bacteria (Eaton et al., 2008; CDC, 2011) and further complications arising from the use of antibiotics in the chemotherapy of verotoxic infections calls for more investigations into alternative, more effective agents (Doughari et al., 2009).

Curtisia dentata C.A Smith (Cornaceae or dogwood family) or assegai (English common name) is a traditional medicinal plant that has been employed in the treatment of diarrhoea and related stomach ailments in South Africa (Notten, 2004). In South Africa and other parts of Southern Africa, the common names include: assegaai (Afrikaans), uSirayi, umGxina (Xhosa), umLahleni (Xhosa, Zulu), uMagunda, uMaginda, umBese, umPhephelelangeni (Zulu), iliNcayi, isiNwati (Stwanee), modula-tshwene (Northern Sotho), musangwe, mufhefhera (Venda) and modula-shtwene (Pede) (Notten, 2004; Shai et al., 2008). Of the 15 plant genera found in the Cornaceae family, only the *Curtisia* genera are found in Africa (Shai et al., 2008).

Traditionally, the plant concoction is used as an aphrodisiac, a blood purifier and for treatment of heart-water in cattle, various stomach ailments, pimples and diarrhoea (Pujol, 2000; Dold and

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http://dx.doi.org/10.1016/j.jep.2012.03.051

Cocks, 2001; Shai et al., 2008). The ethanol and aqueous extracts of the plant have been reported to exhibit antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aerugenosa*, *Enterococcus faecalis* as well as *Candida albicans* (McGaw et al., 2000; Shai et al., 2009). Shai et al. (2008) also reported its inhibition of motility in some parasitic and free living nematodes. Despite the medicinal potentials of *Curtisia dentata*, there is paucity of reports of phytochemical, pharmacological and biological investigations of the plant. This study reports on the antioxidant potential of the roots, stem bark and leaves, and the antimicrobial and antiverotoxic potentials of stem bark extracts of *Curtisia dentata* against *Escherichia coli* and *Acinetobacter* spp.

2. Materials and methods

2.1. Collection and processing of plant sample

Curtisia dentata was authenticated and donated by Prof. Charles Laubscher from his plant collections (voucher CD/CP01122) in the Glass House of the Horticulture Department, Faculty of Applied Sciences of the Cape Peninsula University of Technology, Cape Town, South Africa. The fresh parts (stem bark, leaves and roots) were dried to a constant weight in the oven at 45 °C for 24–48 h, grated and reduced to powder and then stored in amber-coloured bottles at ambient conditions until use (Doughari and Obidah, 2008). For this work, all three plant parts were used for the determination of antioxidant activity, total phenolic content as well as reducing power, while only stem bark extract was used in the determination of antiverotoxic and antimicrobial activity.

2.1.1. Extraction and determination of phytoconstituents

Plant parts were exhaustively extracted by sonicating 5 g ground plant parts for 30 min in 200 ml of solvent (dichloromethane, hexane, acetone and ethanol in this order), alongside aqueous extraction using distilled water followed by filtration; this procedure was repeated three times per extractant by replacing the solvent after each extraction. The filtrates from any one solvent was combined, and dried under vacuum at 25 °C and percentage yield of the extracts obtained [hexane (42.68%, w/w), dichloromethane (18.73%, w/w), acetone (22.64%, w/w) ethanol (38.72%, w/w) and water extracts (58.82%, w/w)] used to screen for the presence of phytoconstiuents (Doughari and Iioryue, 2009) and some organic compounds.

2.1.2. Test for saponins

Two grammes (2 g) of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. To the filtered sample (10 ml), distilled water (5 ml) was added, shaken vigorously and observed for a stable persistent frothing. The frothing suspension was mixed with 3 drops of olive oil and shaken vigorously and observed for the formation of emulsion.

2.1.3. Test for tannins and phenolics

Dried powdered sample (0.5 g) was boiled in water (20 ml) in a test tube and then filtered. 1 ml of 0.1% ferric chloride was added and observed for brownish green or a blue–black colouration.

2.1.4. Test for alkaloids

Aqueous extracts (1 ml) was mixed with picric acid solution (2 ml) in a test tube and observed for the formation of orange colouration.

2.1.5. Test for glycosides

To coarse plant material (1 g) 5 ml each of dil. H_2SO_4 or water was added in two sets of beakers, heated for 3 min and filtered. To the filtrates, 1 ml of $NaOH_{(aq)}$ was added, heated with 5 ml of Fehling's solution for 3 min and observed for the appearance of a reddish-brown precipitate.

2.1.6. Test for anthraquinones

Powdered plant (3 g) was soaked into benzene (10 ml) in a conical flask and allowed to stand for 10 min then filtered. To the filtrate, 5 ml of 10% ammonia solution was added, shaken for 30 s, and observed for the appearance of a pink, red or violet colour in the ammonia phase.

2.1.7. Test for flavonoids

Equal volumes (5 ml) of dil. $NH_{3(aq)}$ and the aqueous extract filtrate were mixed with 2–3 drops of conc. H_2SO_4. The formation of a yellow colouration indicated the presence of flavonoids.

2.1.8. Test for steroids

Acetic anhydride (2 ml) was added to 0.5 g of extracts followed 2 ml dil. H_2SO_4. Colour change from violet to blue or green showed the presence of steroids.

2.1.9. Determination of amines

Phenolphthalein (1 drop) was added to 20 ml each of 4 M HCl solution and plant extract in a conical flask and shaken to mix until a pink to brown colour was formed. The presence of an offensive (cartbolic) odour signified the presence of amines (Kenner and Obrien, 1997).

2.1.10. Determination of carboxylic acids

Phenolphthalein (1 drop) was added to 25 ml each of plant extract, and standard solution of $K_3Mn_5O_7$ in a conical flask. The appearance of a faint pink colour which disappeared after 30 s indicated the presence of carboxylic acids (Kenner and Obrien, 1997).

2.1.11. Determination of phenols

To 20 ml each of plant extract and 2 M sodium hydroxide (NaOH) solution in a conical flask, phenolphthalein (1 drop) was added, and the mixture gradually shaken to mix and observed for the appearance of a purple colour within 30 s (Kenner and Obrien, 1997).

2.1.12. Determination of anthocyanins

Briefly, 1 ml of boiling water, 0.5 ml of 37% HCl to 10 mg of dry extract were mixed in a test tube and mixture heated at 100 °C, cooled and 0.4 ml of amylic alcohol added and observed for colour change to dark blue (Rojas et al., 2006).

2.1.13. Determination of quinones

Quinones were identified by extracting 10 ml of the aqueous extract with dichloromethane, evaporating the organic phase, and adding 5 ml of ethanol, 1 ml of 5% H_2O_2 and 1 ml of 50% H_2SO_4. The mixture was heated, cooled, extracted with benzene and 1 ml of NH_4OH added. The quinone extracts was then separated from the benzene and NH_3 phase by careful decantation (Rojas et al., 2006).

2.2. Effect of plant extracts on bacterial beta-lactamase and verocytotoxin production

For the purpose of this study, 5 ml trypton soy broth (TSB) culture of the bacteria was centrifuged at 2000 rpm for 10 min. The supernatant was decanted and the sediment (bacterial cells) was twice washed with normal saline by centrifuging at 2000 rpm for 10 min and the cells made up to 10 ml with normal saline. After standardizing the cells to 0.5 McFarland standard (equivalent to 10^8 cfu/ml), equal volume (5 ml) was mixed with 30 mg/ml crude extract, adequately shaken to mix and held at room temperature (28 ± 2 °C) for 6 h and then incubated at 37 °C for 18 h. After

incubation, a loopful of bacterial culture from the surviving bacteria after exposure to extracts was inoculated onto trypton soy agar (TSA) and further incubated at 37 °C for 18 h. A loopful of surviving bacteria was then suspended in sterile distilled water and 1 ml inoculated into TSB and then incubated for 18–20 h at 37 °C while shaking at 120–150 rpm to allow for toxin secretion into broth medium. Bacterial suspension was then centrifuged for 20 min at 4000 rpm and 4 °C. Supernatant was transferred to new tubes and then screened for verotoxin production using Duopath▽® verotoxin latex reagent (Merck, SA) as described by the manufacturer. The non-pathogenic strains *Escherichia coli* ATCC 25922 and *Acinetobacter haemolyticus* 19002 were used as controls. To test for beta-lactamase production, 1 ml of the supernatant was inoculated into 5 ml of Muller–Hinton broth (MHB) and incubated for 6 h at 37 °C then subcultured onto Muller Hinton agar (MHA) plates onto which two discs, ceftazidime and cefotaxime (30 μg in each case) were then placed. The culture plates were incubated at 37 °C for 18 h and extended beta-lactamase production (ESBL) production was determined by the measurement of zone diameters of inhibition (≤22 mm for ceftazidime and ≤27 mm for cefotaxime) against the test bacterial growths using vernia calipers.

2.3. Quantification of extract-induced cationic leakage from bacterial cell wall

The cation (Na^+ and K^+) leakage assay was used for this purpose. Na^+ and K^+ leakage from 5 strains of *Escherichia coli* and 4 each of *Acinetobacter lwoffii* and *Acinetobacter haemolyticus* was determined after exposure to 30 mg/ml of the crude ethanol plant extracts for 1 h. The bacteria were first exposed to salt solutions of Na^+ and K^+ separately by mixing equal volumes (5 ml) each of 25 ppm each of NaCl and KCl with a broth culture (5 ml) of the test bacteria (0.5 McFarland standard) and incubating at intervals of 0, 10, 20, and 30 min at 37 °C. The cells were then centrifuged at 2000 rpm for 10 min, the supernatant decanted and the sediment washed twice in distilled water by centrifuging at 2000 rpm for 10 min. To 1 ml of this washed bacterial test suspension, 1 ml of the 30 mg/ml of crude extract was added in different sets of sterilized curvets and incubated at ambient conditions for 1 h. Curvets containing test bacteria, extract or 25 ppm Na^+ or K^+ only were used as controls. The non-pathogenic strains *Escherichia coli* ATCC 25922 and *Acinetobacter haemolyticus* 19002 were used as controls, while salt-treated *Acinetobacter lwoffii* RWW1i unexposed to extract was used as control for *Acinetobacter lwoffii* strains. Presence of Na^+ or K^+ were determined spectrophotometrically from each cell suspension and the controls according to their respective incubation periods by placing the curvets in an atomizer orifice and taking readings at 266 nm.

2.4. Determination of antioxidant activity using the DPPH radical scavenging system

The hydrogen or electrons donation ability of the extracts was measured from bleaching of purple methanol solution of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) free radical (Changwei et al., 2008; Hazra et al., 2008). A 2-ml aliquot of a suspension of the ethanol extracts was mixed with 1 ml of 0.5 mM DPPH solution and 2 ml of 0.1 M sodium acetate buffer (pH 5.5), properly shaken and incubated at ambient temperature in the dark for 30 min, following which the absorbance was measured at 517 nm using a UV-160A spectrometer. Extracts suspension only, re-dissolved in ethanol was used as negative control. Radical scavenging activity expressed as the inhibition percentage was calculated as described by Abe et al. (1998) using the formula:

$$\%\ \text{radical scavenging activity} = \left[\frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}}\right] \times 100$$

where A_{control} is the absorbance of the control (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution plus antioxidant).

2.5. Determination of reducing power of extracts

Reaction mixture containing plant extract at different concentrations (10–100 μl) in phosphate buffer (0.2 M, pH 6.6) and equal amounts of 1% (w/v) potassium ferricyanide, was incubated at 50 °C for 20 min. The reaction was terminated by the addition of equal volumes of 10% (w/v) tricarboxyllic acid (TCA) solution and the mixture centrifuged at 3000 rpm for 20 min. The supernatant was mixed with equal volume of distilled water and 0.1% (w/v) ferric chloride solution and the absorbance measured at 700 nm. Increased absorbance of the mixture with concentration indicated the reducing power of the extract.

2.6. Determination of total phenolic content

Stock solution (0.5 mg/ml) of plant extracts was prepared and further diluted to five different concentrations (0.4, 0.3, 0.2, 0.1, and 0.05 mg/ml). 0.1 ml of 2 N Folin–Ciocalteu reagent (Sigma–Aldrich) was added to the extract concentrations in different sets of test tubes, shaken thoroughly, and left to stand for 1 min. 2.8 ml of 10% $NaHCO_3$ was then added and the mixture once again allowed to stand for 30 min after which the absorbance (725 nm) was measured spectrophotometrically and the total phenolic content (TPH) was expressed as mg equivalent of Gallic acid (mg GAE) (0.05–0.5 mg/ml as control/blank) per gram dry weight of the extract (Djeridane et al., 2006).

2.7. Antimicrobial susceptibility test of plant extracts

Briefly, 0.5 ml McFarland turbidity standard of test bacteria (14 strains of *Acinetobacter haemolyticus*, 29 strains of *Acinetobacter lwoffii* and 69 strains of *Escherichia coli* as test bacteria, with *Escherichia coli* ATCC 25922 and *Acinetobacter haemolyticus* 19002 as control strains) was seeded on to sterile MHA plates, spread out using sterile glass rod in order to achieve confluent growth and the plates left on the table for 5 min to dry. Sterile filter paper discs (4 mm in diameter) soaked in the extract solution at different concentrations (5.0, 10.0–300 mg/ml/disc) were placed on the different MHA plates preseeded with different test organisms and the plates were then incubated at 37 °C for 24 h. Filter papers soaked in ethanol and ampicillin (10 μg/ml) were used as negative and positive controls respectively. Antibacterial activity was determined by measurement of zone diameter of inhibition (mm) against each test bacteria (Doughari and Obidah, 2008). The antimicrobial activity (expressed as percentage relative inhibition zone diameter) was calculated by applying the expression:

$$\%\ \text{RIZD} = \frac{\text{IZD sample} - \text{IZD negative control}}{\text{IZD antibiotic standard}} \times 100$$

where RIZD is the percentage of relative inhibition zone diameter and IZD is the inhibition zone diameter (mm). The equation compensates the possible effect of the solvent (blank) other than water on the IZD. The test was considered negative (−) when the IZD of the sample equaled to the IZD of the controls (Rojas et al., 2006).

2.8. Determination of minimum inhibitory concentration (MIC) plant extracts

The MIC was carried out on extracts that showed antimicrobial activity (RIZD % of 1 and above) using the broth dilution method. The organisms (14 strains of *Acinetobacter haemolyticus*, 29 strains of *Acinetobacter lwoffii* and 69 strains of *Escherichia coli* as test bacteria, with *Escherichia coli* ATCC 25922 and *Acinetobacter haemolyticus* 19002 as control strains) were inoculated into test tubes containing varying concentrations (100–3000 μg/ml and 2.5–200 mg/ml/disc) of plant extract and 1 ml of nutrient broth (NB) added. A loopful of the test bacteria previously diluted to 0.5 McFarland turbidity standard, was introduced into each broth sample. The procedure was repeated on the test organisms in test tubes containing NB only and the standard antibiotic ampicillin (10 μg) as negative and positive controls respectively. All the culture tubes were then incubated at 37 °C for 24 h. After incubation, they were examined for bacterial growth by observing/measuring of turbidity. The MICs for verotoxin inhibition at these same extract concentrations were also determined as earlier described.

2.9. Bacterial strains

A total of 112 stock cultures of test bacteria (14 strains of *Acinetobacter haemolyticus*, 29 strains of *Acinetobacter lwoffii* and 69 strains of *Escherichia coli* as test bacteria, with *Escherichia coli* ATCC 25922 and *Acinetobacter haemolyticus* 19002 as control strains) serotypes obtained variously from treatment plant wastewater, abattoir wastewater and from Rivers Berg and Plankenberg all in Cape Town, South Africa. We previously isolated these strains from the various water sources and characterized them using standard methods for verotoxins (Doughari et al., 2012) in the Microbiology Laboratory, Department of Biotechnology, Faculty of Applied Sciences Cape Peninsula University of Technology, Cape Town, South Africa. The veropositive isolates were used to test for antiverotoxic activity of the plant extracts. The non-pathogenic strains of *Escherichia coli* ATCC 25922 and *Acinetobacter haemolyticus* 19002 were used as controls. The bacteria were previously maintained on TSA slants at 4 °C were subcultured onto plates of MHA and incubated at 37 °C for 18 h before use.

2.10. Statistical analysis

Results are given as mean ± SEM values while relationships between antibacterial activity and test bacteria and plant extracts were determined using the Student *t*-test of the SIGMAPLOT at $P \leq 0.05$.

3. Results

Phytochemical and other organic compounds analysis of *Curtisia dentata* showed that the solvent extracts of stem bark contain differing classes of compounds. Ethanol extracts (ET) contain the highest classes, followed by dichloromethane (DCM), acetone (AC) and hexane (HX). Distilled water (DW) extracts contained the least number of phytochemicals in all the plant parts. Phytochemicals and organic compounds detected include anthraquinones, alkaloids, essential oils, glycosides, phenols, steroids, saponins and tannins and the organic compounds quinones, anthocyanins, amines and carboxylic acids (Table 1).

Results of antimicrobial potentials of *Curtisia dentata* against *Escherichia coli* serotypes as relative inhibition zone diameters (RIZD) of the extracts ranged between 8 and 28% (MIC, 100–2500 μg/ml) against *Acinetobacter lwoffii* and *Acinetobacter haemolyticus*, 10–28% (MIC, 100–850 μg/ml) against *Acinetobacter lwoffii* and 6–28% (MIC 150–2500 μg/ml) against *Acinetobacter haemolyticus* (Table 2). Results of antiverotoxic activity showed that the extracts demonstrated inhibitory activity against both Vtx1 and Vtx2 production. The ethanol extracts (ET) demonstrated the highest inhibitory action against 82.61% of the verotoxic *Escherichia coli* serotypes, followed by the dichloromethane extracts (DCM, 71.01%), hexane (HX, 44.93%), chloroform (CHL, 34.78%) and acetone (AC, 27.54%). Water extracts did not show any antiverotoxic activity against the test bacteria. Extracts also inhibited the vtx gene expression for verotoxin production at MIC range of 100–2500 μg/ml for both *Escherichia coli* (Table 2) and *Acinetobacter* spp. (Table 3).

Results of effect of extracts on bacterial cell wall revealed the presence of sodium and potassium cations in the tested medium. While isolates incubated for 30 min generally showed higher ODs, *Acinetobacter haemolyticus* isolates showed lower OD values (0.172–54.44 for Na^+ and 0.572–102.78 for K^+) (Fig. 3) compared to the *Acinetobacter lwoffii* (0.432–184.45 for Na^+ and 0.76–367.27 for K^+) (Fig. 2) and *Escherichia coli* (12.06–334.67 for Na^+ and 22.36–596.55 for K^+) isolates (Fig. 1).

The DPPH radical scavenging activities, total phenolic content (TPH) and reducing power (RP) of the different extracts are shown in Table 4. The ethanol root bark extracts consistently showed the highest DPPH radical scavenging activity (62.43%), TPH (57.62 mg GAE/g) and RP (41.32%) followed by those of the stem bark extracts with the respective values of 54.68%, 37.77 mg GAE/g and 21.83%. Water extracts (DW) showed the least values in all the test results. Among the solvents, ethanol demonstrated the highest values for DPPH, TPH and RP followed by DCM, HX, AC and DW in this order.

4. Discussion

The presence of phytoconstiuents in various parts of *Curtisia dentata* confirms its potential as source of antimicrobial substances. Generally, anthraquinones, alkaloids, essential oils, glycosides, phenols, steroids, saponins and tannins observed in this study are reported to confer innate defence mechanisms against invading bacteria, fungi, pests and diseases (Fink-Gremmels, 2010). Individually, alkaloids have been employed therapeutically as antimicrobials, analgesics/narcotics, mydriatics, miotics, hypertensives, hypotensives, bronchodilators, stimulants or antileukaemic agents (Pengelly, 2004). Anthraquinones as laxatives for the treatment of constipation and their antiseptic effects deter the growth of enteric pathogens. Some anthraquinones and napthaquinones significantly inhibit Epstein-Barr virus early antigen activation at low doses. Essential oils (or volatile oils) have stimulant, decongestant, antiviral, antitumour, antimicrobial, antiseptic, tonifying, spasmolytic, anti-inflammatory or antiviral potential (Pengelly, 2004). Tannins exert astringent activity via precipitation of proteins, thereby protecting the underlying tissue leading to improvement of wound healing (Tyler et al., 1998; Madziga et al., 2010). Awosika (1991) also reported that tannins inhibit microbial proliferation by denaturation of enzymes involved in microbial metabolism and their potential as antiviral, antibacterial, antiparasitic and anticancer effects have also been reported (Akiyama et al., 2001). Saponins have been associated with anaesthetic or CNS stimulant potentials and thus have been applied as local analgesics and as antimalarials. Steroids on the other hand have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness, inhibit growth of tumours and to reduce blood cholesterol (Pengelly, 2004; Aliu and Nwude, 1982). Therapeutic effects of flavonoids such as the antiallergic, antioxidant, antiviral, hepatoprotective, antiatheromatous, anti-inflammatory, antimicrobial and anti-cancer activity and antihypertensive have been widely reported (Yamamato and Gaynor, 2002; Pengelly, 2004; Stauth, 2007). Cardiac glycosides have been used in the treatment of

Table 1
Phytochemicals and organic compounds present in extracts of *Curtisia dentata* parts.

Phytochemical	Plant part/solvent														
	Root bark extract					Stem bark extract					Leaf extract				
	DW	DCM	HX	AC	ET	DW	DCM	HX	AC	ET	DW	DCM	HX	AC	ET
Anthraquinones	–	–	–	+	+	+	–	–	+	+	–	–	–	+	+
Alkaloids	–	–	+	+	+	–	–	–	–	+	–	–	+	–	+
Essential oils	–	–	–	–	+	–	–	–	–	+	–	+	–	–	+
Flavonoids	+	+	+	–	–	+	+	+	+	–	+	+	+	–	–
Glycosides	+	+	–	–	–	+	+	–	–	+	+	+	–	+	–
Phenols	+	+	–	–	+	+	+	+	–	+	+	+	–	–	+
Steroids	–	–	–	–	+	–	–	–	–	+	–	–	+	–	+
Saponins	–	+	–	+	+	+	+	–	+	+	–	+	–	+	+
Tannins	–	–	+	+	+	+	+	+	+	+	–	–	–	+	+
Quinones	–	+	–	+	+	–	+	–	–	+	–	+	–	–	+
Anthocyanins	+	+	–	+	+	+	+	–	+	+	+	–	–	+	+
Amines	–	+	+	–	+	–	+	+	–	+	–	–	+	–	+
Carboxyllic acids	–	+	+	+	+	–	+	+	+	+	–	–	+	+	+

Solvents: DW, distilled water; DCM, dichloromethane; HX, hexane; AC, acetone; ET, ethanol.

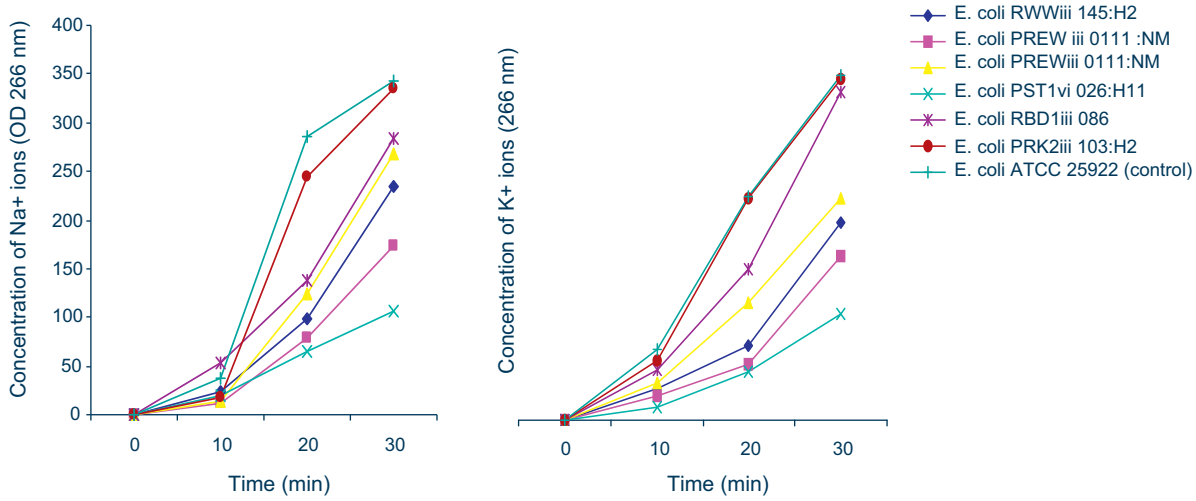

Fig. 1. Leakage of Na^+ and K^+ ions from *Escherichia coli* isolates by stem bark ethanol extracts of *Curtisia dentata* (*Escherichia coli* isolates from various samples as RWW, raw wastewater; PREW, primary raw abattoir effluent; PST, primary settled wastewater; RBD, River Berg downstream; PRK, Plankenberg river water).

congestive heart failure, constipation, edema and microbial infections (Robinson, 1967; Frantisck, 1991). Saponins have expectorant and antibacterial properties and have been employed in the treatment of upper respiratory tract and other microbial infections (Birk and Petri, 1980; Trease and Evans, 1984). Presence of these various phytochemicals in the extracts of *Curtisia dentata* and demonstration of activity of these extracts against various *Escherichia coli* and *Acinetobacter* spp. provides the possibility of sourcing a wide range drugs and antibacterial substances against these various ailments and infections associated with these bacteria.

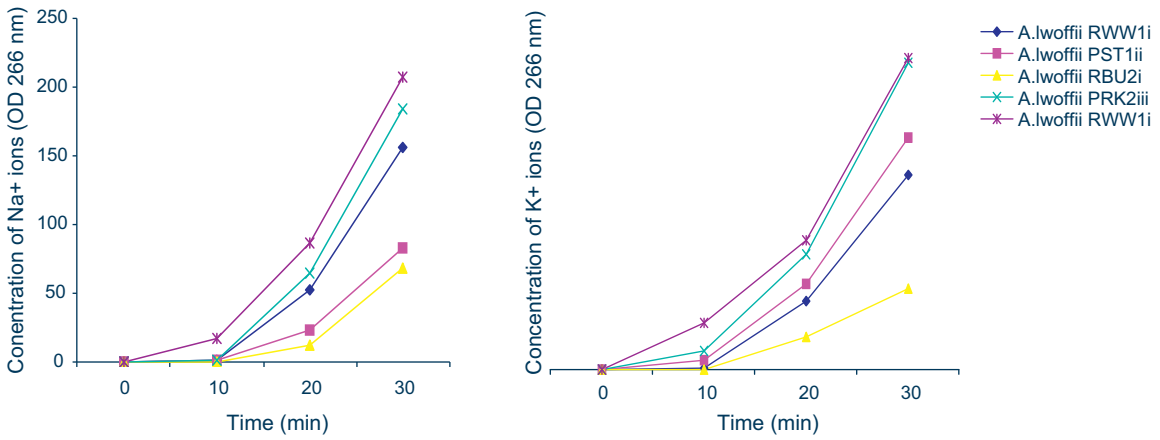

Fig. 2. Leakage of Na^+ and K^+ ions from *Acinetobacter lwoffii* isolates by stem bark ethanol extracts of *Curtisia dentata* (*Acinetobacter lwoffii* isolates from various samples as RWW, raw wastewater; PST, primary settled wastewater; RBU, River Berg upstream; PRK, Plankenberg river water).

Table 2
Relative inhibition zone diameters (%), minimum inhibitory concentration (MIC) (μg/ml) and antiverotoxic effect of stem bark ethanol extracts of *Curtisia dentata* on various environ mental *Escherichia coli* serotypes.

Sample number (mean pH/Temp. °C)	Isolate/serotype	RIZD values (%)	MIC (μg/ml) to *Curtisia dentata*	Vtx & ESBL status before treatment	Vtx & ESBL status after treatment					
					DW	DCM	HX	CHL	AC	ET
Wastewater (n = 18) (6.4/17.8)	*Escherichia coli* RWW1i O103:H2	10.00	650.00	Vtx1	Vtx1	–	Vtx1	Vtx1	Vtx1	–
	Escherichia coli RWW1ii O86	16.00	250.00	Vtx1, Vtx2	Vtx1, Vtx2	–, Vtx2	–, Vtx2	Vtx2	–	–
	Escherichia coli RWW1iii O145:H2	14.00	350.00	Vtx1	Vtx1	Vtx1	–	Vtx1	Vtx1	–
	Escherichia coli RWW1iv O96:H9	8.00	750.00	Vtx1	Vtx1	–, Vtx2	–	Vtx1	Vtx1	–
	Escherichia coli RWW1v O126	14.00	200.00	Vtx1	Vtx1	–	Vtx1	Vtx1	Vtx1	Vtx1
	Escherichia coli RWW1vi O4	16.00	250.00	Vtx1	Vtx1	Vtx2	–	–	Vtx1	–
	Escherichia coli RWW1vii O55	14.00	400.00	Vtx1, Vtx2	Vtx1, Vtx2	–	–, Vtx2	–, Vtx2	–, Vtx2	–
	Escherichia coli RWW1viii O111:NM	22.00	150.00	Vtx1, Vtx2	Vtx1, Vtx2	–	–	–, Vtx2	–	–
	Escherichia coli RWW2i O96:H9	8.00	1000.00	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	–
	Escherichia coli RWW2ii O124	14.00	400.00	Vtx1	Vtx1	–, Vtx2	–	–	–	–
	Escherichia coli PSW1i O96:H9	16.00	200.00	Vtx1	Vtx1	–	Vtx1	Vtx1	Vtx1	–
	Escherichia coli PSW1ii O145:NM	22.00	150.00	Vtx2	Vtx2	Vtx1, –	Vtx2	Vtx2	Vtx2	–
	Escherichia coli PSW1iii O96:H9	16.00	250.00	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, –	Vtx1, Vtx2	Vtx1, Vtx2	–	–
	Escherichia coli PSW1iv O111:NM	24.00	150.00	Vtx1, Vtx2	Vtx1, Vtx2	–	–	–	–	–
	Escherichia coli PSW2i O86	14.00	200.00	Vtx1, Vtx2	Vtx1, Vtx2	Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, –
	Escherichia coli PSW2ii O96:H9	10.00	550.00	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1	–, Vtx2	–	Vtx1, Vtx2	–
	Escherichia coli PSW2iii O103:H2	14.00	300.00	Vtx1	Vtx1	–	–	–	–	–
	Escherichia coli FEW1i O111:NM	18.00	200.00	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	–
	Escherichia coli FEW1ii O103:H2	14.00	400.00	Vtx1	Vtx1	–	Vtx1	Vtx1	Vtx1	–
	Escherichia coli FEW1iii O124	14.00	350.00	Vtx1	Vtx1	–	–	Vtx1	–	–
	Escherichia coli FEW1iv O44	20.00	200.00	Vtx2	Vtx2	–, Vtx2	Vtx2	Vtx2	Vtx2	Vtx2
	Escherichia coli FEW2i O124	20.00	150.00	Vtx2	Vtx2	–, Vtx2	Vtx2	Vtx2	Vtx2	–
	Escherichia coli FEW2ii O103:H2	24.00	100.00	Vtx2	Vtx2		–	–	Vtx2	–
	Escherichia coli FEW2iii O145:NM	18.00	250.00	Vtx1, Vtx2	Vtx1, Vtx2		–, Vtx2	–, Vtx2	–, Vtx2	–
	Escherichia coli FEW2iv O145:NM	14.00	400.00	Vtx1, Vtx2	Vtx1, Vtx2		–, Vtx2	–, Vtx2	–, Vtx2	–
Abattoir water (n = 18) (6.4/17.8)	*Escherichia coli* PRE1i O4	6.00	2000.00	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2
	Escherichia coli PRE1ii O145:H2	6.00	2500.00	Vtx1	Vtx1	–	Vtx1	–	Vtx1	–
	Escherichia coli PRE1iii O111:NM	10.00	600.00	Vtx1	Vtx1	Vtx1	Vtx1	Vtx1	Vtx1	Vtx1
	Escherichia coli PRE1iv O86	8.00	800.00	Vtx2	Vtx2	–	–	Vtx1	Vtx2	–
	Escherichia coli PRE1v O4	16.00	250.00	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	–
	Escherichia coli PRE1vi O111:NM	10.00	500.00	Vtx1, Vtx2	Vtx1, Vtx2	–, Vtx2	–, Vtx2	–, Vtx2	–, Vtx2	–, Vtx2
	Escherichia coli PRE2i O103:H2	28.00	100.00	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	–
	Escherichia coli PRE2ii O4	20.00	250.00	Vtx1, Vtx2	Vtx1, Vtx2	–	–	–	–	–
	Escherichia coli FSE1i O113	20.00	150.00	Vtx2	Vtx2	–	Vtx2	Vtx2	Vtx2	–
	Escherichia coli FSE1ii O145:H2	22.00	250.00	Vtx2	Vtx2	–	Vtx2	Vtx2	Vtx2	–
	Escherichia coli FSE1iii O86	12.00	500.00	Vtx2	Vtx2	Vtx2	–	Vtx2	Vtx2	Vtx2
	Escherichia coli FSE1iv O111:NM	6.00	900.00	Vtx2	Vtx2	–	Vtx2	Vtx2	Vtx2	–
	Escherichia coli FSE1v O96:H9	8.00	750.00	Vtx2	Vtx2	Vtx2	Vtx2	–	Vtx2	–
	Escherichia coli FSE1vi O4	20.00	200.00	Vtx2	Vtx2	–	–	–	–	–
	Escherichia coli FSE2i O111:NM	12.00	500.00	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	–
	Escherichia coli FSE2ii O103:H2	8.00	850.00	Vtx2	Vtx2	–	Vtx2	Vtx2	Vtx2	–
	Escherichia coli PST1i O145:H2	10.00	500.00	Vtx1, Vtx2	Vtx1, Vtx2	–, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	–
	Escherichia coli PST1ii O26:H11	14.00	300.00	Vtx1, Vtx2	Vtx1, Vtx2	–	–, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	–
	Escherichia coli PST1iii O113	20.00	150.00	Vtx1, Vtx2	Vtx1, Vtx2	–	–	–	–	–
	Escherichia coli PST1iv O4	10.00	600.00	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2
	Escherichia coli PST1v O96:H9	4.00	950.00	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	–
	Escherichia coli PST1vi O26:H11	18.00	250.00	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, –	Vtx1, Vtx2	Vtx1, Vtx2	–	–
	Escherichia coli PST2i O124	24.00	2500.00	Vtx1, Vtx2	Vtx1, Vtx2	–	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	–
	Escherichia coli PST2ii O124	10.00	700.00	Vtx1, Vtx2	Vtx1, Vtx2	–	–	–	–	–
River Berg (n = 13) (7.2/17.3)	*Escherichia coli* RBU1i O86	4.00	2500.00	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	–
	Escherichia coli RBU2i O113	12.00	400.00	Vtx2	Vtx2	–	Vtx2	–	Vtx2	–
	Escherichia coli RBU2ii O145:H2	20.00	200.00	Vtx2	Vtx2	–	–	Vtx2	Vtx2	–
	Escherichia coli RBU2iii O113	12.00	450.00	Vtx2	Vtx2	–	–	Vtx2	Vtx2	–
	Escherichia coli RBD1i O113	22.00	150.00	Vtx1	Vtx1	Vtx1	Vtx1	Vtx1	Vtx1, Vtx1, Vtx2	–

Table 2 (*Continued*)

Sample number (mean pH/Temp. °C)	Isolate/serotype	RIZD values (%)	MIC (μg/ml) to *Curtisia dentata*	Vtx & ESBL status before treatment	Vtx & ESBL status after treatment					
					DW	DCM	HX	CHL	AC	ET
	Escherichia coli RBD1ii O4	16.00	300.00	Vtx1, Vtx2	Vtx1, Vtx2	–	–	–	Vtx1, Vtx2	Vtx1, –
	Escherichia coli RBD1iii O86	22.00	150.00	Vtx1, Vtx2	Vtx1, Vtx2	–, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	–
	Escherichia coli RBI1i O4	28.00	100.00	Vtx1, Vtx2	Vtx1, Vtx2	–, Vtx2	Vtx1, –	–	Vtx1, Vtx2	–
	Escherichia coli RBI1ii O103:H2	8.00	750.00	Vtx1, Vtx2	Vtx1, Vtx2	–	Vtx1, Vtx2	Vtx1, Vtx2	Vtx2	–
	Escherichia coli RBI2i O124	18.00	250.00	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	–
	Escherichia coli RBI2ii O86	22.00	200.00	Vtx2	Vtx2	–	–	Vtx2	Vtx2	–
	Escherichia coli RBI2iii O96:H9	12.00	350.00	Vtx2	Vtx2	Vtx2	Vtx2	–	Vtx2	Vtx2
	Escherichia coli RBI2iv O145:H2	22.00	200.00	Vtx1	Vtx1	–	–	Vtx2	Vtx2	–
	Escherichia coli RBI2v O113	14.00	300.00	Vtx2	Vtx2	–	Vtx2	Vtx2		–
River Plankenberg (n = 13) (7.2/17.3)	*Escherichia coli* PRK1i O4	20.00	150.00	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2
	Escherichia coli PRK1ii O26:H11	26.00	100.00	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, –	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	–
	Escherichia coli PRK2i O145:H2	16.00	300.00	Vtx1, Vtx2	Vtx1, Vtx2	–, Vtx2	–, Vtx2	–	–	–, Vtx2
	Escherichia coli PRK2ii O86	24.00	150.00	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	Vtx2	–
	Escherichia coli PRK2iii O4	12.00	350.00	Vtx1, Vtx2	Vtx1, Vtx2	–	Vtx1, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	–
	Escherichia coli PRK2iii O103:H2	14.00	450.00	Vtx1, Vtx2	Vtx1, Vtx2	–	–	–	–	–
	Escherichia coli ATCC 25922	18.00	250.00	–	–	–	–	–	–	–

DW, distilled water extract; DCM, dichloromethane extract; HX, hexane extract; CHL, chloroform extract; AC, acetone extract; ET, ethanol extract; ESBL, extended spectrum beta lactamase; Vtx, veortoxin.

The organic chemical components such as quinones, anthocyanins, amines and carboxylic acids, have formed bases for many synthetic antibiotics including ubiquinol and pycnogenol (Pengelly, 2004). Quinones form an important component of the electron-transport system in plants and mammals. Ubiquinol, the reduced form of coenzyme Q10, and menaquinone (vitamin K) have significant antioxidant properties, playing a major role in protecting cells from free-radical damage. Pycnogenol® is the proprietary name for oligomeric procyanidins (OPCs) extracted commercially from grape seeds and pine bark, and are responsible for many of the benefits associated with red wines, including treatment of cardiovascular and cerebrovascular diseases. Amines and carboxylic acids are used in the hydrolytic synthesis of amide drugs such as acetaminophen, a well-known anti-inflammatory drug – a simple amide formed from 4-hydroxyphenylamine and acetic acid. Such amide functional groups so formed, are quite resistant to hydrolysis, and amide linkages between amino acids and peptides are essential to the stability of proteins. The presence of these organic compounds in *Curtisia dentata* offers very promising sources of chemical backbones for antioxidant therapeutic drugs.

There were differences in concentration of the chemical components on different parts of the plant as observed from this study. Mountousis et al. (2006) had earlier reported differences in chemical component concentration from one plant part to the other, depending on their degree of maturity. Care must therefore be taken in the choice of plant part in medicinal plant drug research. The MICs were generally low, and since the plant extracts were in crude form, this outcome is promising. Low MIC values indicate potentially high efficacy of the extracts as antimicrobial agents (Doughari et al., 2008; Doughari and Iioryue, 2009; Sharma et al., 2010). Also, higher antiverotoxic potentials against the test bacteria demonstrated by ethanol extracts might be as a result of higher concentration of phytoconstituents in this solvent compared to the other solvents used. Absence of antiverotoxic activity from aqueous extracts however does not rule out the presence of such activity, but the phytoconstituents may be occurring in very low ineffective concentrations. The inhibition of the expression of both Vtx1 and Vtx2 genes in both *Escherichia coli* and *Acinetobacter* spp. is a very significant finding as it provides a gateway for the development of very effective antiverotoxic drugs. Currently, antibiotic treatment induces the release of more of the toxins into the protoplasm resulting in further complications. Recently *Escherichia coli* O104:H4 was implicated in a fatal foodborne illness resulting in 882 people contracting hemolytic uremic syndrome (HUS) with 32 deaths in Europe and 1 death in America within just 2 months (CDC, 2011). This, in addition to the emergence of some verotoxic strains of *Acinetobacter* spp. underline the significance of findings of this study and the need to continue searching for potential control agents. Though this study did not establish the toxic effect of this plant to human cells, the plant has demonstrated potential as source of novel antimicrobial agents for the treatment of verotoxic bacterial infections. Furthermore, the study represents the first report of antiverotoxic activity of *Curtisia dentata* extracts against various Vtx genes from bacteria. Future research work to determine the possible impact on human cells should be carried out.

The presence of Na^+ and K^+ ions in the medium indicates leakage of these ions through the bacterial cell walls. Therefore, this is an indication that the extracts are capable of causing damage to bacterial cell walls, thereby causing leakage of protoplasmic contents – one of several mechanisms of actions of antimicrobials. The differences in Na^+ and K^+ ion leakage rates might be due to differences in ionic sizes of the two metal ions. Though both have an equivalent number of charges, the greater leakage rate observed for K^+ might be as a result of its higher molecular size and atomic mass compared to that of Na^+ ions. However, this does not imply that cell wall

Table 3
Relative inhibition zone diameters (%), minimum inhibitory concentration (MIC) (μg/ml) and antiverotoxic effect of stem bark ethanol extracts of *Curtisia dentata* on various environmental isolates of *Acinetbacter* spp.

Sample number (mean pH/Temp. °C)	Isolate/serotype	RIZD values (%)	MIC (μg/ml) to *Curtisia dentata*	Vtx & ESBL status before treatment	Verotoxim status after treatment					
					DW	DCM	HX	CHL	AC	ET
Wastewater (n = 18) (6.4/17.8)	*Acinetobacter lwoffii* RWW1i	14.00	750.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii RWW1ii	10.00	1500.00	–	–	–	–	–	–	–
	Acinetobacter haemolyticus RWW1v	8.00	1000.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii RWW1vi	24.00	250.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii RWW2i	28.00	100.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii RWW2ii	20.00	350.00	Vtx1	–	–	–	–	–	–
	Acinetobacter lwoffii PSW1i	22.00	200.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii PSW1ii	26.00	150.00	–	–	–	–	–	–	–
	Acinetobacter haemolyticus PSW2i	14.00	700.00	–	–	Vtx1	Vtx1	Vtx1	Vtx1	–
	Acinetobacter haemolyticus PSW2ii	6.00	2000.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii FEW1i	26.00	250.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii FEW2i	28.00	150.00	–	–	–	–	–	–	–
	Acinetobacter haemolyticus FEW2iv	24.00	250.00	–	–	–	–	–	–	–
Abattoir water (n = 18) (6.4/17.8)	*Acinetobacter lwoffii* PRE1i	28.00	150.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii PRE1ii	26.00	200.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii PRE2i	18.00	450.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii PRE2ii	22.00	250.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii FSE1i	24.00	200.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii FSE1ii	26.00	150.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii FSE1iii	28.00	200.00	–	–	–	–	–	–	–
	Acinetobacter haemolyticus FSE1iv	28.00	150.00	Vtx1, Vtx2	Vyx1	–	–, Vtx2	Vtx1, Vtx2	Vtx1, Vtx2	–
	Acinetobacter haemolyticus FSE1v	28.00	250.00	Vtx2	VTx2	–	Vtx2	Vtx2	Vtx2	–
	Acinetobacter lwoffii FSE2i	28.00	100.00	–	Vtx2	–	–	–	–	–
	Acinetobacter lwoffii FSE2ii	26.00	150.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii PST1i	22.00	200.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii PST1ii	12.00	850.00	–	–	–	–	–	–	–
	Acinetobacter haemolyticus PST1i	6.00	2500.00	Vtx1	–	Vtx1	Vtx1	Vtx1	Vtx1	–
	Acinetobacter haemolyticus PST2i	24.00	200.00	–	–	–	–	–	–	–
	Acinetobacter haemolyticus PST2ii	22.00	200.00	–	–	–	–	–	–	–
River Berg (n = 13) (7.2/17.3)	*Acinetobacter lwoffii* RBU1i	26.00	150.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii RBU2i	12.00	750.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii RBU2ii	30.00	100.00	–	–	–	–	–	–	–
	Acinetobacter haemolyticus RBD1i	26.00	150.00	Vtx1	Vtx1	–	–	–	–	–
	Acinetobacter haemolyticus RBD1ii	24.00	150.00	–	–	–	–	–	–	–
	Acinetobacter haemolyticus RBD1iii	10.00	900.00	–	–	–	–	–	–	–
	Acinetobacter haemolyticus RBI1i	28.00	150.00	–	–	–	–	–	–	–
	Acinetobacter haemolyticus RBI2i	12.00	600.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii RBI2ii	24.00	200.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii RBI2iii	24.00	250.00	Vtx1, Vtx2	– Vtx2	Vtx1	Vtx1	Vtx1	Vtx1	Vtx1
River Plankenberg (n = 13) (7.2/17.3)	*Acinetobacter lwoffii* PRK2i	26.00	150.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii PRK2ii	28.00	150.00	–	–	–	–	–	–	–
	Acinetobacter lwoffii PRK2iii	22.00	200.00	–	–	–	–	–	–	–
	Acinetobacter haemolyticus 19002	20.00	200.00	–	–	–	–	–	–	–

DW, distilled water extract; DCM, dichloromethane extract; HX, hexane extract; CHL, chloroform extract; AC, acetone extract; ET, ethanol extract; ESBL, extended spectrum beta lactamase; Vtx; veortoxin.

leakage is the sole mechanism of action of this plant given the variety of compound classes present in the crude extract. Although actual microscopic damage to the bacterial cell walls was not determined, results of the study indicate that damage to bacterial cell wall could be one of several mechanisms of action of the plant extract. *Acinetobacter haemolyticus* isolates showed low OD values compared to *Acinetobacter lwoffii* and *Escherichia coli* isolates. Extract impurity might be accountable for this low activity in addition to possible innate immunity to antibiotic-like compounds.

There was a correlation between total phenolic content and high antioxidant activity as well as reducing power of extracts. Tawaha et al. (2007) reported a similar correlation between phenolic content with antioxidant activity from plant extracts. Among natural antioxidants, plant polyphenols are especially important

Table 4
Antioxidant activity, total phenol content and reducing power of extracts of *Curtisia dentata*.

Plant part	DPPH (% at 0.1 mg/ml, 517 nm)						Total Phenolic content (TPH) (725 nm)						Reducing Power (RP)					
	DW	DCM	HX	CHL	AC	ET	DW	DCM	HX	CHL	AC	ET	DW	DCM	HX	CHL	AC	ET
Control	12.68	12.68	12.68	12.68	12.68	12.68	14.52	14.52	14.52	14.52	14.52	14.52	3.27	3.27	3.27	3.27	3.27	3.27
SBE	14.68	50.34	41.47	38.55	26.28	54.68	11.32	18.22	16.61	30.22	24.25	37.77	11.83	21.15	16.18	14.21	12.26	21.83
RBE	32.43	56.67	42.82	38.52	24.51	62.43	17.44	42.36	28.71	51.12	21.32	57.62	22.32	32.74	36.42	27.21	21.28	41.32
LE	18.45	40.34	38.45	28.37	18.26	44.56	8.64	18.83	15.18	12.58	8.84	24.73	1.62	3.61	3.84	3.22	1.07	4.62

SBE, stem bark extract; RBE, root bark extracts; LE, leaf extracts; DW, distilled water; DCM, dichloromethane; HX, hexane; CHL, chloroform; AC, acetone; ET, ethanol.

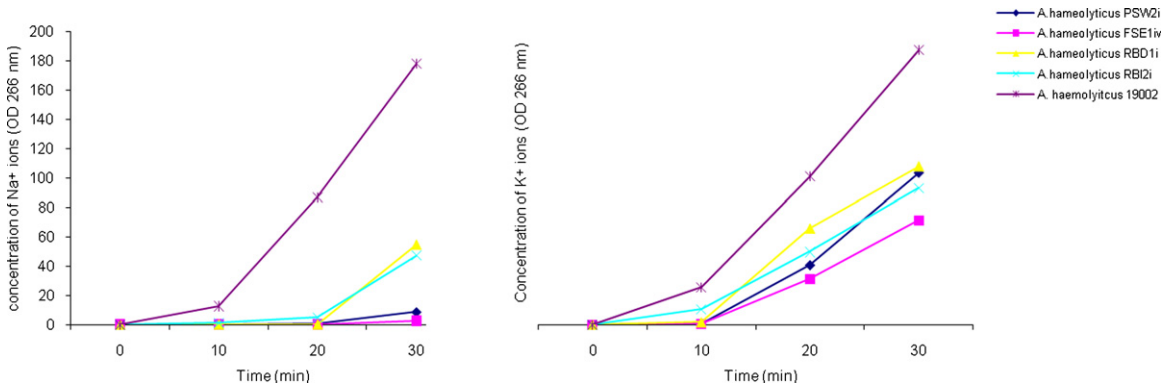

Fig. 3. Leakage of Na^+ and K^+ ions from *Acinetobacter haemolyticus* isolates by stem bark ethanol extracts of *Curtisia dentata* (*Acinetobacter haemolyticus* isolates from various samples as PSW, primary settled wastewater; FSE, final settled effluent; RBD, River Berg downstream; RBI, River Berg Influent).

(Kalim et al., 2010). The exhibition of high DPPH radical scavenging activity, total phenol content and reducing power by the extracts is an indication that drugs useful as antioxidants can be sourced from *Curtisia dentata*. Results also showed differences in extraction efficiency by the various solvents with highest DPPH, TPH and RP values followed by dichloromethane, hexane, acetone and distilled water. Differences in antioxidant activity between the various solvents may due to variation in polyphenol concentration extracted. Different solvents have different degrees of solubility depending on their polarity (Doughari, 2006). DPPH assay has been commonly employed in screening antioxidant activity of plant extracts. Radical scavenging activity potential of *Curtisia dentata* observed in this study is a promising outcome for possible control of many oxidative stress-related diseases. Recently, much attention has been directed towards the development of ethnomedicines with strong antioxidant properties but low cytotoxicity. It has been estimated that approximately two-thirds of anticancer drugs approved worldwide up to 1994 were derived from plant sources (Kalim et al., 2010). The demonstration of antioxidant activity by extracts of *Curtisia dentata* is an indication that the plant can serve as a useful source for chemical substances for development of novel drugs.

5. Conclusion

The study revealed the presence of a wide range of phytochemicals in *Curtisia dentata* extracts, as well as the possession of antioxidant, antimicrobial and antiverotoxic activity against strains of *Escherichia coli* and *Acinetobacter* spp. The study also revealed the possible damaging effect of the ethanol extracts on the bacterial cell walls an indication of the possible mechanism of action of the plant. Toxicological studies and further purification of *Curtisia dentata* extracts for possible structural elucidation of the phytochemical compounds to enable possible sourcing of antibiotic substances should be carried out. This might serve as a milestone for the development of novel antibiotic substances for treatment of verotoxic as well as nosocomial infections associated with these bacterial strains.

Acknowledgement

The study was supported by the Cape Peninsula University of Technology, Cape Town, South Africa through the Research Fund RP03.

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