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*

James Hamuel Doughari*, Patrick Alois Ndakidemi, Izanne Susan Human, Spinney Benade

Faculty of Applied Sciences, Cape Peninsula University of Technology, P.O. Box 652, Cape Town 8000, South Africa

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

* Corresponding author. Tel.: +27 0733 55 0274/234 0703 5599 712.
E-mail addresses: jameshamuel@yahoo.com, dougharijames@gmail.com (J.H. Doughari).

0378-8741/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
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