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South African Journal of Botany 72 (2006) 295 – 297
SOUTH AFRICAN JOURNAL OF BOTANY
www.elsevier.com/locate/sajb

Short communication

Emodin, an antibacterial anthraquinone from the roots of *Cassia occidentalis*

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Received 29 April 2005; accepted 4 August 2005

Abstract

The antibacterial activity of the ethanolic root extract of *Cassia occidentalis* was examined. A biologically active component was isolated and identified as emodin by spectroscopic analysis. The bioactive Minimum Inhibitory Concentration (MIC) values of emodin were 7.8×10^{-3} and 3.9×10^{-3} mg ml^{-1} against *Bacillus subtilis* and *Staphylococcus aureus*, respectively. It was not active against two Gram-negative bacteria (*Klebsiella pneumoniae* and *Escherichia coli*) at the highest concentration (5.0×10^{-1} mg ml^{-1}) tested.

Keywords: *Cassia occidentalis*; Emodin; Antibacterial

Cassia occidentalis L. (Leguminaceae) is used in traditional medicine in the tropical areas of the world. In Peru, the roots are used as a diuretic and the decoction is made to treat fevers (Soukup, 1970). The seeds are brewed into a coffee-like beverage to treat asthma and the flower infusion is used for bronchitis in the Peruvian Amazon (Rutter, 1990). In Brazil the roots are considered to be a tonic, febrifuge and diuretic, and are used against fevers, tuberculosis, anaemia, liver complaints and as a reconstituent for general weakness and illness (Coimbra, 1994). In southeastern Nigeria the leaves are used for fever (Chukwujekwu et al., 2005). Different parts of this plant have been reported to possess anti-inflammatory (Kuo et al., 1996), antihepatotoxic (Saraf et al., 1994), antibacterial (Samy and Ignacimuthu, 2000) and antiplasmodial activities (Tona et al., 2004). A wide range of the chemical constituents have been isolated from *C. occidentalis*, including sennoside (Christ et al., 1978), anthraquinone glycosides (Lal and Gupta, 1974; Chauhan et al., 2001), fatty oils, flavonoid glycosides (Purwar et al., 2003), gallactomannan, polysaccharides and tannins (Kudav and Kulkarni, 1974).

Although *C. occidentalis* is indigenous to Brazil, it is a naturalized plant in Nigeria where it is widespread and grows profusely. It is well incorporated into traditional medicinal practices in the West African region, where it is extensively used against fever. It has been reported to show good antibacterial activity (Chukwujekwu et al., 2005). The aim of this study was to isolate the antibacterial agent(s) from the *C. occidentalis* roots.

Roots of *C. occidentalis* L. were collected between May and June 2002 from southeastern Nigeria. The voucher specimen was deposited in the Enugu State Herbarium (voucher number-EFH9009). Plant material was air-dried for two weeks, powdered and stored in paper containers for a week prior to extraction.

The air-dried roots (190 g) were extracted with 1 L 80% aqueous ethanol with sonication for 1 h and then left overnight on a magnetic stirrer. The extract was filtered through a Büchner funnel using Whatman No. 1 filter paper. The extraction and filtration were subsequently repeated three times with 0.5 L 80%

Table 1
Minimum inhibitory concentrations (mg ml^{-1}) of emodin isolated from *C. occidentalis* roots against some selected bacteria

Compound tested	Bacterium[a]			
	K.p.	*E.c.*	*B.s.*	*S.a.*
Emodin	>0.5	>0.5	7.8×10^{-3}	3.9×10^{-3}
Neomycin	1.6×10^{-3}	1.6×10^{-3}	7.8×10^{-4}	6.3×10^{-3}

[a] Abbreviations: *K.p.* — *K. pneumoniae; E.c.* — *E. coli; B.s.* — *B. subtilis; S.a.* — *S. aureus*.

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doi:10.1016/j.sajb.2005.08.003

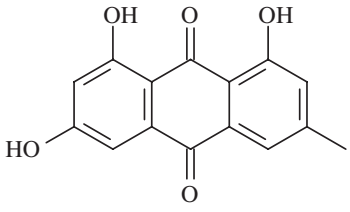
aqueous ethanol, and the solvent was evaporated under reduced pressure at 30 °C. Vacuum liquid chromatography (VLC) (Merck 9385, 150 g, 6 × 30 cm) of the ethanolic extract (15 g), using a CH_2Cl_2 : MeOH step gradient (9 : 1 to 0 : 1), produced sixteen fractions of which fractions 6 (177 mg) and 7 (190 mg) eluted with CH_2Cl_2 : MeOH (4 : 1 and 7 : 3), respectively, showed good antibacterial activity in the bioautographic assay. Further purification by gravity column chromatography (Merck 9385, 127 g, 2.5 × 73 cm) using a CH_2Cl_2 : MeOH step gradient (9 : 1 to 0 : 1) afforded 68 mg of active fraction. This was finally separated by preparative TLC (Merck glass plates, 20 × 20 cm, silica gel 60 F_{254}, 0.25 mm thickness) using CH_2Cl_2 : MeOH (15 : 1) as the mobile phase. The active compound(s) (14 mg, appearing as a dark brown band under UV_{366}) was scraped off the TLC plates, eluted from the silica with absolute ethanol, and filtered through Millipore filters (0.45 and 0.22 μm) to remove residual silica. The purity of the isolate was confirmed by TLC using various solvent systems.

Throughout the isolation procedure antibacterial activity was determined by the direct bioautographic assay (Hamburger and Cordell, 1987) using Gram-positive *Staphylococcus aureus* (ATCC 12600) as the test organism. The inhibition of bacterial growth by compounds isolated was indicated as white spots against a deep red background. The microtitre bioassay (Eloff, 1998) was used to determine the Minimum Inhibitory Concentration (MIC), against *S. aureus* (ATCC 12600), *Escherichia coli* (ATCC 11775), *Klebsiella pneumoniae* (ATCC 13883) and *Bacillus subtilis* (ATCC 6051). Neomycin (100 μg ml^{-1}) served as a positive control for each bacterium, with solvent (25% aqueous ethanol) and bacteria free wells being included as blank controls. The experiment was repeated twice with two replicates each.

The identity of the isolate was achieved by comparison of its ^1H and ^{13}C NMR spectral data with literature values (Wang et al., 1996; Fujimoto et al., 1998), and by analysis of its 2D (HSQC, HMBC, COSY and NOESY) NMR spectra. These data confirmed the compound as 4,5,7-trihydroxy-2-methylanthraquinone.

Emodin has previously been found in a wide variety of botanical and fungal species (Turner and Aldridge, 1983; Wang et al., 1996; Fujimoto et al., 1998), including other plant species belonging to the genus *Cassia* (Kelly et al., 1993). It has not hitherto been reported from *C. occidentalis*. It has been shown to possess both monoamine oxidase (Fujimoto et al., 1998) and tyrosine kinase (Jayasuriya et al., 1992; Kumar et al., 1998) inhibitory activity and was reported to act as an antimicrobial, antineoplastic and cathartic agent (Dictionary of Natural Products, 2004).

The MIC results obtained for emodin are presented in Table 1. Due to the limited amount of the compound obtained, it was dissolved in 25% aqueous ethanol at a final or stock concentration of 2 mg ml^{-1}. At this concentration, there was no bacteriostatic effect on the Gram-negative bacteria. This was not surprising since the crude extract did not exhibit any bacteriostatic effect on these Gram-negative bacteria in our previous study (Chukwujekwu et al., 2005). However, emodin exhibited a remarkable bacteriostatic effect on the Gram-positive bacteria tested, especially *S. aureus*. It had significantly higher activity when compared with the results obtained for the crude extract (Chukwujekwu et al., 2005). Emodin also exhibited a higher bacteriostatic effect against *S. aureus* when compared to the standard neomycin (Fig. 1).



emodin

Fig. 1.

Thus, while our isolate is not a new compound, this is the first report of its isolation from *C. occidentalis*.

Acknowledgements

We thank the National Research Foundation, Pretoria, and the University of KwaZulu-Natal Research Fund for financial support.

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