African Journal of Biotechnology Vol. 5 (11), pp. 1092-1094, 2 June 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Antibacterial activity of aloe emodin and aloin A isolated from *Aloe excelsa*

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Accepted 27 March, 2006

Different extracts of leaves of *Aloe excelsa* were prepared and two compounds; 1,8-dihydroxy-3-hydromethyl-9,10-antracenedione and 10-*C*-β-D-glucopyranosyl-1,8-dihydroxymethyl-9-anthracenone, were isolated and tested for antibacterial activities against four Gram negative and five Gram positive bacterial strains. The structures of the two compounds were determined by chemical spectroscopy

Key words: *Aloe excelsa*, Aloaceae, antibacterial, aloe emodin, aloin A.

INTRODUCTION

Aloe excelsa is one of the larger species of aloes from the family Aloaceae (Glen et al., 1997). As with most Aloe species (Van Wyk et al., 1997), *A. excelsa* have been used extensively as a traditional remedy. The use of herbal remedies for treatment of various diseases is still vital to the provision of health care in Southern Africa and to the continent as a whole Ndubani and Hojer 1999). *A. excelsa* also referred to as the Nobel Aloe is widespread in Africa with great abundance in Zimbabwe. Aloe emodin and aloin A are known to occur in both commercial viable species of aloes (*Aloe vera* and *A. ferox)*, but has not previously been isolated from *A. excelsa*. Although extensive research had been done with most Aloe species including chemical work (Speranza et al., 1986; Speranza et al., 1990; Koyama et al., 1994), little or no work in terms of isolation and antibacterial activities have been performed in *A. excelsa*.

MATERIALS AND METHODS

Melting points were determined on a Gallenhamp melting point apparatus and were uncorrected. The UV and IR spectra were recorded on Beckman DU-7400 and Perkin Elmer FT-IR spectrometers respectively. ^{1}H (400 MHz) and ^{13}C (100.60) NMR as well as 2D NMR spectra were recorded on a Bruker AMX 400 instrument with chemical shift data reported in parts per million (ppm) relative to the solvent used with field gradient BBI (inverse) probe. Mass spectra were recorded on Micromass 70-70E mass spectrometer. FABMS spectra were obtained with m-nitrobenzyl alcohol matrix. Vacuum liquid chromatography (VLC) and column chromatography (CC) were performed on silica gel 60 H (15um) and silica gel (0.063 – 0.2 mm), respectively. Silica gel F254 60 coated on aluminium plates for thin layer chromatography (TLC) and silica gel F254 60 coated on glass plates (20cm X 20 cm) for preparative thin layer chromatography (PTLC), all were supplied by Merck. Sephadex LH – 20 (25 -100um) for gel filtration chromatography (GFC) was obtained from Fluka.

Plant material

Approximately 1 kg of collected plant material was dried in the sun over a period of a month. The dried material was finely ground and prepared for extraction.

Extraction of pure compounds

Approximately 1000 g of ground material was extracted by shaking at room temperature for 72 h. The extract was filtered and evaporated under reduced pressure to give 52.4 g. Partitioning of the extract was done between n-hexane and water. The aqueous part was further partitioned between ethyl acetate (EtOAc) and water.

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The n-hexane portion (4.9 g) was further fractionated by VLC over silica gel using solvents of increasing polarity (0 – 100% EtOAc in n-hexane) and a total of 29 fractions was collected. The fraction obtained with 45 – 50% EtOAc in n-hexane was subsequently subjected to gel filtration (Sephadex LH – 20), eluted with $CHCl_3$ followed by MeOH : $CHCl_3$ (5:95), producing a total of 36 fractions. Fractions 21 – 29, amounted to 0.24 g, were combined and subjected to column chromatography over silica gel using EtOAc : n-hexane (60:40, v/v). A total of 15 fractions were collected. Yellow fractions, 4 – 12 were combined (75 mg) and further subjected to column chromatography on a silica gel column using 2% MeOH in $CHCl_3$ to give aloe emodin.

The EtOAc extract, amounted to 8.2 g, was subjected to a similar process as described above, using solvent system MeOH : EtOAc (0 – 30%). A total of 48 fractions (20 ml each) were collected. The fractions eluted with $CHCL_3$: MeOH (90:10, v/v) to give a total of 48 fractions. Fractions 1 – 9 were combined (0.2 g) and further fractionated by column chromatography over silica gel using $CHCl_3$: MeOH (90:10 v/v) to yield aloin A (51 mg).

RESULTS

Aloe emodin

Yellow crystal form when subjected to MeOH : $CHCl_3$ (5:95, v/v), mp, IR and UV data agreed with literature for other *Aloe species* (Rizk et al., 1972). ^{1}H NMR ($CDCl_3$): δ 4.65 (2H, s, 3-CH_2OH), 7.74 (1H dd, J=1.5, 8.4 Hz H-5), 7.69 (1H, s, H-4), 7.60 (1H, dd, J=8.4 Hz, H-6), 7.26 (1H, s, H-2), 7.22 (1H, dd, J=1.5, 8.4 Hz, H-7). ^{13} C NMR ($CDCl_3$): δ 162.5 (C-1) 121.2 (C-2), 152.5 (C-3) 119.9 (C-4), 117.7 (C-5), 136.9 (C-6), 124.5 (C-7), 162.0 (C-8), 192.4 (C-9), 182.0 (C-10), 114.9 (C-1a), 133.3 (C-4a), 133.4 (C-5a), 115.7 (C-8a), 63.1 (3-CH_2OH).

Aloin A

Yellow crystals from 15% MeOH in CHCl3, UV λ_{max} (MeOH) nm: 211, 264, 361. ^{1}H NMR (CD_3OD): δ 7.47 (1H, t, J=8.0 Hz, H-6), 7.03 (1H, s, H-4), 7.02 (1H, d, J=8.8 Hz, H-5), 6.86 (1H, s, H-2), 6.83 (1H, t, J=8.o, H-7), 4.64 (2H, d, J=3.6 Hz, 3-CH_2-OH), 4.56 (1H, s, H-10), 3.38 (1H, dd, J=9.2, 2.0 Hz, H-1'), 3.01 (1H, t, J=9.2, H-2'), 3.23 (1H, t, J=8.8 Hz, H-3'), 2.89 (1H, t, J=8.8 Hz, H-4'), 2.91 (1H, m, H-5'), 3.54 (1H, dd, J=1.6, 11.6 Hz, H-6'), 3.40 (1H, dd, J=4.0, 9.6 Hz, H-6). ^{13}C NMR (CD_3OD): δ 163.4 (C-1), 114.5 (C-2), 151.5 (C-3), 64.6 (3-CH_2-OH), 119.2 (C-4), 119.9 (C-5), 137.0 (C-6), 116.8 (C-7), 162.9 (C-8), 195.6 (C-9), 45.9 (C-10), 117.7 (c-1a), 143.3 (C-4a), 146.6 (C-5a), 118.7 (C-8a), 86.7 (C-1'), 71.9 (C-2'), 80.0 (C-3'), 72.1 (C-4'), 81.7 (C-5'), 63.3 (C-6').

Antibacterial assay

Gram positive and gram negative bacterial strains of *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Micrococcus kristinae, Staphylococcus epidermidis, Escherichia coli, Proteus vulgaris, Enterobacter aerogenes* and *Shigella sonnei* were used in the antibacterial assay. Bioautographic assay (Slusarenko et al, 1989) was performed on TLC plates using *B. subtilis* simultaneously with the extractions. An inoculated layer of agar was sprayed with fresh culture bacteria over a developed TLC plate and incubated for 24 hours at 37°C. 0.2 mg/ml *p*-iodonitrotetrazolium (INT) solution was sprayed over the plates and incubated at 37°C for 30 min to indicate bacterial growth. The inhibition of bacterial growth by compounds separated on the TLC plate was visible as white spots.

The minimum inhibitory concentration (MIC) values of the pure compounds were determined with microplate dilution method against five Gram-positive (*B. cereus, B. subtilis, M. kristinae, S. aureus* and *S. epidermidis*) and four Gram-negative bacteria (*E. coli, P. vulgaris, E. aerogenes* and *S. sonnei*) using 96-well micotiter plates. Each test organism was prepared by diluting 24 h old broth cultures with sterile nutrient broth. The cultures were then further diluted to give approximately 10^6 bacteria ml^{-1}. The microtiter plates were prepared using serial dilution (Eloff, 1998) and incubated for 24 – 48 h at 37°C. As an indicator of bacterial growth, 40 µl of 0.2 mg/ml *p*-iodonitrotetrazolium (INT) solution was added to each well and incubated at 37°C for 30 min. The colourless tetrazolium salts gave off a red product due to the biological activity of the organism, thereby making the inhibition of bacterial growth visible as clear wells. Each treatment was replicated three times. Streptomycin, chloramphenicol, solvents and sample free solutions were used as standard and blank controls.

DISCUSSION

A. excelsa is indigenous to Zimbabwe and has been utilized by the indigenous people as a source of remedy for various ailments. There exist many similarities in the traditional medicinal uses between *A. excelsa* and other members of the genus, example *A. ferox* and *A. vera* (Newton and Chan, 1998). Isolation and purification of many compounds have been successfully accomplished from the latter two species (Dagne et al., 2000). This research on *A. excelsa* is an attempt to isolate compounds and possibly obtain purified, if not novel compounds. The two compounds isolated and purified have been known to occur in both *A. ferox* and *A. vera.* However, isolation of these two compounds, although not novel compounds, have been accomplished in this work for the first time in *A. excelsa.*

The two compounds; 1,8-dihydroxy-3-hydroxymethyl-9,10-anthracenedione (aloe emodin) and 10-C-β-D-glucopyranosy l-1,8-dihydroxy-3- hydroxymethyl-9-

Table 1. Antibacterial activity of isolated compounds of *Aloe excelsa*.

Bacteria	Gram +/-	Compounds		Streptomycin	Chloramphenicol	DMSO
		1	2			
B. subtilis	+	125*	62.5	4	7.8	>250
M. kristinae	+	250	125	4	4	>250
B. cereus	+	62.5	62.5	4	4	>250
S. aureus	+	125	62.5	2	7.8	>250
S. epidermidis	+	250	125	2	4	>250
E. coli	-	62.5	125	4	4	>250
P. vulgaris	-	62.5	125	4	4	>250
E. aerogenes	-	125	250	4	4	>250
S. sonnei	-	250	250	4	4	>250

1 = aloe emodin, 2 = aloin A
*Minimum inhibitory concentration (µg/ml)

antracenone (aloin A) were isolated from the leaves of *A. excelsa*. Aloe emodin showed inhibitory activity against all test organisms with MIC ranging from 62.5 µg/ml in *B. subtilis* and *E. coli* to 250 µg/ml in *S. epidermidis* and *S. sonnei* (Table 1). Hatano et al. (1999) reported MIC's of aloe emodin against *E. coli* to be greater than 128µg/ml. Aloe emodin has been reported to be an anticancer agent with selective activity against neuroectodermal tumors (Pecere et al., 2000). Generally, both aloe emodin and Aloin A are known to be of medicinal values including functioning as laxative (Van Wyk, 1997).

In conclusion, the isolation of the two compounds as well as the action against Gram-positive and Gram-negative bacteria has demonstrated great potential of the plant as an antimicrobial agent. *A. excelsa* can be used in treatment of various ailments such as skin treatment, stomach ailments, and as a laxative and can be comparable to both *A. vera* and *A. ferox* which are currently being commercially exploited.

ACKNOWLEDGEMENT

The authors are grateful to the NRF, South Africa for the financial support in this project. The authors are also grateful to Professor K. Dzama, Department of Agriculture, Faculty of Science and Agriculture, University of Fort Hare, for providing the specimens.

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