

## Short Communication

# Antibacterial activity of chrysophanol isolated from *Aloe excelsa* (Berger)

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**Extraction of the yellow colour compounds of leaves of *Aloe excelsa* were performed and 1,8-dihydroxy-3-methylanthracenedione (chrysophanol) was isolated and tested for antibacterial activities against four gram negative and five gram positive bacterial strains. The structures of chrysophanol was determined by chemical spectroscopy**

**Key words:** *Aloe excelsa*, Aloaceae, antibacterial, chrysophanol.

## INTRODUCTION

Nobel Aloe of Zimbabwe, a member of the family Aloaceae (Glen et al., 1997), has extensive traditional uses (Van Wyk et al., 1997). Herbal remedies have currently been acknowledged as an alternative health care option in Southern Africa and to the continent as a whole (Ndubani and Hojer, 1999). Isolation and identification of Aloin A and Aloe emodium has already been achieved in *A. excelsa* (Cooposamy and Magwa, 2006) Chrysophanol is known to occur in both commercial viable species of aloes (*Aloe vera* and *A. ferox*), but has not previously been isolated from *A. excelsa*.

## MATERIALS AND METHODS

### Measurements

Melting points were determined on a Gallenhampt melting point apparatus and were uncorrected. The UV and IR spectra were recorded on Beckman DU-7400 and Perkin Elmer FT-IR spectrometers respectively. <sup>1</sup>H (400MHz) and <sup>13</sup>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 (15µm)

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 -100µm) 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

One kilogram (1 kg) 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.

The EtOAc extract amounted to 8.2 g was further fractionated by VLC over silica gel using solvents of increasing polarity EtOAc : n-hexane (0 – 100%) and then MeOH : EtOAc(0 – 30%). The eluate obtained from 30 – 40% EtOAc in n-hexane (0.9 g) was column chromatographed over silica gel and eluted with EtOAc: n-hexane (60:40, v:v). A total of 48 fractions (20 ml each) were collected. Fractions 10–18 (0.11 g) were combined and further chromatographed over silica gel using EtOAc : n-hexane (80:20, v:v) to give chrysophanol (22 mg).

### Antibacterial assay

Gram positive and gram negative bacterial strains of *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus*

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

Bacteria	Gram +/-	Chrysophanol extract	Streptomycin	Chloramphenicol	DMSO
<i>B. subtilis</i>	+	>250	4	7.8	>250
<i>M. kristinae</i>	+	>250	4	4	>250
<i>B. cereus</i>	+	>250	4	4	>250
<i>S. aureus</i>	+	>250	2	7.8	>250
<i>S. epidermidis</i>	+	31.25	2	4	>250
<i>E. coli</i>	-	125	4	4	>250
<i>P. vulgaris</i>	-	125	4	4	>250
<i>E. aerogenes</i>	-	>250	4	4	>250
<i>S. sonnei</i>	-	>250	4	4	>250

\* Minimum inhibitory concentration ( $\mu\text{g/ml}$ ).

*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 h 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 microtiter 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  $\text{ml}^{-1}$ . 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  $\mu\text{l}$  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.

## RESULTS AND DISCUSSION

### Chrysophanol

Yellow crystals from 5% MeOH in  $\text{CHCl}_3$ , mp, IR and UV data agreed with literature (Rizk et al., 1972; Huneck and Yoshimura, 1996).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.81 (1H, dd,  $J=2$ , 7.8 Hz, H-5), 7.67 (1H, m, H-6), 7.64 (1H, s, H-4), 7.52 (1H, dd,  $J=2$ , 8.8 Hz, H-7), 7.09 (1H, s, H-2), 2.47 (3H, s, 3- $\text{CH}_3$ ), 12.1 (1-OH), 12.0 (8-OH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  22.3 (3- $\text{CH}_3$ ), 162.7 (C-1), 124.4 (C-2), 149.3 (C-3), 119.9 (C-4), 121.4 (C-5), 136.9 (C-6), 124.5 (C-7), 162.4 (C-8), 192.5 (C-9), 181.9 (C-10), 113.7 (C-1a), 133.3 (C-4a), 133.6 (C-5a), 115.9 (C-8a).

*A. excelsa* has been utilized by the indigenous people as a source of remedy for various ailments, many of

which shows similarities in the traditional medicinal uses between *A. excelsa* and other members of the genus (Newton and Chan, 1998). Successful isolation many compounds have been accomplished in other species of Aloes (Dagne et al., 2000). Chrysophanol isolation and purification have been known to occur in both *A. ferox* and *A. vera*. However, isolation of this compound, although not novel compound, have been accomplished for the first time in *A. excelsa*.

1,8-dihydroxy-3-methylanthracenedione (chrysophanol) was isolated from the leaves of *A. excelsa*. Chrysophanol was active against *B. subtilis*, *S. epidermidis* and *E. coli* (Table 1). Hatano et al. (1999) reported MIC's of chrysophanol against *E. coli* to be greater than 1024  $\mu\text{g/ml}$ , while the MIC of chrysophanol was 256  $\mu\text{g/ml}$  against methicillin resistant *Staphylococcus aureus*.

*A. excelsa* have is well known for its traditional values in treatment of various ailments. Isolation and identification of compounds present in extracts could be useful in understanding the relations between traditional cures and current medicines. Isolation of chrysophanol as well as the action against Gram-positive and Gram-negative bacteria has demonstrated great potential of the plant as an antimicrobial agent.

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