Short Communications

Comparative antibacterial activity of two *Helichrysum* species used in male circumcision in South Africa.

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The leaves of *Helichrysum pedunculatum* and *H. longifolium* are used for the treatment of wounds arising from male circumcision by the Xhosas and the Pondos of South Africa, respectively. The antibacterial activity of these herbs was compared by direct bioautography using *Staphylococcus aureus*. Extracts from the leaves of *H. pedunculatum* showed more activity against the bacterium than those from *H. longifolium*. Heating the extracts from the latter, further reduced their activity against *S. aureus*. The traditonal heating of the leaves of this plant over hot ash before use is, therefore, likely to reduce their activity against infection.

Keywords: Antibacterial, circumcision, *Helichrysum*, medicinal, Pondos, Xhosas.

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Traditional male circumcision is a common practice among the indigenous people of South Africa especially the Xhosas and the Pondos. The practice involves the surgical removal of the foreskin of the male reproductive organ with a sharp knife followed by a period of seclusion for several weeks during which the wounds are treated with herbs. Usually, the whole ceremony takes place in the bush and the patients, who are mainly teenagers, are kept far away from families and friends throughout the period.

Circumcision performed in the bush has a high risk of infection. Information obtained from various local communities has revealed a high incidence of complications arising from wound contaminations. Cases of complications requiring hospital admission, according to hospital sources, ranged from 105 to 135 during an initiation ceremony, depending on the community. While reports of contamination leading to complications are common among the Pondos which, in most cases, have resulted in the death of many patients, such reports are rare among the Xhosas.

The authors observed that different species of *Helichrysum* are used by the different communities for the treatment of the wounds after circumcision. The Xhosas use *H. pedunculatum* Hilliard & Burtt while the Pondos use *H. longifolium* DC for the treatment of the wounds. Both species are perennial herbs and are widely distributed in southern Africa (Hilliard 1983). Traditionally, the Pondos heat the leaves of *H. longifolium* over very hot ash before using them as a bandage for the treatment of wounds after circumcision. Extracts from the shoots of *H. pedunculatum* have been reported to be active against a number of bacterial species (Meyer and Dilika 1996). However, no information is available on the antimicrobial property of *H. longifolium*. The aim of this study was to compare the antibacterial activities of *H. pedunculatum* and *H. longifolium* by direct bioautography using *Staphylococcus aureus*. *S. aureus* is one of the common organisms usually isolated from such wounds. The effect of temperature on the antibacterial activity of *H. longifolium* was also examined.

Air dried leaves of *H. pedunculatum* and *H. longifolium* were collected from the Eastern Cape province of South Africa in September 1996 and confirmed with voucher specimens Dilika 299 and Lubbe 128 deposited in the H.G.W.J. Schweickerdt Herbarium (PRU), Pretoria.

Leaves from *H. pedunculatum* and *H. longifolium* (8 g each) were shaken in acetone for 5 min and the resultant extracts were filtered separately and concentrated to dryness under reduced pressure giving 92.4 and 18.7 mg respectively. The remaining leaf material was then homogenised in acetone, filtered and concentrated to dryness yielding 97.9 and 70.0 mg of dry extract respectively. The four dry extracts were each dissolved in acetone to a final concentration of 20 $mgml^{-1}$

For direct bioassay on thin layer chromatography (TLC), 10 µl of each extract was spotted on silica gel 60 plates (Merck) and developed in chloroform-benzene (60:40). A suspension of 24 h old *S. aureus* cultured in nutrient agar was sprayed onto the TLC plates (Meyer and Dilika 1996), and incubated at 37°C for 24 h. After incubation, the plates were sprayed with an aqueous solution of p-iodonitrotetrazolium violet and reincubated at 37°C for 3 h (Lund and Lyon 1975).

The extract obtained from the leaves of *H. longifolium* shaken in acetone was divided into four parts. Three portions were heated at 60°C, 80°C and 100°C respectively in closed glass tubes for 15 min, while the fourth part was left unheated and served as a control. The four treatments were then subjected to direct bioautography as described above.

Considering their positions and colours on the TLC plates under UV light at 254 and 366 nm, many similar compounds were detected in the extracts obtained from shaken and homogenised leaves of *H. pedunculatum* and *H. longifolium* (Figure 1A). This might be of taxonomic interest since Hilliard (1983) placed these species in different groups. According to Gershenzon and Mabry (1983), individual or whole classes of secondary compounds are frequently restricted to groups of plant taxa that are considered to be related on many grounds.

Clear (white) zones on the TLC plates indicated antibacterial activity of the extracts. *H. pedunculatum* extracts from both shaken and homogenised plant material exhibited activity against *S. aureus*, whereas only the shaken extract from *H. longifolium* showed activity using direct bioassay (Figure 1B). Although direct bioassay on TLC plates is not an ideal method for the quantification of bioactivity, it could be safely stated that the acetone extract from the leaves of *H. pedunculatum* has more activity against *S. aureus* than *H. longifolium*. This is further supported by the fact that when equal amounts of leaves of these two plants were extracted, *H. pedunculatum* yielded about 5 times more of crude extract than that of *H. longifolium*. Meyer and Dilika (1996) have found the MIC of the dichloromethane extract from *H. pedunculatum* against *S. aureus* to be 35 $mgml^{-1}$. The antibacterial activity of the shaken extract from *H. longifolium* decreased with increase in temperature as indicated by the relative areas of the clear zones on the TLC plate (Figure 1C). The highest activity was observed in the control (unheated) extract, while the extract heated at 100°C showed little activity.

Although many factors may be responsible for the occurrence of complications following male circumcision among the Pondos, it is plausible to assume that the lower antibacterial activity of *H. longifolium*, coupled with the traditional method of heating the leaves over hot ash before use, may be two of the factors. Since *H. pedunculatum* also grows in the area where the Pondos live, it is recommended for use rather than *H. longifolium*. Heating of the plant should be discouraged as it appears to reduce the activity of the leaf extract against *S. aureus*.

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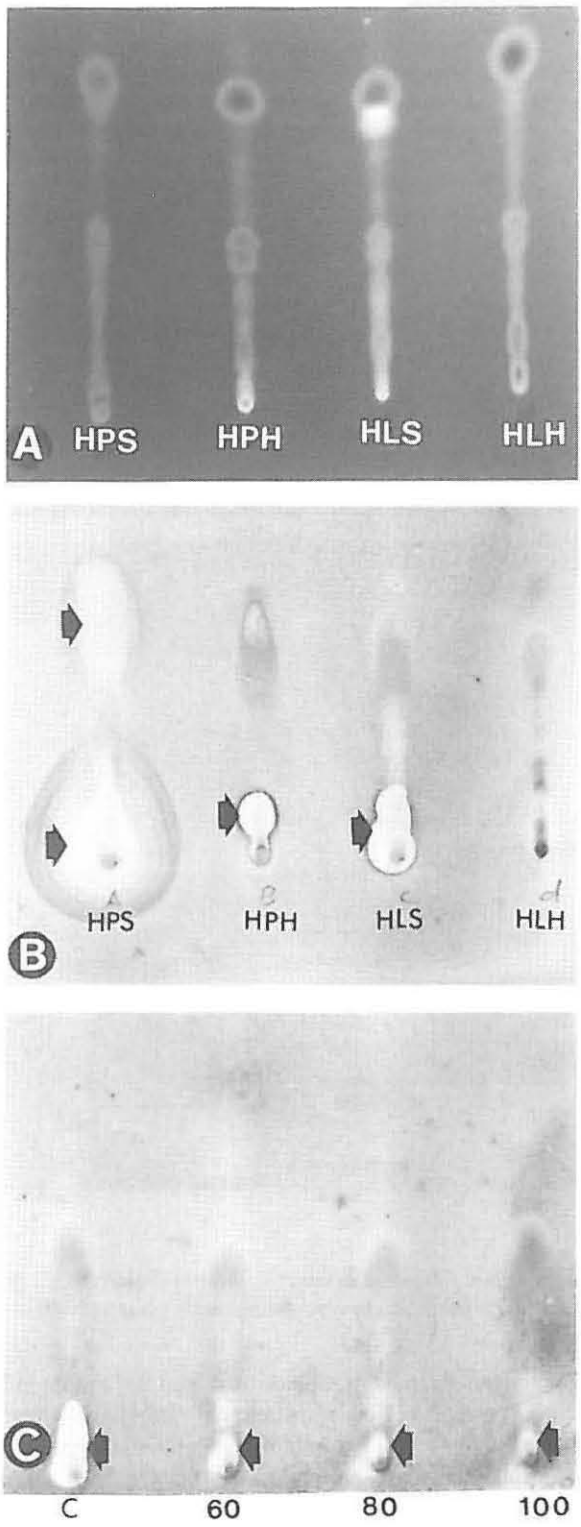

Figure 1 Direct bioautographic assay of extracts from *Helichrysum pedunculatum* and *H. longifolium* using *Staphylococcus aureus*. A. TLC plate of extracts from leaves of both plants under UV light at 366 nm. (HPS, extract from leaves of *H. pedunculatum* shaken in acetone; HPH, extract from the homogenised leaves of the same plant; HLS, extract from the leaves of *H. longifolium* shaken in acetone; HLH, extract from the homogenised leaves of the same plant). Note the similarity in colour and positions of the compounds. B. Activity of the extracts against *Staphylococcus aureus* shown as clear zones (arrowed) after spraying with p-iodonitrotetrazolium violet. C. Effect of temperature on the activity of the shaken extract of *H. longifolium* against *S. aureua*. Extracts were heated at 60°, 80° and 100°C for 15 min. The control (C) was not heated.

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In vitro propagation of *Veltheimia bracteata* and *V. bracteata* 'Lemon Flame'

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In vitro adventitious bud formation was initiated using leaf and bulb explants of both *Veltheimia bracteata* and *V. bracteata* 'Lemon Flame'. The initiation medium used consisted of Murashige and Skoog (MS) solidified with 0.8% agar, and was further supplemented with 100 mgl^{-1} myo-inositol, 25 mgl^{-1} NaFeEDTA, 0.5 mgl^{-1} thiamine-HCl and 3% sucrose. Adventitious bud initiation was stimulated by a combination of BA (2 mgl^{-1}) and NAA (0.1 mgl^{-1}) Buds were transferred to MS medium supplemented with 100 mgl^{-1} myo-inositol, 3% sucrose and IBA (2 mgl^{-1}) for root initiation. Plantlets were successfully hardened off in vermiculite under misthouse conditions over a 4 week period. These plants were subsequently transferred to a greenhouse and watered twice weekly. There was 98% survival of plantlets. Explants from the cultivar 'Lemon Flame', however produced 50% less plantlets *in vitro*.

Keywords: *Veltheimia bracteata*, micropropagation, tissue culture, conservation, horticulture.

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The genus *Veltheimia* (Family Hyacinthaceae), a small genus of bulbs indigenous to southern Africa, is characterised by large mesomorphic leaves and a variegated scape (Dahlgren *et al.*, 1985). The plants have a dense cluster of broad leaves with an attractive wavy edge, and grow to a height of approximately 450 mm. The tubular, drooping flowers are produced on a spike in early spring. *V. bracteata* Harv. ex Baker produces dusky pink flowers tipped with green, while *V. bracteata* 'Lemon Flame' bears pale yellow flowers tipped with green (Figure 1A). The plants prefer well drained soils in frost free areas and are suitable as pot plants, giving them great potential in the horticultural market. Propagation is largely from seed and offsets. This is, however, slow and yields few plants (Pienaar, 1984). The genus was cultivated extensively in Victorian times, with several strains showing variations in flower colour, including deep red and yellow spotted with red. There are thus considerable possibilities for the development of a wider range of colours through hybridisation (Bryan, 1989). It was thus decided to generate an efficient protocol for the mass propagation of plantlets.

Leaf and bulb material from adult specimens of *V. bracteata* and *V. bracteata* 'Lemon Flame' were used as explant sources.