APPENDIX 3

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Antibacterial activity of South African *Helichrysum* species

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Received 27 April 1998; revised 20 June 1998

Acetone extracts of *Helichrysum callicomum, H. glomeratum, H. hypoleucum, H. odoratissimum, H. pilosellum* and *H. rugulosum* were investigated for antibacterial activities against ten bacteria using the agar diffusion method. Epicuticular (shaken) and homogenized extracts of *H. hypoleucum, H odoratissimum* and *H. rugulosum* significantly inhibited the growth of *Bacillus cereus, B. pumilus, B. subtilis, Micrococcus kristinae* and *Staphylococcus aureus* (all Gram-positive bacteria) and *Enterobacter cloacae* (Gram-negative) at a concentration range of 0.01 to 1.0 mg/ml. In addition, the epicuticular extract of *H. hypoleucum* was active against *Escherichia coli* and *Pseudomonas aeruginosa* whereas the homogenized extract only had activity against *P. aeruginosa*. None of the other six extracts inhibited the growth of *E. coli, Klebsiella pneumoniae, P. aeruginosa* and *Serratia marcescens*, all Gram-negative bacteria. The extracts of *H. glomeratum* and *H. pilosellum* had no activity against any of the organisms tested. Shaken extracts proved to be more bioactive than homogenized extracts.

Keywords: Antibacterial, Asteraceae, *Helichrysum*, traditional medicine.

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Introduction

There are 500 *Helichrysum* species of which 245 occur in South Africa. The South African species display great morphological diversity and are, therefore, classified into 30 groups (Hilliard 1983). *Helichrysum* species have been reported to have antimicrobial activities (Rios *et al.* 1988; Tomas-Barberan *et al.* 1988, Tomas-Barberan *et al.* 1990; Tomas-Lorente *et al.* 1989; Cosar & Cubukcu 1990; Meyer & Afolayan 1995; Meyer & Dilika 1996). Not much information on the bioactivity of compounds isolated from these species is available. *In vitro* antimicrobial screening methods provide the required preliminary observations to select among crude plant extracts those with potentially useful properties for further chemical and pharmacological investigations. In this study we investigated the antibacterial activities of crude acetone extracts (shaken and homogenized) of six *Helichrysum* species against ten bacteria species.

Materials and Methods

Plant material

Plants were collected from the Drakensberg Mount-aux-Sources area in QwaQwa (Witsieshoek, South Africa). Their identity was verified by a taxonomist, Prof. R.O. Moffett, and voucher specimens were deposited in the herbarium of the Department of Botany, University of the North, QwaQwa Branch, South Africa.

H. callicomum Harv., (herbarium voucher specimen M5054) Group 2 (Hilliard 1983), is a tufted perennial herb with short woody stems and greyish-white clustered leaves, often forming large patches in overgrazed grasslands. Basothos in the QwaQwa district use leaf infusions for colds, body pains and as ingredients in an enema for colic (Watt & Breyer-Brandwijk 1962).

H. odoratissimum (L.) Sweet., (M5061) Group 4 (Hilliard 1983), is a much branched aromatic perennial herb with greyish-white leaves, forming large patches on grassy or rocky slopes. It is used to relieve abdominal pains, heartburn, coughs, colds, and treats female sterility, eczema and wounds (Van Puyvelde *et al.* 1989).

H. glomeratum Klatt, (M5055) Group 6 (Hilliard 1983), is a rhizomatous perennial herb with densely rosetted leaves, found in large colonies in open grassland. Leaf infusions are administered to children with stomach ache and burnt parts used as an ingredient in a colic remedy by the Basothos (Watt & Breyer-Brandwijk 1962).

H. rugulosum Less., (M5060) Group 9 (Hilliard 1983), is a perennial herb with a creeping rootstock and a tufted flowering stem. Plants grow in poor sand or stony grasslands, invading over-grazed and road-side areas. A decoction of the root is administered to children suffering from diarrhoea and to fumigate huts. It is similarly used as an ingredient in an enema for colic (Watt & Breyer-Brandwijk 1962).

H. hypoleucum Harv., (M5056) Group 16 (Hilliard 1983), is a soft-wooded shrub with white, hairy leaves, growing in tangled clumps on forest margins on steep grassy mountain slopes. It is used by Basothos as a tea, emetic and for chest problems. It is also used as a poultice for swelling and as an antibiotic (Hutchings & Van Staden 1994).

H. pilosellum (L.f.) Less., (M5059) Group 23 (Hilliard 1983) is a perennial herb with silky hairy leaves and is widespread in the grasslands. Leaf infusions are administered for stomach ache, coughs and colds and is also used as an ingredient in colic remedies by the Basothos (Hutchings & Van Staden 1994).

Preparation of extracts

Shoots (excluding flowers) of the plants were air dried at room temperature. Each plant (80 g) was shaken for five minutes in acetone and filtered through Whatman No 2 filter paper under suction. This filtrate will be referred to as the 'shaken extract'. The residue was then homogenized in acetone, also filtered through Whatman No 2 filter paper and both extracts concentrated to dryness under reduced pressure at 40°C, with a rotary evaporator. After determining the yields, the extracts were stored at 4°C until further use.

Bacteria

Ten bacterial species (Table 1) were obtained from the Department of Microbiology and Plant Pathology, University of Pretoria. Each organism was maintained on nutrient agar (Biolab) and an inoculum was recovered for testing by growth in nutrient broth No 2 (Biolab) for 24 hours. Before streaking, each culture was diluted 1:10 with fresh sterile nutrient broth.

Preliminary screening for antibacterial activities

The plant extracts (sterilized by filtering through a 0.22 µm filter) were added to 5 ml of nutrient agar medium in Petri dishes and swirled carefully before congealing. An aliquot of each extract was serially diluted (ten fold) to obtain a concentration range of 1.0 to 0.01 mg/ml in 2% acetone. The organisms were then streaked in radial patterns on agar plates (Mitscher *et al.* 1972). Plates were

Table 1 Antibacterial activity (MIC) of *Helichrysum* extracts

		MIC (mg/ml)									
	Yield	Gram-positive bacteria[b]					Gram-negative bacteria[b]				
Extracts[a]	% w/w[c]	*B.cer*	*B. pum*	*B. sub*	*M. kri*	*S. aur*	*E. clo*	*E. col*	*K. pne*	*P.aer*	*S. mar*
H. cal S[d]	3.5	1.0	1.0	1.0	1.0	1.0	1.0	na[f]	na	na	na
H[e]	1.9	1.0	1.0	1.0	na	na	na	na	na	na	na
H. glo S	4.0	na	na	na	na	na	na	na	na	na	na
H	1.5	na	na	na	na	na	na	na	na	na	na
H. hyp S	2.3	0.10	1.0	1.0	1.0	1.0	1.0	1.0	na	1.0	na
H	1.2	0.10	0.10	0.10	1.0	na	1.0	na	na	1.0	na
H. odo S	6.0	0.01	0.01	0.01	0.01	0.01	0.01	na	na	na	na
H	4.0	1.0	1.0	1.0	1.0	na	na	na	na	na	na
H. pil S	3.0	na	na	na	na	na	na	na	na	na	na
H	1.8	na	na	na	na	na	na	na	na	na	na
H. rug S	4.2	0.10	0.10	0.10	0.10	1.0	0.10	na	na	na	na
H	1.2	1.0	1.0	na	na	na	1.0	na	na	na	na

[a] *H. cal (Helichrysum callicomum), H. glo (H. glomeratum), H. hyp (H. hypoleucum), H. odo (H. odoratissimum), H. pil (H. pilosellum* and *H. rug (H. rugulosum)*

b *B. cer (Bacillus cereus), B. pum (B. pumilus), B. sub (B. subtilis), M. kri (Micrococcus krtistinae, S. aur (Staphylococcus aureus), E. clo (Enterobacter cloacae), E. col (Escherichia coli), K. pne (Klebsiella pneumoniae), P.aer (Pseudomonas aeruginosa)* and *S. mar (Serratia marcescens)*

[c] % w/w (% residue mass/fresh mass)

[d] S (shaken extract)

[e] H (homogenised extract)

[f] na (not active)

incubated at 37°C and examined after 24 and 48 hours. Complete inhibition of growth was required for an extract to be declared bioactive. A blank plate containing only nutrient agar and a second containing nutrient agar and 2% acetone served as controls (Meyer & Afolayan 1995). Each treatment was performed in triplicate.

Results and Discussion

Four of the plant species tested exhibited some degree of antibacterial action (Table 1). Their extracts were active against all the Gram-positive bacteria tested. *H. glomeratum* and *H. pilosellum* had no activity against any of the organisms.

The epicuticular extract of *H. callicomum* had no effect on *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens* (all Gram-negative bacteria), but was active against *Bacillus cereus, B. pumilus, B. subtilis, Micrococcus kristinae* and *Staphylococcus aureus* (all Gram-positive) as well as *Enterobacter cloacae* (Gram-negative). The homogenized extract was active against *B. cereus, B. pumilus* and *B. subtilis*. The MIC of all the extracts of this species was 1.0 mg/ml.

The epicuticular extract of *H. hypoleucum* showed strong activity against all Gram-positive bacteria tested and in addition inhibited the growth of the Gram-negative bacteria *E. cloacae, E. coli* and *P. aeruginosa*. The shaken extract had an MIC of 1.0 mg/ml whereas the homogenized extract was active at 0.10 mg/ml against *B. cereus, B. pumilus* and *B. subtilis*; it was in addition active against *M. kristinae, E. coli* and *P. aeruginosa* at 1.0 mg/ml.

Both extracts of *H. odoratissimum* did not inhibit the growth of *E. coli, K. pneumonia, P. aeruginosa* and *S. marcescens* all Gram-negative bacteria, but had activity against all Gram-positive bacteria at the low MIC of 0.01 mg/ml, the highest dilution used in this study. The homogenized extract inhibited the growth of four of the five Gram-positive bacteria at a MIC of 1.0 mg/ml.

None of the extracts of *H. rugulosum* showed activity against *E. coli, K. pneumoniae, P. aeruginosa* and *S. marcescens*, but the shaken extract was active against *E. cloacae* at a MIC of 0.1 mg/ml and the homogenized extract at 1.0 mg/ml. The shaken extract was active against all Gram-positive bacteria at a MIC of 0.10 mg/ml, except for *S. aureus* which had an 1.0 mg/ml MIC. The homogenized extract was only active against *B. aureus, B. pumilus* and *E. cloacae*.

Infections caused by *P. aeruginosa* are amongst the most difficult to treat with conventional antibiotics (Levinson & Jawetz 1992). The growth of *P. aeruginosa* was inhibited by extracts of *H. hypoleucum* at a MIC of 1.0 mg/ml and this plant may, thus, be a source which could yield drugs that could improve the treatment of infections caused by this organism.

The activity of *H. callicomum, H. hypoleucum, H. odoratissimum* and *H. rugulosum* against *S. aureus*, another human pathogen, qualifies these plants for further investigation of their bioactive compounds.

Most *Bacillus* species are regarded as having little pathogenic potential, however, both *B. cereus* and *B. subtilis* have been known to act as a primary invader or secondary infectious agents in a number of diseases and have been implicated in some cases of food poisoning (Turnbull & Kramer 1991). Four of the *Helichrysum* species investigated in this study inhibited the growth of these bacilli.

In view of the fact that Gram-negative organisms are in general terms more resistant than Gram-positive ones to anti-

microbial agents from plants (Stickler & King 1992), it is significant that *E: cloacae, E. coli* and *P. aeruginosa* were inhibited at concentrations of 1.0 mg/ml or lower by some of the plant species investigated. These are much lower MIC's than those found by authors investigating other plant species (Rios *et al.* 1990; Meyer & Dilika 1996; Navarro *et al.* 1996; Salie *et al.* 1996).

Research is underway to isolate and identify the active compounds from some of the species that showed high inhibitory activity.

Acknowledgements

Financial support for this research was provided by the Foundation for Research Development.

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