CHAPTER 2

ANTIBACTERIAL ACTIVITY OF *HELICHRYSUM* SPECIES

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2.1 Introduction

South Africa has a rich flora of *Helichrysum* (everlastings / sewejaartjies) species that are widely distributed throughout the country. They produce many unusual secondary products, which are biologically active. *Helichrysum* species have been used in folk medicine for thousands of years in areas as far apart as Europe, Egypt, North America, China, Australia and South Africa (Balick, 1990; Cosar *et al.*, 1990; Farnsworth, 1988; Farnsworth, 1990; Jakupovic, 1989), and are included in many pharmacopoeias. South African species are generally used for infectious diseases and antibiotic activity has been demonstrated for a number of species (Hutchings and Van Staden, 1994; Watt and Breyer-Brandwijk, 1962). Extracts of *H. armenium, H. gaveolens and H. plicatum* have been reported to be active against *Staphylococcus albus* and *S. aureus* as well as a number of other Gram-positive and Gram-negative bacteria (Cosar and Cubukcu 1990; Mathekga and Meyer, 1998). Tomas-Barberan *et al.,* (1988, 1990) and Tomas-Lorente *et al.,* (1989) have respectively reported antibacterial activity of *H. decumbens* and *H. nitens*.

The antibacterial activity of 28 species reported to be used for various diseases in traditional Sotho, Xhosa and Zulu medicine are investigated in this study. Most of the plant preparations are snuffed or taken as decoctions.

The genus *Helichrysum* belongs to the tribe Inuleae in the Asteraceae family and is known for its aromatic and therapeutic properties. It is a large family of about 500 species worldwide, with 245 species indigenous to South Africa (Hilliard, 1983). The South African *Helichrysum* species display great morphological diversity and are, therefore, classified into 30 groups (Hilliard, 1983), occurring in or along forest margins of woodlands, while some species occur in drier regions or rocky outcrops or even in open grassland. There is a high concentration (83 species) in the Free State east of the Drakensberg escarpment in the former Basotho QwaQwa homeland, where they are

subjected to a high altitude, moist and warm climate, and conditions conducive for the proliferation of most microorganisms. *Helichrysum* species are annual or perennial herbs, with hairy leaves, and stems. Bright and variously coloured flowers, with a stereome (the thickened region in the lower part of an involucral bract), a structure which has been found to be of considerable value in the classification of the genera (Hilliard, 1983).

Some members of genus *Helichrysum* have been well characterized with respect to their secondary metabolites, largely dominated by alkaloids, flavonoids, phloroglucinols and tannins (Dekker *et al.*, 1983; Jakupovic *et al.*, 1989) with antibacterial properties.

In Table 2.1, the medicinal uses of some species are listed, as obtained from a review of the available literature and interviews with local traditional healers.

Not much information on the antibacterial activity of compounds isolated from these species is available. With the knowledge that antibacterial phloroglucinols and flavonoids have been found in *Helichrysum* species, this study was undertaken to screen as many indigenous species as available for antibacterial activity present in crude shoot extracts. In the present study 28 species were screened by the agar dilution bioassay method (Mitscher *et al.*, 1972) to determine the minimum inhibition concentration (MIC).

In vitro antibacterial screening provide the required preliminary observation to select among crude plant extracts those with potentially useful properties for further chemical and pharmaceutical investigation. In this investigation we studied the antibacterial activity of crude acetone extracts (epicuticular and homogenized) against five Gram-positive and five Gram-negative bacteria

Table 2.1. Medicinal uses of some *Helichrysum* species

Helichrysum species	Traditional use
H. caespititium (DC.) Harv.	Drunk by Bakwena and Bakgatla in the treatment of gonorrhoea. Basotho in addition to inhaling the smoke from burning the plant for the relief of head and chest colds, use the slightly warmed plant as wound dressing in male circumcision rites, and for 'internal wounds' (? intestinal ulceration) (Watt and Breyer-Brandwijk, 1962)
H. calocephalum Klatt.	Given to children suffering from diarrhoea (Hutchings, 1996)
H. imbricatum Less.	Taken as a tea, and an infusion as a demulcent in coughs and in pulmonary affections (Pappe, 1857)
H. kraussii Sch. Bip.	Smoked in a pipe for the relief of cough and as a remedy for pulmonary tuberculosis (Watt and Breyer-Brandwijk, 1962)
H. lepidissimum S. Moore	Basotho commonly use it as a body perfume in the form of a powder or an ointment (Hutchings, 1996)
H. nudifolium Less.	Basotho and Xhosa use the leaf as a remedy for cough and colds. Also as demulcent and an infusion used in catarrh, phthisis and other pulmonary affections, and in wound dressing (Pappe, 1857)

Helichrysum species	Traditional use
H. crispum Less.	Used in the Western Cape for heart trouble, backache, kidney disease, coronary thrombosis and hypertension (Petrie, 1913)
H. pedunculare DC.	Applied by Xhosa and Fengu as a dressing after circumcusion.The root is also used for coughs and colds (Hutchings, 1996)
H. psilolepis Harv.	Basotho use it as remedy for painful menstruation (Watt and Breyer-Brandwijk, 1962)
H. setosum Harv.	Basotho use it to fumigate huts. Zulu use a decoction of the leaf to swab the skin in acute dermatoses. The ash is dissolved in beer and taken as a cure for epilepsy (Hume, 1954, Hutchings, 1996).

2.2 Material and Methods

Plants were collected form the Drakensberg, Mont-aux-sources area in QwaQwa (Free State, South Africa). A taxonomist, Prof. R.O. Moffett, verified their identity and voucher specimens were deposited in the herbarium of the Department of Botany, University of the North, QwaQwa Branch, South Africa and the National Botanical Institute herbarium, Pretoria.

2.2.1 Extract Preparation

Shoots (excluding flowers) of the plants were air dried at room temperature. Each plant (80g) was shaken for five minutes in acetone and filtered through Whatman No 2 filter paper under suction to obtain an epicuticular extract. The residue was then homogenized

in acetone, and also filtered through Whatman No 2 filter paper under suction. Both extracts were concentrated to dryness under reduced pressure at 45^0C with a rotary evaporator. After determining the yields, extracts were stored at 4^0C until further use.

2.2.2 Bacterial strains

Ten bacteria species (Table 2.2) 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.

2.2.3 Antibacterial bioassay

The plant extracts (sterilized by filtering through a 0.22 mm 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 acetone. The organisms were then streaked in radial patterns on agar plates (Mitscher *et al.*, 1972). Plates were incubated at 37^0C and examined after 24 and 48 hours. Complete inhibition of growth was required for an extract to be declared bioactive. A blank containing only nutrient agar and a second containing nutrient agar and 2% acetone served as controls (Meyer and Afolayan, 1995).

2.3 Results

Twenty-three of the plant species tested exhibited significant antibacterial activity while, 17 out of the 28 plant extracts screened for antibacterial activity had a significant inhibitory effect on all Gram-positive bacteria tested (Table 2.2). Gram-negative bacteria were resistant to most extracts tested. Of the Gram-negative bacteria tested, five extracts significantly inhibited the growth of three bacteria, one extract inhibited two bacteria and six extracts inhibited only one bacterial strain. None of the plant extracts inhibited the growth of *Klebsiella pneumoniae* and *Serratia marscecens* at this range of testing. The epicuticular (shaken) extracts of *H. chionosphaerum*, *H. longifolium* and *H. chionosphaerum* showed no activity against *Bacillus cereus* and *Micrococcus kristinae*, respectively.

The epicuticular extracts of *H. candolleanum, H. herbaceum, H. melanacme, H. psilolepis, H. rugulosum, H. simillimum* and *H. umbraculigerum* and the homogenized extracts of *H. decorum* and *H. melanacme* significantly inhibited growth of the organisms at the low MIC of 0.10 mg/ml level. The shaken and homogenized extracts of *H. odoratissimum* did not inhibit the growth of *Escherichia coli., K. pneumoniae, P. aeruginosa* and *S. marscence,* all Gram-negative bacteria, but had a noticeably higher level of activity against the other bacteria, than most extracts tested, at the low MIC of 0.01 mg/ml, the highest dilution used in this study. The homogenized extracts inhibited the growth of four of the five Gram-positive bacteria at a MIC of 1.0 mg/ml. Epicuticular (shaken) extracts proved to be more bioactive when compared to the macerated (homogenized) extracts.

Table 2.2 Antibacterial activity (MIC) of the crude extracts of the aerial parts of *Helichrysum* species

Helichrysum Species (Voucher No.)		MIC (mg/ml)[a]									
		Gram-positive[b]					Gram-negative[c]				
Bacterial Species		*B. cer*	*B. pum*	*B. sub*	*M. kri*	*S. aur*	*E. clo*	*E. col*	*K. pne*	*P. ear*	*S. mar*
H. appendiculatum	S[d]	1.0	1.0	1.0	1.0	1.0	na[f]	na	na	na	na
(M5135)	H[e]	1.0	1.0	1.0	1.0	1.0	na	na	na	na	na
H. argyrosphaerum	S	na	1.0	0.01	1.0	1.0	na	na	na	1.0	na
(M5080)	H	na	1.0	0.01	1.0	na	na	na	na	1.0	na
H. aureonitens	S	1.0	1.0	1.0	1.0	1.0	na	1.0	na	na	na
(M5096)	H	1.0	1.0	1.0	1.0	1.0	na	1.0	na	na	na
H. bellum	S	1.0	1.0	1.0	1.0	1.0	1.0	na	na	1.0	na
(M5178)	H	1.0	1.0	1.0	1.0	1.0	1.0	na	na	1.0	na
H. caespititium	S	1.0	1.0	1.0	1.0	1.0	1.0	1.0	na	1.0	na
(M0011)	H	1.0	1.0	1.0	1.0	1.0	1.0	1.0	na	1.0	na

Helichrysum Species (Voucher No.)		MIC (mg/ml)[a]									
		Gram-positive[b]					Gram-negative[c]				
H. callicomum (M5054)	S	1.0	1.0	1.0	1.0	1.0	1.0	na	na	na	na
	H	1.0	1.0	1.0	na	na	na	na	na	na	na
H. candolleanum (M3078)	S	0.10	0.10	0.10	0.10	na	na	na	na	0.10	na
	H	na	1.0	1.0	1.0	na	na	na	na	na	na
H.chionosphaerum (M5111)	S	na	1.0	1.0	na	1.0	na	na	na	na	na
	H	na	na	na	na	na	na	na	na	na	na
H. decorum (A0006)	S	1.0	0.10	0.01	0.10	0.10	na	na	na	na	na
	H	0.10	0.10	0.10	na	na	na	na	na	na	na
H. glomeratum (M5055)	S	na	na	na	na	na	na	na	na	na	na
	H	na	na	na	na	na	na	na	na	na	na
H. herbaceum (M5272)	S	1.0	1.0	1.0	1.0	1.0	1.0	1.0	na	1.0	na
	H	1.0	1.0	1.0	1.0	1.0	1.0	1.0	na	1.0	na
H. hypoleucum (M5056)	S	1.0	1.0	1.0	1.0	1.0	1.0	1.0	na	1.0	na
	H	1.0	0.10	0.10	1.0	na	1.0	na	na	1.0	na
H. kraussii (M5173)	S	1.0	1.0	1.0	1.0	1.0	na	na	na	1.0	na
	H	na	1.0	1.0	1.0	na	na	na	na	1.0	na
H. longifolium (M5109)	S	na	1.0	1.0	1.0	na	na	na	na	1.0	na
	H	na	1.0	1.0	1.0	na	na	na	na	na	na
H. melanacme (M5110)	S	0.10	0.10	0.10	0.10	0.10	0.10	na	na	na	na
	H	0.10	0.10	0.10	0.10	0.10	0.10	na	na	na	na
H. microniifolium (5100)	S	1.0	1.0	1.0	1.0	1.0	na	na	na	na	na
	H	na	na	na	na	na	na	na	na	na	na
H. montanum (M3707)	S	na	na	na	na	na	na	na	na	na	na
	H	na	na	na	na	na	na	na	na	na	na
H. monticola (M5177)	S	na	na	na	na	na	na	na	na	na	na
	H	na	na	na	na	na	na	na	na	na	na
H. nudifolium (M3708)	S	1.0	1.0	1.0	na	1.0	1.0	1.0	na	1.0	na
	H	na	na	1.0	1.0	1.0	1.0	1.0	na	na	na
H. odoratissimum (M5061)	S	0.01	0.01	0.01	0.01	0.01	0.01	na	na	na	na
	H	1.0	1.0	1.0	1.0	na	na	na	na	na	na

Helichrysum Species (Voucher No.)		MIC (mg/ml)[a]									
		Gram-positive[b]					Gram-negative[c]				
H. oreophilum (M5097)	S	na	na	na	na	na	na	na	na	na	na
	H	na	na	na	na	na	na	na	na	na	na
H. pilosellum (M5059)	S	na	na	na	na	na	na	na	na	na	na
	H	na	na	na	na	na	na	na	na	na	na
H. psilolepis (M5081)	S	0.10	0.10	0.10	0.10	0.10	na	na	na	na	na
	H	1.0	1.0	1.0	1.0	1.0	na	na	na	na	na
H. rugulosum (M5060)	S	0.10	0.10	0.10	0.10	1.0	0.10	na	na	na	na
	H	1.0	1.0	na	na	na	1.0	na	na	na	na
H. simillimum (M0001)	S	0.10	0.10	0.10	0.10	0.10	na	na	na	0.10	na
	H	1.0	1.0	1.0	1.0	1.0	na	na	na	na	na
H. sutherlandii (M5179)	S	1.0	1.0	1.0	1.0	1.0	na	na	na	na	na
	H	na	na	na	na	na	na	na	na	na	na
H. trilineatum (M5172)	S	1.0	1.0	1.0	1.0	1.0	1.0	1.0	na	1.0	na
	H	1.0	1.0	1.0	1.0	1.0	1.0	1.0	na	1.0	na
H.umbraculigerum (M5174)	S	0.10	0.10	0.10	0.10	0.10	na	na	na	na	na
	H	1.0	1.0	1.0	1.0	1.0	na	na	na	na	na

[a] Minimum inhibition concentration

[b] *B. cer* (*Bacillus cereus*), *B. pum* (*Bacillus pumilus*), *B. sub* (*Baccilus subtilis*), *M. kri* (*Micrococus kristinae*), *S. aur* (*Staphylococcus aureus*)

[c] *E. clo* (*Enterobacter cloacae*), *E. col* (*Escherichia coli*), *K. pne* (*Klebsiella pneumoniae*), *P. aer* (*Pseudomonas aeruginosa*) and *S. mar* (*Serratia marcescens*)

[d] Shaken extract

[e] Homogenized extract

[f] Not active

herbaceum, H. melanacme, H. psilolepis, H. rugulosum, H. simillimum and *H. umbraculigerum* and the homogenized extracts of *H. decorum* and *H. melanacme* significantly inhibited growth of the organisms at the low MIC of 0.10 mg/ml level. The shaken and homogenized extracts of *H. odoratissimum* did not inhibit the growth of *Escherichia coli., K. Pneumoniae, P. aeruginosa* and *S. marscence,* all Gram-negative bacteria, but had a noticeably higher level of activity against the other bacteria, than most extracts tested, at the low MIC of 0.01 mg/ml, the highest dilution used in this study.

Discussion

Twenty-three (82%) of the *Helichrysum* species showed inhibition against the Gram-positive bacteria tested. The negative results obtained against Gram-negative bacteria were not unexpected as, in general, this class of bacteria are more resistant than the Gram-positive bacteria (Tomas-Barberan *et al.,* 1983). A novel phloroglucinol, isolated from the aerial parts of *H. caespititium* (Dekker *et al.,* 1983), showed significant inhibition against Gram-positive bacteria, but also had no observable effect against the Gram-negative bacteria.

Extracts are generally richest in antibacterial agents after the flowering (sexual) stage of their growth is complete, and plants taken from stressful environments were particularly active (Mitscher *et al.*, 1972). Antibacterial extracts from tested species can be assumed to be useful to the producing plant in warding off infectious diseases. The infecting microorganisms are usually the same as those infecting higher animals (Turnbull and Kramer, 1991), and there is therefore compelling reason to suppose that antiifective agents could be active against human or veterinary pathogens. It is comforting, to find that the spectrum of activity of these plant extracts is broad enough to include human pathogens, as was suggested by folkloric and historical accounts. A number of examples are included in Table 2.1 in which one sees a number of applications that could be interpreted as related to infectious disease.

These results are consistent with previous reports (Tomas-Barberan *et al.,* 1990, Dekker *et al.,* 1983) on related species against Gram-negative bacteria. Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria (Burn, 1988). Access of most

compounds to the peptidoglycan layer of the cell wall is hindered by the outer lipopolysaccharide layer. This explains the resistance of Gram-negative strains to the lytic action of most extracts exhibiting activity.

Infections caused by *P. aeruginosa* are among the most difficult to treat with conventional antibiotics (Levison and Jawetz, 1992). The growth of *P. aeruginosa* was inhibited at 0.1 mg/ml by three crude extracts. These plants may, thus, be a source which could yield drugs that could improve the treatment of infections caused by this organism.

The activity of most extracts against *S. aureus*, another human pathogen, qualify these plants for further investigation of their bioactive compounds. Strains of *E. coli* have been identified which are capable of colonizing the gastrointestinal tract and producing potent enterotoxins (Kwon-Chung and Bennett, 1992). The pathogenesis of the resulting illness resembles that of cholera. Outbreaks of *E. coli* are characterized by prolonged illness, high mortality and morbidity and by the ease and rapidity with which infection spreads (Turnbull and Kramer, 1991).

Bacillus species are common microbes found in most natural environments including soil, water, plant and animal tissues. While most *Bacillus* species are regarded as having little pathogenic potential, both *B. cereus* and *B. subtilis* have been known to act as primary invaders or secondary infectious agents in a number of diseases and have been implicated in some cases of food poisoning (Turnbull and Kramer, 1991). Some species of *Helichrysum* in the food and medicine of the indigenous people of South Africa may have helped to combat these microbes.

Different *Helichrysum* species produce different secondary metabolites (acetophenones, chalcones, flavonoids, phloroglucinols, tannins, etc) as a biochemical defence mechanism (Tomas-Barberan *et al.*, 1990). This indicates the use of different metabolic pathways to produce chemical barriers, which has a single ecological defence against bacteria and other pathogens. The antibacterial compounds harvested from these species may inhibit bacteria by a different mechanism than the presently used antibiotics and may have clinical value in the treatment of resistant microbial strains.

2.5 Conclusion

On the basis of the results obtained, we conclude that the crude extracts of these *Helichrysum* species exhibit significant antibacterial activity and properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents. This probably explains the use of extracts from these species by the indigenous people of South Africa against a number of infections for generations. Consequently, we propose a detailed study of these plants in order to determine their pharmacological effects, active compounds as well as their mechanism of action.

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