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Antimicrobial activity of *Eriocephalus* L. species

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The genus *Eriocephalus,* commonly known as 'wild rosemary', 'Cape snow bush', 'kapokbos' or 'asmabossie', belongs to the family Asteraceae, of the tribe Anthemideae. It is endemic to southern Africa and is comprised of 32 species, of which several are economically important as traditional herbal remedies and as perfumes in fragrance industries. The species may be an important potential source for new and novel drugs for the treatment of various diseases, hence warrants further research. An investigation into the antimicrobial activity of the genus *Eriocephalus* using the disc diffusion assay against a range of Gram-positive and Gram-negative bacteria as well as a few selected fungi was carried out. The study included 15 *Eriocephalus* species with 113 essential oil and acetone leaf extract samples. Preliminary screening was carried out using 16 test pathogens: *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus* (four strains), *S. epidermidis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Salmonella typhimurium*, *S. enteriditis*, *Proteus vulgaris*, *Serratia odorifera*, *Enterococcus faecalis*, *Cryptococcus neoformans*, *Candida albicans* and *Alternaria alternata.* From the preliminary screening, the most susceptible test pathogens selected for further study were: *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus* (one strain), *Klebsiella pneumoniae*, *Escherichia coli*, *Cryptococcus neoformans* and *Candida albicans*. The Gram-positive bacteria and two fungal pathogens showed inhibition for most of the essential oils and the leaf extracts while there was very little activity noted on the Gram-negative bacteria. Intra- and inter-population variation as well as inter-specific variation was observed in the antimicrobial activity for some species of *Eriocephalus*. The major variation was mainly observed in the activity of the essential oils and the leaf extracts against the yeast, *Cryptococcus neoformans* and the Gram-positive bacteria, *Bacillus cereus, B. subtilis* and *Staphylococcus aureus*. From the results obtained from the disc diffusion assay, the most active species were selected to determine the minimum inhibitory concentration against two Gram-positive and two Gram-negative bacteria and two fungal strains. The acetone extracts of *E. aromaticus* from Swartberg produced the most promising activity for all species studied with MIC values of 400µg ml^{-1} and 200µg ml^{-1} for *B. cereus* and *S. aureus* respectively.

Abbreviations: AE = acetone extract, ATCC = American Type Culture Collection, *Bc* = *Bacillus cereus*, *Bs* = *Bacillus subtilis*, *Ca* = *Candida albicans*, CFU = colony forming units, *Cn* = *Cryptococcus neoformans*, *Ec* = *Escherichia coli*, EO = essential oil, *Kp* = *Klebsiella pneumoniae*, MIC = minimum inhibitory concentration, NCTC = National Collection of Type Cultures, R = resistant, *Sa* = *Staphylococcus aureus*

Introduction

Traditional herbal medicine plays a vital role in the provision of primary health care, especially for the rural folk. Herbal remedies are widely used in South Africa and it is estimated that 70–80% of the people use plants for therapeutic purposes (Dyson 1998). The cost of manufactured drugs has continued to escalate, thus becoming unaffordable for many citizens. It is therefore important to investigate the plants used traditionally for potential novel antimicrobial compounds and confer credibility or establish the 'rational usage' upon what healers have known and used for centuries in traditional therapy, as noted by Hammer *et al*. (1999) and Swanepoel (1997). Infectious and inflammatory diseases are among those treated using herbal remedies Shale *et al*. (1999) and many people are reverting back to the traditional use of plants for treatment of such and other ailments (Dorman and Deans 2000).

The genus *Eriocephalus,* commonly known as 'wild rosemary', 'Cape snow bush', 'kapokbos' or 'asmabossie' belongs to the family Asteraceae, of the tribe Anthemideae, (Adamson and Salter 1950) and is characterised by aromatic and highly dissected leaves. It is comprised of 32 species endemic to southern Africa (Müller *et al.* 2001). The Griqua and Nama used some of the members of the genus as a diuretic and diaphoretic, a tincture for heart troubles, a colic remedy and for treatment of oedema and stomach ache. The species used include *E. africanus*, *E. ericoides*, *E.*

racemosus and *E. punctulatus.* Leaf infusions of *E. africanus*, decoctions and tinctures are used to treat coughs, flatulence and delayed menstruation, as well as for swelling and pain arising from gynaecological conditions. The plants are also popular ingredients for footbaths and as a hair rinse to treat dandruff and itchy scalps. They are also used to treat inflammation of the skin and for chest complaints, hence the name 'asmabossie' or 'asthma bush'. An infusion of *E. africanus* and *Rosmarinus officinalis* is used for bathing to invigorate the skin and hair, as recorded in Watt and Breyer-Brandwijk (1962), Salie *et al*. (1996), Van Wyk *et al.* (1997), Dyson (1998) and Van Wyk and Gericke (2000). *E. punctulatus* is used by the southern Sotho with *Metalasia muricata* to fumigate the hut of a person suffering from a cold or after the death of a person.

The chemistry of most *Eriocephalus* species is poorly studied, with the exception of *E. punctulatus* (Mierendorff *et al*. 2003), *E. africanus* and a few other species endemic to Namibia (Zdero *et al.* 1987). Some of the major compounds reported to occur in the species include various terpenoid aliphatic esters, camphor, linalyl acetate, nerolidol, spathulenol and several sesquiterpene lactones. Since the focus of this study was on the antimicrobial activity, the chemistry of the species will be addressed elsewhere (Njenga *et al.* in prep.).

As some of the conditions mentioned above may be microbe-related, this study is aimed at investigating the potential antimicrobial properties of the species of *Eriocephalus* and to verify the rationale for their use in traditional herbal remedies by *in vitro* screening.

Materials and Methods

Preparation of plant material

The 15 species tested in this study and their localities are given in Table 1. The voucher specimens are deposited in the Department of Pharmacy and Pharmacology at the University of the Witwatersrand. The aerial plant parts were collected from natural populations during their growing periods and the fresh material hydrodistilled in a Clevenger apparatus for three to four hours to obtain the essential oils. It should be noted at this juncture that the essential oils yields for several of the species of *Eriocephalus* were relatively low, hence it is only those species which yielded sufficient oil that were considered for the MIC assay. Due to variability aspects, essential oils were distilled from a single plant, thus explaining low yields of the oils. Dried plant material was crushed, weighed (0.5–3.0g) and 30ml of acetone added. The mixture was extracted for four hours in a water bath at 30°C, then filtered and evaporated. The residue was re-suspended in acetone to a concentration of 50mg ml^{-1}.

Disc diffusion assay

Preliminary antimicrobial screening was carried out using 16 test pathogens, and seven of these were selected for further study, based on susceptibility patterns. The selected test pathogens are given in Table 1.

The disc diffusion assay was used to determine the growth inhibition of the bacteria and selected fungi. Tryptone Soya agar was prepared by dissolving 30g of the agar in 750ml of water and autoclaved for 15min at 121°C and cooled to 55°C in a water bath. A base layer of 100ml of agar was poured into the plate and inoculated with a top layer of 100ml of agar containing an inoculum of approximately 1 x 10^6CFU ml^{-1}. Sterilised paper discs (6mm) were saturated with approximately 8µl of either essential oils or the acetone leaf extracts (50mg ml^{-1}) and loaded onto the agar plates. The plates were kept at 4°C for one hour to pre-diffuse the oil and extracts into the agar and then incubated for 24h at 37°C for bacterial isolates. The yeast and mould were incubated for 48h and seven days respectively. Neomycin (30µg) was used as a positive control for the bacterial strains and Nystatin (100IU) as a control for the fungal strains. Activity was measured as growth inhibition zones in millimetres from the edge of the disc (Table 1). Repetitions were made to confirm results.

Minimum inhibitory concentration

Based on the results obtained from the disc diffusion assays, two Gram-positive bacteria (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923), two Gram-negative bacteria (*Klebsiella pneumoniae* NCTC 9633, *Escherichia coli* ATCC 8739) and the yeasts (*Cryptococcus neoformans* ATCC 90112, *Candida albicans* ATCC 10231) were selected for further study. The plant specimens were selected on the basis of activity resulting from the disc diffusion assays and availability of samples, especially the essential oils, most of whose quantities were not sufficient for minimum inhibitory concentration (MIC) determination.

The test cultures were inoculated in Tryptone Soya broth and incubated for 24h. One millilitre of the inoculum was transferred into 100ml of sterile broth. The starting concentration of essential oils was 32mg ml^{-1} and 12.5mg ml^{-1} for the leaf extracts. The 96-well micro titre plates were aseptically prepared and serial dilutions carried out as outlined by Eloff (1998, 1999).

Results and Discussion

The most susceptible pathogens observed from the broad preliminary screening were selected for further study as shown in Table 1. Among the species studied, the essential oils of *E. purpureus* (Nieuwoudtville), *E. ericoides* subsp. *ericoides*, *E. pauperrimus*, *E. microphyllus* (Sutherland), *E. africanus* (Malmesbury), *E. punctulatus* and *E. racemosus* var. *racemosus* exhibited at least 50% activity against the total number of the pathogens tested, though the activity was relatively low. The leaf extracts, however, showed lower activity, ranging from 20–40% against the total number of the test pathogens with an exception of a few, e.g. *E. aromaticus*, *E. microphyllus* (Nieuwoudtville), *E. punctulatus* (Nieuwoudtville population 1 and 2) and *E. africanus* (Melkbosstrand), that showed at least 50% activity overall.

The test pathogens *Staphylococcus aureus* (ATCC 6538, ATCC 12600, methicillin-resistant *Staphylococcus aureus* (clinical strain), *Staphylococcus epidermidis* (ATCC 2223),

Table 1: Antimicrobial activity of essential oils and acetone leaf extracts of *Eriocephalus.* Activity is measured in millimetres (mm) from the edge of the disc

Taxon	Locality	Extract	Activity in mm from edge of the disc						
			Cn	*Ca*	*Bc*	*Bs*	*Sa*	*Kp*	*Ec*
E. africanus L.	Mossel Bay	EO	1.5	<1	R	1	<1	<1	<1
E. africanus*	**Malmesbury**	**EO**	**5**	**2**	**6**	**2**	**2**	**1**	**R**
E. africanus	Melkbosstrand	EO	1	1	3	1	1	R	R
E. africanus	Citrusdal (A)	EO	2	1	2	<1	<1	1	R
E. africanus	Citrusdal (B)	EO	1	R	5.5	2	1	<1	R
E. africanus	Citrusdal (C)	EO	8	1	4	<1	1.5	R	1
E. aromaticus C.A.Sm	Swartberg	EO	R	2	3.5	3	2	<1	<1
E. aromaticus	Ladismith (B)	EO	R	R	3	1	<1	R	R
E. aromaticus	Ladismith (C)	EO	8	R	4	<1	R	R	R
E. brevifolius (DC) M.A.N. Müller	Vergelegen (A)	EO	<1	<1	1.5	1	<1	<1	R
E. brevifolius	Vergelegen (B)	EO	3	<1	3	<1	<1	R	R
E. brevifolius	Vergelegen (C)	EO	5.5	1	4	1	<1	<1	R
E. brevifolius	**Oudtshoorn**	**EO**	**R**	**2**	**5.3**	**4**	**5**	**2**	**2**
***E. capitellatus* DC**	**Swartberg Pass (A)**	**EO**	**4**	**R**	**3.5**	**<1**	**1**	**<1**	**R**
E. capitellatus DC	Swartberg Pass (B)	EO	2	1	3.5	1	<1	1	<1
E. capitellatus	**Swartberg Pass (C)**	**EO**	**3**	**2**	**2.5**	**2**	**R**	**1**	**<1**
E. decussatus Burch	Sutherland	EO	4	2	2	2	3	<1	<1
E. ericoides subsp. *ericoides* (L.F) Druce	Scheepersrust (A)	EO	3	<1	2.5	2	<1	<1	R
E. ericoides subsp. *ericoides*	Scheepersrust (B)	EO	2	1	3	1	1	R	1
E. ericoides subsp. *ericoides*	Scheepersrust (C)	EO	3	1	2	1	1	<1	R
E. ericoides subsp. *ericoides*	Prince Albert-1	EO	R	1	7.25	3	1	<1	<1
E. ericoides subsp. *ericoides*	Prince Albert (A)-2	EO	4.5	1	4	<1	<1	R	R
E. ericoides subsp. *ericoides*	Prince Albert (B)	EO	5	1	3	1.2	1	R	R
E. ericoides subsp. *ericoides*	Prince Albert (C)	EO	3	2	4	3	3	1	1
E. ericoides* subsp. *ericoides	**Bethulie (A)**	**EO**	**9**	**5**	**3**	**2**	**1**	**1**	**1.2**
E. ericoides* subsp. *ericoides	**Bethulie (B)**	**EO**	**4**	**2**	**2**	**3**	**2**	**1**	**1.5**
E. eximius DC	Sutherland (A)	EO	R	R	3	<1	<1	<1	R
E. eximius	Sutherland (B)	EO	5	R	2	<1	1.5	R	R
E. microphyllus DC	Sutherland (A)	EO	R	3	8	2	3	<1	<1
E. microphyllus	Sutherland (B)	EO	6	3	2	1	2	1	R
E. microphyllus	Sutherland (C)	EO	7	3	3	1.5	1.5	1.5	2
E. microphyllus	Nieuwoudtville (B)	EO	4	2	3.5	3	1.5	<1	<1
E. microphyllus	Nieuwoudtville (C)	EO	6	<1	3	R	1	1	1
E. microphyllus	**Kamiesberg**	**EO**	**5**	**1**	**2**	**1**	**<1**	**2**	**1**
E. microphyllus	Spektakel Pass	EO	6	1	4	<1	<1	1.5	R
E. namaquensis M.A.N. Müller	Clanwilliam (A)	EO	10	1	3.5	3	1.5	<1	<1
E. namaquensis	Clanwilliam (C)	EO	6	2	R	R	1	<1	R
E. pauperrimus	Nieuwoudtville (A)	EO	6	3	4	2	2	R	R
E. pauperrimus	Nieuwoudtville (C)	EO	3	2	2	1.5	1.5	R	R
***E. punctulatus* DC**	**Nieuwoudtville (A)-1**	**EO**	**2**	**<1**	**3**	**1**	**1**	**1**	**1.5**
E. punctulatus	Nieuwoudtville (B)	EO	9	2	3	2	1	1	1
E. punctulatus	**Nieuwoudtville (C)**	**EO**	**3**	**5**	**2.5**	**3**	**1**	**1**	**1**
E. punctulatus	Calvinia	EO	R	2	3	4	1.5	<1	<1
E. punctulatus	Nieuwoudtville-2	EO	5	2	5.5	2	1.5	1	2.5
E. purpureus Burch	Nieuwoudtville	EO	5	2	3.5	4	2	<1	<1
E. purpureus	Kamiesberg	EO	5	R	2	1.5	<1	1	1
***E. racemosus* L.**	**Koeberg**	**EO**	**R**	**1**	**1**	**4**	**3**	**1**	**1**
E. racemosus var. *racemosus* L.	Velddrif (A)	EO	2	1	4	1	1	<1	R
E. racemosus var. *racemosus*	Velddrif (B)	EO	2	R	4	1	<1	<1	R
E. racemosus* var. *racemosus	**Velddrif (C)**	**EO**	**5**	**1**	**3.5**	**1**	**1**	**2**	**R**
E. spinescens Burch	Sutherland (B)	EO	2	R	2	<1	<1	R	R
E. spinescens	Sutherland (C)	EO	4	1	4	<1	2	R	R
E. africanus	De Rust	AE	4	R	R	R	R	<1	<1
E. africanus	Malmesbury	AE	3	R	1	2	R	<1	R
E. africanus	Melkbosstrand	AE	3	1	2	2	<1	<1	R
E. africanus	Citrusdal (A)	AE	2	R	1	<1	R	R	R
E. africanus	Citrusdal (B)	AE	1.5	R	1	2	R	<1	<1
E. africanus	Citrusdal (C)	AE	3	R	2	R	<1	R	R
E. africanus var. *paniculatus* (Cass.) M.M, H & K	Sutherland (A)	AE	4	R	R	1	R	<1	<1
E. africanus var. *paniculatus*	Sutherland (B)	AE	5	R	R	R	R	R	R
E. africanus var. *paniculatus*	Sutherland (C)	AE	2	R	<1	R	R	R	R

Table 1: (cont.)

Taxon	Locality	Extract	Activity in mm from edge of the disc						
			Cn	*Ca*	*Bc*	*Bs*	*Sa*	*Kp*	*Ec*
E. aromaticus	**Swartberg**	**AE**	**R**	**R**	**8**	**4**	**5**	**<1**	**<1**
E. aromaticus	**Ladismith (A)**	**AE**	**1**	**<1**	**4**	**2**	**4**	**R**	**<1**
E. aromaticus	**Ladismith (B)**	**AE**	**5**	**R**	**7.3**	**6**	**6**	**R**	**R**
E. aromaticus	Ladismith (C)	AE	1	<1	R	1	4	R	<1
E. brevifolius	Vergelegen	AE	2	R	3	R	R	R	R
E. brevifolius	Oudtshoorn	AE	3	R	R	4	<1	R	R
E. capitellatus	Swartberg Pass-1	AE	R	R	1	1	R	<1	<1
E. capitellatus	Swartberg Pass-2	AE	<1	R	R	<1	<1	R	R
E. decussatus	Sutherland (A)	AE	5	R	R	1	R	<1	<1
E. decussatus	Sutherland (B)	AE	R	R	1	R	R	R	R
E. decussatus	Sutherland (C)	AE	<1	R	1	<1	R	R	<1
E. ericoides subsp. *ericoides*	Scheepersrust	AE	2	R	3	1.5	1	R	R
E. ericoides subsp. *ericoides*	Prince Albert-1	AE	3	R	1	<1	<1	R	R
E. ericoides subsp. *ericoides*	Prince Albert-2	AE	3	R	2	1.5	1	R	R
E. ericoides subsp. *ericoides*	Bethulie (A)	AE	R	R	<1	1	1	1	R
E. ericoides subsp. *ericoides*	Bethulie (B)	AE	R	R	1	1	2	<1	R
E. eximius	Sutherland (A)	AE	R	R	1	<1	R	R	<1
E. eximius	Sutherland (B)	AE	2	R	R	1	R	R	R
E. eximius	Sutherland (C)	AE	2	R	R	1	R	R	R
E. grandiflorus	Laingsburg (A)	AE	R	R	1	2	R	R	R
E. grandiflorus	Laingsburg (B)	AE	3	R	<1	1	<1	R	R
E. grandiflorus	Laingsburg (C)	AE	3	<1	1	1	1	R	R
E. microphyllus	Sutherland (A)	AE	R	R	1	1	R	<1	<1
E. microphyllus	Sutherland (B)	AE	2	R	1	R	<1	R	R
E. microphyllus	Sutherland (C)	AE	2	R	1	R	<1	R	R
E. microphyllus	Nieuwoudtville (A)	AE	4	R	2	3	1	<1	<1
E. microphyllus	Nieuwoudtville (B)	AE	4	R	1	1	R	<1	<1
E. microphyllus	Nieuwoudtville (C)	AE	1	R	2	2	R	R	R
E. microphyllus	Kamiesberg	AE	2	1	<1	1	1	R	R
E. microphyllus	**Spektakel Pass**	**AE**	**3**	**2**	**3**	**1**	**1.5**	**R**	**<1**
E. namaquensis	Clanwilliam (A)	AE	4	R	2	1	<1	<1	<1
E. namaquensis	Clanwilliam (C)	AE	R	R	R	1	R	R	R
E. pauperrimus	Nieuwoudtville (A)	AE	1	R	R	2	R	<1	R
E. pauperrimus	Nieuwoudtville (B)	AE	4	R	R	R	R	<1	R
E. pauperrimus	**Nieuwoudtville (C)**	**AE**	**4**	**R**	**3**	**3**	**3**	**1**	**R**
E. punctulatus	**Nieuwoudtville (A)-1**	**AE**	**6**	**1**	**3**	**2**	**R**	**<1**	**R**
E. punctulatus	Nieuwoudtville (B)	AE	3	R	3	R	<1	R	R
E. punctulatus	Nieuwoudtville (C)	AE	R	R	1	2	2	<1	R
E. punctulatus	Calvinia	AE	3	R	1	2	<1	R	R
E. punctulatus	Nieuwoudtville (A)-2	AE	3	R	R	R	R	<1	R
E. punctulatus	Nieuwoudtville (B)	AE	R	R	R	R	R	<1	R
E. punctulatus	Nieuwoudtville (C)	AE	R	R	1	R	R	R	<1
E. purpureus	Laingsburg (A)	AE	3	R	1	R	R	<1	R
E. purpureus	Laingsburg (B)	AE	5	R	1	1	<1	<1	<1
E. purpureus	Nieuwoudtville-1	AE	3	R	R	1	R	<1	<1
E. purpureus	Nieuwoudtville-2	AE	7	R	1	2	R	<1	1
E. purpureus	**Kamiesberg**	**AE**	**4**	**2**	**4**	**1**	**1.5**	**R**	**<1**
E. racemosus	Koeberg	AE	2	R	1	1	1	R	R
E. racemosus var. *racemosus*	Velddrif (A)	AE	R	R	1	1	R	<1	<1
E. racemosus var. *racemosus*	Velddrif (B)	AE	6	<1	2	R	1	R	R
E. racemosus var. *racemosus*	Velddrif (C)	AE	R	R	2.5	1.5	1	R	R
E. spinescens	Sutherland (A)	AE	R	R	<1	1	R	<1	R
E. spinescens	Sutherland (B)	AE	2	R	2	1.5	1	R	R
E. spinescens	Sutherland (C)	AE	1	R	R	R	R	R	R
Control			11	7	8	6.5	7	3.5	2

*The samples in bold were selected for further MIC assays (Table 2)

*Pseudomonas aeruginosa (*ATCC 9027), *Yersinia enterocolitica* (ATCC 23715), *Salmonella typhimurium* and *Salmonella enteriditis* (clinical strains), *Proteus vulgaris* (clinical strain), *Serratia odorifera* (ATCC 33132), *Enterococcus faecalis (*ATCC 29212) and *Alternaria alternata* (clinical strain) did not show promising results and were not studied further. Of the four strains of *Staphylococcus aureus*, only the most sensitive was selected for further study.

Table 1 presents a summary of the results of essential oil and leaf extracts which exhibited antimicrobial activity against the seven selected test pathogens. This confirms that some species of the genus have antimicrobial properties and supports their use in traditional herbal remedies. The species show variation in activity within individuals of the same species and between different populations of the same species and between the species. Variation in activity between individuals of the same population was observed in several of the taxa studied. This was observed in the essential oils of *E. punctulatus* from Nieuwoudtville (population 1) and *E. brevifolius* from Vergelegen against *Cryptococcus neoformans* where three individuals (A, B and C) had inhibition of 2mm, 9mm and 3mm and <1mm, 3mm and 5.5mm respectively. This pattern was observed among the taxa where three individuals were tested for activity against the test pathogens (Table 1) and the same phenomenon was observed in the leaf extracts. This variation in the antimicrobial activity of essential oils and leaf extracts should be investigated further in reference to the chemical composition of the oils and the extracts.

Variation in sensitivity patterns against the test pathogens was also evident amongst the essential oils and the extracts of populations of the same species as observed in the populations of *E. africanus*, *E. punctulatus*, *E. aromaticus*, *E. microphyllus*, *E. brevifolius*, *E. ericoides* subsp. *ericoides* and *E. racemosus* var. *racemosus.* This intra-specific variation was also observed within the rest of the species of *Eriocephalus* (Table 1).

Essential oils showed antimicrobial activity against most of the test pathogens with the highest activity noted against the Gram-positive bacteria *Bacillus cereus* (8mm in *E. microphyllus* from Sutherland and *E. ericoides* subsp. *ericoides* from Swartberg), and moderate activity against *Bacillus subtilis* and *Staphylococcus aureus*. Little inhibition (1–2.5mm) against the Gram-negative bacteria *Klebsiella pneumoniae* and *Escherichia coli* was noted for all the essential oils. This may be due to the fact that the Gram-negative bacteria are more resistant because of their membrane structure, as mentioned in Martin (1995), Rabe and Van Staden (1998) and Mangena and Muyima (1999).

The essential oils showed relatively good activity against the yeast *Cryptococcus neoformans* with *E. namaquensis* producing a zone of 10mm. The activity of the oils against *Candida albicans* was moderate with the largest zone (5mm) in *E. punctulatus* (Nieuwoudtville population 1) and *E. ericoides* subsp. *ericoides* (Bethulie). This is in agreement with the activity of essential oils against the yeasts where they are reported to be more active than against the bacteria as noted in Bagci and Digrak (1996).

Among the species of *Eriocephalus* studied, some individuals of *E. punctulatus*, *E. ericoides* subsp. *ericoides*, *E. brevifolius*, *E. purpureu*s and *E. microphyllus* showed varying degrees of sensitivity against all the test pathogens.

The leaf extracts were not as active as the essential oils against the Gram-positive *Bacillus subtilis* except *E. aromaticus,* with activity of 2–6mm zone of inhibition. The same species was active against *Bacillus cereus* (4–8mm) and *Staphylococcus aureus* (4–6mm). The extracts showed very low (1mm or less inhibition) or no activity against the Gram-negative bacteria *Klebsiella pneumoniae* and *Escherichia coli.* However, most of the extracts were active against the yeast *Cryptococcus neoformans*, with the highest activity of 7mm noted for *E. purpureus* (Nieuwoudtville), and 6mm for *E. racemosus* var. *racemosus* (from Velddrif) and *E. punctulatus* from (Nieuwoudtville population 1). The extracts were mostly inactive against *Candida albicans* except for some species, namely *E. microphyllus* and *E. purpureus*, which showed some inhibition. *E. aromaticus*, however, was active against at least four out of the seven test pathogens and the same group recorded the highest activity among the extracts of *Eriocephalus* species. Promising results were also observed in the leaf extracts of the following taxa: *E. punctulatus*, *E. africanus*, *E. racemosus* var. *racemosus*, *E. spinescens*, *E. purpureus*, *E. microphyllus* and *E. pauperrimus*, but overall the essential oils were comparatively more active than the extracts (Table 1). This implies that the activity in the members of the genus used in herbal remedies is mainly influenced by the presence of essential oils.

In other studies, Salie *et al.* (1996) reported the petroleum ether stem and methanol root extracts of *E. africanus* to be slightly active against *Staphylococcus aureus.* In this study a similar pattern was observed, as the acetone leaf extracts of *E. africanus* had little or no activity against *Staphylococcus aureus*. The essential oils of the same species showed very low antimicrobial activity against *Staphylococcus aureus*. The essential oils of *E. africanus* were observed to be active against *Candida albicans* but the acetone leaf extracts were not active; however, Salie *et al.* (1996) reported the lipophilic extracts of the same species to be active against *Candida albicans.*

Following the results from the disc diffusion assay, the minimum inhibitory concentration (MIC) was determined for six selected test pathogens (Table 2). The 10 species of *Eriocephalus* comprising 18 samples (Table 2) that showed promising activity in the disc diffusion screening assay (Table 1, species in bold text) and those with sufficient oil quantities were selected for the MIC assay. The antimicrobial effect ranged between 4–32mg ml^{-1} for the Gram-positive bacteria, 8–32mg ml^{-1} for the Gram-negative bacteria and 1–8mg ml^{-1} for the fungal strains for the essential oil (Table 2).

The MIC for the leaf extracts for the Gram-positive bacteria was 0.2–3.1mg ml^{-1} and 0.8–6.3mg ml^{-1} for the fungal strains. The Gram-negative bacteria were not tested for the extracts, as there was no notable activity observed from the disc diffusion assays. It is well documented that testing and evaluation of antimicrobial activity of essential oils is difficult because of their volatility, their water insolubility and their complexity. The results are greatly

Table 2: Minimum inhibitory concentration (mg ml^{-1}) of essential oils and leaf extracts of 10 *Eriocephalus* species

Species	Locality	Extract	Minimum inhibitory concentration					
			Cn	*Ca*	*Bc*	*Sa*	*Kp*	*Ec*
E. africanus	Malmesbury	EO	4	4	8	32	*	16
E. brevifolius	Oudtshoorn	EO	*	8	8	16	>32	*
E. capitellatus	Swartberg Pass (A)	EO	4	*	16	*	*	*
E. capitellatus	Swartberg Pass (C)	EO	4	*	*	*	*	*
E. ericoides subsp. *ericoides*	Bethulie (A)	EO	1	4	4	8	16	*
E. ericoides subsp. *ericoides*	Bethulie (B)	EO	*	4	*	*	8	16
E. microphyllus	Kamiesberg	EO	*	*	*	*	8	16
E. punctulatus	Nieuwoudtville (C)	EO	4	8	*	8	*	*
E. punctulatus	Nieuwoudtville (A)-1	EO	*	*	*	*	*	16
E. racemosus	Koeberg	EO	*	*	*	*	*	16
E. racemosus var. *racemosus*	Velddrif	EO	2	*	16	*	16	*
E. aromaticus	Swartberg	AE	*	*	0.4	0.2	*	*
E. aromaticus	Ladismith (A)	AE	*	*	3.1	0.8	*	*
E. aromaticus	Ladismith (B)	AE	1.6	*	0.8	0.4	*	*
E. microphyllus	Spektakel Pass	AE	6.3	1.6	3.1	0.8	*	*
E. pauperrimus	Nieuwoudtville	AE	*	*	*	1.6	*	*
E. punctulatus	Nieuwoudtville-1	AE	0.8	*	0.8	*	*	*
E. purpureus	Kamiesberg	AE	*	1.6	*	0.8	*	*

* Not determined due to insufficient sample or lack of activity

influenced by the choice of assay technique, growth medium, the test pathogen and the oil extract (Janssen *et al*. 1986). Studies to establish if there is any correlation between the inhibition diameters and MIC values for essential oils have been carried out and it is evident that qualitative screening methods and quantitative minimum inhibitory concentration methods are not necessarily comparable, as indicated in Janssen *et al*. (1986). The nature of diffusion of the leaf extracts and the essential oil in water or culture medium differs considerably. Hence, the results obtained may vary qualitatively and quantitatively. In this study, the same phenomenon was observed with the results obtained for the MIC test not confirming or tallying with those obtained for inhibition diameters in the disc diffusion assay as mentioned in Brantner and Grein (1994).

In herbal remedies, the species of *Eriocephalus* are mainly used for treatment of respiratory-related ailments, skin inflammation, stomach disorders and as diuretics and diaphoretics. From the broad screening of the taxa in the genus it was observed that most of the essential oils were active against the respiratory pathogen *Cryptococcus neoformans. Eriocephalus racemosus* var. *racemosus* and *E. ericoides* subsp. *ericoides* had an MIC of 2mg ml^{-1} and 1mg ml^{-1} respectively, compared to the rest of the species tested (Table 2). The leaf extracts of *E. punctulatus* and *E. aromaticus* had an MIC of 0.8mg ml^{-1} and 0.2–1.6mg ml^{-1} respectively. The MIC of *E. ericoides* subsp. *ericoides* and *E. microphyllus* was 8–16mg ml^{-1} for the former and 8mg ml^{-1} for the latter for the essential oils against *Klebsiella pneumoniae*. This supports the use of the species of *Eriocephalus* for treatment of respiratory-related ailments.

Most of the species studied showed activity in the essential oils and the leaf extracts against *Bacillus cereus* and *Staphylococcus aureus*, which may be associated with dermal infections. The essential oils of *E. punctulatus*, *E. ericoides* subsp. *ericoides*, *E. africanus* and *E. brevifolius* had an MIC of between 4mg ml^{-1} to 8mg ml^{-1} for effective inhibition of the test pathogen. For the leaf extracts, *E. aromaticus*, *E. punctulatus*, *E. microphyllus* and *E. purpureus* had an MIC range of 0.2mg ml^{-1} to 0.8mg ml^{-1} (Table 2).

For gastro-intestinal disorders or infections, *E. punctulatus*, *E. microphyllus*, *E. racemosus*, *E. brevifolius* and *E. ericoides* subsp. *ericoides* indicated potential, as these species showed activity against *Escherichia coli*, while the rest showed activity against *Candida albicans* (Tables 1 and 2).

From the results obtained from the disc diffusion screening, the essential oils of *E. punctulatus*, *E. ericoides* subsp. *ericoides*, *E. purpureus*, *E. microphyllus*, *E. decussatus* and *E. brevifolius* are active against nearly all the test pathogens and can be used to treat respiratory-related ailments, dermal infections and gastro-intestinal disorders recorded for the traditional herbal remedies. The other notably biologically active species include *E. pauperrimus*, *E. microphyllus*, *E. racemosus* and *E. capitellatus* and are therefore potentially useful as a source of herbal remedies. *E. punctulatus*, *E. africanus* and *E. racemosus* are traditionally used for treatment of respiratory, skin and stomach problems and the results from the disc diffusion assay and the MIC values obtained in this study confirm their efficacy in traditional uses.

This study confirms that *Eriocephalus* species have broad and varied antimicrobial activity within their essential oils and leaf extracts. The results obtained from the broad screening with various test pathogens confirm their use in traditional herbal remedies. The essential oils have proved to be more antimicrobially active in comparison to the leaf extracts. This study showed antimicrobial activity for selected test pathogens, which clearly indicates that there are more potentially active species of the genus not initially documented. It should also be noted that nearly all the essential oils and most of the leaf extracts were active against the yeast *Cryptococcus neoformans.* This forms a

basis for an alternative source of remedies for treatment of fungal infections. More research will be carried out to isolate the active compound(s) by bioassay-guided fractionation for some of the species like *E. aromaticus*, which showed high inhibitory activity in the preliminary screening. However, if these species are to be used for medicinal purposes, their chemical composition and issues of safety and toxicity will need to be investigated further.

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