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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),