THE CHEMOTAXONOMY, PHYLOGENY AND BIOLOGICAL ACTIVITY OF THE GENUS *ERIOCEPHALUS* L. (ASTERACEAE).

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ABSTRACT

The genus *Eriocephalus* commonly known as 'wild rosemary', 'Cape snow bush', or 'kapokbos' is a member of the family Asteraceae (tribe Anthemideae). The genus is endemic to southern Africa, with the highest concentration of species in the Western and Northern Cape. The genus comprises 32 species and a total of 42 taxa, which are distributed in South Africa, Namibia, Botswana, and Lesotho. The characters used in species delimitation are purely based on morphological variation in floral and foliar parts and are highly homoplastic due to phenotypic plasticity. In many cases these features are not sufficiently distinctive, as some taxa tend to exhibit dimorphism in some character states such as the presence of opposite and alternate leaves. In some species there is extensive intergrading of the major diagnostic characters leading to uncertainty in species delimitation. Both chemical and molecular characters were used in this study in an attempt to evaluate current species delimitations in the genus, along with species-level relationships and affinities. The genus is also economically important with some of its members used as medicinals, fodder, perfumes, and cosmetics. This warrants investigation into the phytochemistry and biological activity of these species in order to determine a scientific rationale for their traditional uses. For this reason, the antimicrobial, antiinflammatory, antioxidant activities, and inhibition of acetylcholinesterase by the volatile oils and leaf extracts of the genus, which are relatively unknown for most members of the genus, were also investigated.

Representatives of 22 species of the genus, eight of which were from Namibia and 14 from South Africa were collected from wild populations. In most cases multiple collections per population per species were considered. Aerial plant parts were hydrodistilled to obtain the essential oils, and phenolics were extracted from leaves using acetone. Essential oils were analysed by thin layer chromatography (TLC), gas chromatography (GC), gas chromatography coupled to mass spectroscopy (GC/MS), and phenolics were analysed using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC/UV). Biological assays were carried out using the 5-lipoxygenase enzyme to evaluate antiinflammatory activity; disc diffusion and microtitre plate dilution assays were used to assess antimicrobial activities of selected fungi and bacteria; the TLC-DPPH and DPPH-microtitre methods were used to investigate antioxidant activities and a TLC-bioautographic assay was used for testing the inhibition of the acetylcholinesterase enzyme. Total genomic DNA was extracted from silica dried leaf material. The non-coding plastid DNA regions, the *psb*A-*trn*H intergenic spacers and the internal transcribed spacer (ITS) region of nuclear ribosomal DNA were amplified, and sequenced and analysed using the parsimony algorithm.
The essential oils are largely comprised of acyclic, monocyclic, and bicyclic regular and irregular mono- and sesquiterpenes of various structural groups. Two hundred compounds were

noted in the essential oils with some of the common constituents being; α- and β-pinene, yomogi alcohol, ρ-cymene, 1,8-cineole, camphor, 4-terpineol, spathulenol, caryophyllene oxide, α-copaene and β-caryophyllene. Most of the species have a relatively high content of 1,8-cineole and camphor. Twenty-two chemotypes were noted and the potential for commercial development in the flavour, fragrance and pharmaceutical industries has been recorded. Among the favourable chemotypes noted includes the camphor, 1,8-cineole, bisabolol oxide B and nerolidol rich oils. However, due to the extensive variability in the essential oil profiles, standardization of oils in commercial development is crucial.

The leaf extracts comprised of flavonoids with the flavones and flavanones as the major structural types present in most species. The terpene and flavonoid chemistry of the genus is highly divergent even among multiple individuals of the same species and hence not a good taxonomic marker for specific delimitation as no coherent groups was evident although some phytochemical congruence has been noted between some of the taxa.

The DNA sequence data revealed lack of variability in the non-coding regions *psb*A-*trn*H and *trn*L-F among species of the genus. The nuclear DNA region (ITS) was variable but the number of characters separating taxa was too few for resolution of relationships between taxa. Presence of highly divergent paralogous repeats of ITS were also noted in some taxa. The combination of molecular and chemical data did not resolve the species delimitation problems due to the highly variable distribution of characters within a single species. The patterns of variation observed in the genus may be attributed to chemical convergence, divergence, hybridisation, differential gene expression, polymorphism and allelochemical diversification among other factors. The lack of coherence in the phylogenetic and phenetic groupings of the various taxa implies that the current species boundaries may not be a true reflection of natural taxonomic entities. The use of multiple taxa in taxonomic studies is strongly recommended due to the extensive variability noted in the chemical profiles of the taxa that is also depicted in the phylogenetic histories. It also implies that caution should be taken in bioprospecting for new natural products for commercial development, as plant chemical profiles especially from the same species can be very variable. This implies carrying out exhaustive population and genetic studies for evaluation of diversity in the study group.

In the antimicrobial assay, the oils were more active against the Gram-positive bacteria (2-16 mg/ml) and yeasts (1-16 mg/ml). *Bacillus cereus* and *Cryptococcus neofomans* were the most susceptible pathogens to the oils. The extracts exhibited low activity against the test pathogens except *E. aromaticus* and *E. pinnatus* with activity of 0.2 mg/ml against *Staphylococcus aureus* and *Bacillus cereus* respectively. The susceptibility of the fungal pathogens *Cryptococcus neoformans* and *Candida albicans* and the Gram-positive bacteria *Bacillus cereus* to the oils and extracts is an indication of the potential for use of the members of the genus as natural antibiotics. The essential oils exhibited antiinflammatory activities with IC_{50} values ranging between 19.0-98.6 μg/ml. The oils did not show antioxidant activity at the starting concentration of 100 μg/ml but the acetone leaf extracts exhibited antioxidant activities with IC_{50} values ranging between 21.5-79.6 μg/ml. The essential oils showed inhibitory activity against acetylcholinesterase enzyme. The biological activity of the oils indicates that most of the traditional uses are influenced by the presence of the oils. The *in vitro* biological activity of the essential oils and extracts against the test pathogens provides a scientific basis for the use of

some of the members in traditional herbal remedies and validates the use of some of the members of the genus for treatment of respiratory tract infections, gastro-intestinal disorders, mental conditions, dermal infections, and inflammation. The study records the biological activities for some of the species for the first time and their potential for use in flavourings, perfumery, cosmetics, as sources of antimicrobial drugs, permeability enhancers in pharmaceutical formulations and for use as industrial oils.