EAPAS
East African Journal of Pure and Applied Science

East African Journal of Pure and Applied Science Vol. 2 (1):141 -152 (2012)

THE BIOLOGICAL PROPERTIES OF THE GENUS *ERIOCEPHALUS* L. (ASTERACEAE)

Njenga E. W[1], Viljoen A. M[2], Vuuren S. V.[3]

[1]Department of Biological Sciences, Chepkoilel University College, P.O.Box 1125-30100, Eldoret, Kenya.

[2]Department of Pharmaceutical Sciences, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa.

[3]Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Park town, 2193, South Africa.

ABSTRACT

The genus *Eriocephalus* belonging to the family Asteraceae is economically important with some of its members used as traditional herbal remedies, perfumes and in cosmetics. Most of the uses of members of the genus as traditional herbal remedies have not been validated and therefore this study seeks to determine their biological properties. The biological activities of some of the species were studied to determine the scientific rationale for the use of members of the genus in traditional herbal remedies. Antimicrobial, anti-inflammatory, antioxidant activities and qualitative screening for inhibition of acetyl cholinesterase of oils and leaf extracts were investigated. Aerial parts were hydro distilled to obtain essential oils and leaves were soaked in acetone, filtered and dried for further use. The essential oils were active against the gram +ve bacteria *Bacillus cereus* with a minimum inhibitory concentration of 0.2-16mg/ml and the yeast *Cryptococcus neoformans* 0.5-16mg/ml. The oils showed anti-inflammatory properties with IC_{50} of 30.2-98.9µg/ml but did not show antioxidant properties. The essential oils showed inhibitory properties against acetylcholinesterase. The leaf extracts had antioxidant activity with IC_{50} of 21.8-58.8 µg/ml. The biological activity of the oils indicates that most of the traditional uses are influenced by their presence. The *in vitro* results provide a scientific basis for use of the members of the genus in traditional herbal remedies.

Key words: minimum inhibitory concentration (MIC), inhibition concentration (IC_{50}) antimicrobial, anti- inflammatory, antioxidant

[1]Corresponding author E-Mail address: njengae@yahoo.com
 ISSN 2070 0903

Introduction

The genus *Eriocephalus* L. commonly known as 'wild rosemary' (Merle *et al.,* 2007) or 'Cape snow bush' belongs to the family Asteraceae (tribe Anthemideae). The genus is characterized by presence of aromatic terpenes found in the highly dissected leaves. Thirty-two endemic species and forty two other taxa of the genus are reported to occur in South Africa, Namibia, Botswana and Lesotho (Adamson and Salter; 1950; Müller *et al*., 2001; Njenga *et al*., 2005). The genus derives its name from the Greek word *'erion'* for wool and *'cephale'* for head (Adamson and Salter, 1950). The members have radiate or disciform capitula and grow in varied habitats.

The genus is also economically important as a source of Cape chamomile oil obtained from *E. punctulatus* DC. In recent studies by Sandasi *et al*., (2011) it is indicated that E. *tenuifolius* DC is the source of the Cape chamomile and not the former. Some of the species of the genus are used in traditional herbal remedies for the treatment of respiratory tract infections, gastro-intestinal disorders, dermal infections and as anti-inflammatory agents (Watt and Breyer-Brandwijk, 1962; Zdero *et al*., 1987; Van Wyk *et al*., 1997; Dyson, 1998; Eloff, 1998; Van Wyk and Gericke, 2000; Merle *et al.,* 2007; Van Wyk, 2008a; Van Wyk, 2008b). This paper describes a screening of 22 species of *Eriocephalus* for antimicrobial, antioxidant and anti-inflammatory activities and provision of a scientific rationale for the uses of the species in the genus as herbal remedies.

Materials and methods

Plant material: The aerial parts of the selected *Eriocephalus* species were collected during the flowering stage from various natural populations. Locality data for the representative species studied are given in Table I. Multiple collections were made and voucher specimens prepared. The plants were identified and authenticated at South African National Biodiversity Institute (SANBI) Pretoria, Compton Herbarium (Kirstenbosch) and National Botanical Research Institute (NBRI) Windhoek. The voucher specimens were deposited in the Herbarium at the Department of Pharmacy and Pharmacology, University of the Witwatersrand, South Africa. The species from Namibia were likewise deposited at National Botanical Research Institute (NBRI) Windhoek.

Extraction of plant materials

Hydrodistillation

The plant material was hydrodistilled immediately upon arrival from the collection sites. Between 20-750g of the aerial plant parts (wet or dry material) were hydrodistilled for four hours using a Clevenger apparatus. The distillate was collected in a pre-weighed amber vial, which was later, weighed, capped (Teflon cover) and the percentage yields tabulated. The oils were labeled accordingly, weighed to obtain percentage yield and the color of the oil noted (Table 1). The samples were refrigerated at 4 ^{o}C for further analyses.

Table 1. Voucher specimens, localities, oil colour and percentage yields of essential oils and acetone leaf extract of 22 species of *Eriocephalus*. Unless indicated essential oils values are based on wet weight and acetone leaf extracts on dry weight.

Species	Source/locality	Voucher number	Oil Colour	% yield EO	% Yield AE*
E. africanus L	Malmesbury	AV 444	Yellow	0.18	8.26
	Schakalsberge (ex NBRI)	AV 868	Nd	Nd	1.0
E. aromaticus C.A.Sm.	Ladismith/Seweweekspoort	AV 521B	Clear	0.03	11.1
E. brevifolius (DC) M.A.N. Müller	Oudtshoorn	AV 483	Yellow	0.11	6
E. capitellatus DC	Swartberg Pass	AV 497A	Pale yellow	0.41	3.7
E. decussatus Burch	Sutherland/Kamiesberg	AV 836	Deep blue	0.21	4.3
E. dinteri S. Moore	Near Aus(ex NBRI)	AV 871	Pale	0.19*	2.8
E.ericoides subsp. *ericoides* (L.F.) Druce	Windhoek dist. (ex NBRI)	AV 866	Pale yellow	0.23*	5.9
E. eximius DC	Sutherland/Kamiesberg	AV 837	Deep blue	0.04	2.1
E. grandiflorus M.A.N. Müller	Laingsburg/Matjiesfontein	AV 525 A	Clear	0.07	1.5
	Neiaab Mountain(ex NBRI)	AV 870	Greenish yellow	0.17*	3.9
E. luederitzianus O.Hoffm	12 km E of Windhoek(ex NBRI)	AV 865 A	Pale yellow	0.06*	2.5
E. merxmuelleri M.A.N. Müller	Buschmanberge(ex NBRI)	AV 869	Deep blue	0.16*	2.8
E. microphyllus DC	Kamiesberg	AV 794	Blue	0.24	5.7
E. namaquensis M.A.N. Müller	Clanwilliam/Farm Perdefontein	AV 545 A	Pale yellow	0.05	1.4
E. pauperrimus Merx & Eberle	Nieuwoudtville/Loeriesfontein	AV 539 A	Cloudy white	0.15	1.4
E. pinnatus O. Hoffm	Brandberg (ex NBRI)	AV 864	Greenish yellow	0.09*	3.7
E. punctulatus DC	Nieuwoudtville	AV 439 A	Greenish blue	0.15	6.4
E. purpureus Burch.	Kamiesberg	AV 796	Pale yellow	0.49	11
E. racemosus	Velddrif	AV 457C	Deep yellow	0.18	8.1
E. scariosus L.	Near Aus(ex NBRI)	AV 872	Pale yellow	0.42*	5.4
E. spinescens Burch	Sutherland/Ceres	AV 517	Clear	0.03	2.9

The letters A, B, and C represent individuals of a species from the same population. Nd-not determined; EO-essential oil; AE- acetone extract. *-Values based on dry weight.
*ex-NBRI-National Botanical Research Institute Namibia

Solvent extraction of non-volatile compounds

Between 0.5-9.4 g of air-dried plant parts (ground or whole) were weighed and 30ml of acetone added. The mixture was left to extract in a water bath (37 ^{o}C) for four hours. The extract was filtered using cotton wool and pipetted into pre-weighed polytubes. The solvent was evaporated and the residue resuspended in methanol and was later passed through a Sephadex LH 20 column rinsed with methanol to remove the overwhelming terpenoids (Wollenweber and Mann, 1989). The mixture was left to evaporate and the residue weighed and refrigerated at 4 ^{o}C.

Antimicrobial activity.

The disc diffusion and minimum inhibitory concentration assays were used to determine the antimicrobial activities of the oils and non volatile leaf extracts.

Disc diffusion assay

The antimicrobial screening was carried out on 7 reference cultures. The disc diffusion assay based on Kirby-Bauer method was carried out using five bacterial reference strains namely: *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 25923),

Klebsiella pneumoniae (NCTC 9633), *Escherichia coli* (ATCC 8739) and two yeast strains: *Cryptococcus neoformans* (ATCC 90112) and *Candida albicans* (ATCC 10231). Tryptone Soya agar was prepared by dissolving 30g of the agar in 750 mL of water and autoclaved for 15 min at 121°C and cooled to 55°C in a water bath. A base layer of 100 mL of agar was poured into the plate and inoculated with a top layer of 100 mL of agar containing an inoculum of approximately 1 x 10^6 CFU/mL. Sterilized paper discs (6 mm) were saturated with approximately 8 µL of each of the oils and loaded onto the agar plates. The plates were kept at 4°C for one hour to pre-diffuse the oil and then incubated for 24 hours at 37°C for bacterial isolates. The yeasts were incubated for 48 hours. Neomycin (30 µg per disc) was used as a positive control for the bacterial strains and Nystatin (100 IU per disc) as a control for the fungal strains. Activity was measured as growth inhibition zones in millimeters from the edge of the disc. Replicates were made to confirm results (Hudzicki, 2009)

Minimum inhibitory concentration (MIC)
The oil yields were relatively low hence only those species with sufficient oils and with notable activity from the disc diffusion assay were included in this assay. The test was carried out using the *p*- iodonitrotetrazolium violet (INT) microplate method. The oils with a starting concentration of 128 mg/ mL were transferred into the first well in the microtitre plates and serially diluted. The test cultures yielding an inoculum of approximately 1 x 10^6 CFU/mL were added to the wells and incubated at 37°C for 24 h for bacterial strains and 48 h for the yeast strains. The controls were Ciprofloxacin (0.01mg/mL stock solution) for bacterial strains and Amphotericin B (0.01 mg/mL stock solution) for the yeast strains. Culture growth was visualized by transferring 40 µL of 0.2 mg/mL INT to all the wells and examining them to determine the color change after 6 h for bacterial strains and 24 h for yeasts. The tests were done in triplicate (Eloff, 1998; Njenga, 2005).

Anti-oxidant assay
The radical scavenging activity of the acetone leaf extracts was determined spectrophotometrically using a modified version of 2, 2, diphenyl-1-picryhydrazyl (DPPH) according to Cuendet *et al*., (1997) and Mambro *et al*., (2003). The stock solution was made by dissolving DPPH (Fluka) in analytical grade methanol to obtain a 96.2 µM solution. The extracts (5 mg) were dissolved in 500 µl of dimethyl sulfoxide (DMSO, Saarchem) to give an initial stock of 10,000 µg ml^{-1}. The mixture was vortexed to dissolve the extract. 50 µl of the stock solution was diluted (1:1 dilution) with 950µl of DMSO. Then 50 µl of this stock solution was pipetted into a 96 well micro-titre plate in triplicate (Lourens *et al*., 2004). The plates were shaken on an automated micro-titre plate reader (Labsystems Multiskan RC) for 2 minutes and then kept in the dark at room temperature for 30 min. The changes in colour from deep violet to light yellow were measured at 550 nm on an UV/visible light spectrophotometer (Labsystems Multiskan RC) linked to the computer equipped with GENESIS® software. The radical scavenging activity was measured as the decolourization percentage of the test sample. All determinations were done in triplicate. Ascorbic acid was used as the positive control. The IC_{50} which is the concentration at which there is 50% decolourization of the DPPH by the test sample was determined using the Enzfitter® 1.05 version software where the decolourization (%) was determined using following formula:

% Decolourisation = (Av controls- (Av $sample_{DPPH}$- Av $sample_{MEOH}$)) x 100

Controls

Where Av controls = average absorbance of all DPPH control wells-average absorbance of all methanol control wells; Av $sample_{DPPH}$ average absorbance of sample wells with DPPH and Av $sample_{MEOH}$ = average absorbance of sample wells with methanol.

Anti-inflammatory assay

Possible inhibition of 5-lipoxygenase activity was determined following published protocols (Sircar *et al*., 1983; Evans, 1987). All concentrations refer to final concentrations in 3mL cuvettes maintained at 25°C in a thermostated bath. The standard assay mixture contained 10µL of each oil dissolved in Dimethyl Sulfoxide (DMSO) and Tween 20. A 0.1M potassium phosphate buffer (pH 6.3, 2.95mL) was prepared with analytical grade reagents and 100 uM linoleic acid (≥99%). The reaction was initiated with the addition of 100 U isolated 5-lipoxygenase and diluted with an equal volume of potassium phosphate buffer maintained at 4°C. The increase in absorbance at 234 nm was recorded for 10 min with a single beam spectrophotometer (Analytikjena Specord 40) linked to a PC by the Winaspect software. Increasing amounts of oils were added and the initial reaction rate was determined from the slope of the straight line portion of the curve. The percentage inhibition of enzyme activity was calculated by comparison with the negative control (DMSO and Tween 20). Nordihydroguaiaretic acid (NDGA) represented the positive control. Percentage enzyme activity was plotted against concentration of each oil. The concentration of each oil that caused 50% enzyme inhibition (IC_{50}) was determined using Enzfitter version 1.05 software.

Preliminary screening for detection of acetyl cholinesterase

Preliminary screening for the presence of inhibitors of acetylcholinesterase was carried out on 17 species of *Eriocephalus* using Thin Layer Chromatography (TLC) bioautography. Essential oils were diluted 1:7 with hexane. The acetone leaf extracts were resuspended in acetone to a concentration of 50 mg/ml.

TLC bioautography

Acetylcholinesterase (1000 U) was dissolved in 150 ml 0.05 M Tris-hydrochloric acid buffer at pH 7.8; bovine serum albumen (150 mg) was added to the solution in order to stabilize the enzyme during the bioassay. The stock solution was kept at 4 ^{o}C.

TLC plates were eluted with appropriate solvent (acetone or isopropanol) in order to wash them, and were thoroughly dried just before use. Three microlitres of essential oils and 10µl acetone leaf extracts were loaded on separate plates and eluted with a solvent system comprising toluene (93): ethyl acetate (7) and toluene (90): dioxane (25): acetic acid (5) respectively. After migration of the sample in the above solvent systems, (or direct deposition of sample), the TLC plate was dried with a hair drier for complete removal of the solvent.

For incubation of the enzyme, the plate was laid flat on plastic plugs in a plastic tank containing a little water to avoid the plate coming into contact with the water but the atmosphere was kept humid. The tank was covered and incubation performed at 37 ^{o}C for 20 minutes (Perry *et al*., 1999; Marston *et al.*, 2002). The enzyme had satisfactory stability under these conditions. For detection of the enzyme, solutions of 1- naphthyl acetate (250 mg) in ethanol (100 ml) and of fast blue B salt (400 mg) in distilled water (160 ml) were prepared immediately before use (to prevent

decomposition). After incubation of the TLC plate, 10 ml of naphthyl acetate solution and 40 ml of fast blue B salt solution were mixed and sprayed onto the plate to give a purple coloration after 1-2 minutes.

Results and Discussion

The *in vitro* antimicrobial activities of the essential oils and the extracts against selected test pathogens were assessed qualitatively and quantitatively by sizes of inhibition zones and MIC values respectively. The nature of diffusion of the leaf extracts and the essential oil in water or culture medium differed 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 inhibition diameters obtained in the disc diffusion assay (Brantner and Grein, 1994; Lourens *et al.*, 2004). However, the antimicrobial assays indicated some activity of the essential oils and leaf extracts against gram positive and negative bacteria and two yeast species (Table 2).

The most susceptible pathogens were *Bacillus cereus* (0.2-16mg/ml) and yeasts *Cryptococcus neoformans* (0.5-16mg/ml). This confirms that some species of the genus have antimicrobial properties and supports their use in traditional herbal remedies (Watt and Breyer-Brandwijk, 1962; Van Wyk *et al*., 1997; Dyson, 1998; Van Wyk and Gericke, 2000; Merle *et al.*, 2007; Van Wyk, 2008a and 2008b). In herbal remedies, species of *Eriocephalus* are mainly used for treatment of respiratory related ailments, skin inflammation, stomach disorders and as diuretics and diaphoretics (Watt and Breyer-Brandwijk, 1962; Van Wyk *et al*., 1997; Dyson, 1998; Van Wyk and Gericke, 2000; Van Wyk, 2008a). From the broad screening of taxa in the genus it was observed that most of the essential oils were active against the respiratory pathogen *Cryptococcus neoformans* while both essential oil and the leaf extracts were active against *Bacillus cereus* and *Staphylococcus aureus* (Table 2), both of which may be associated with dermal infections (Van Wyk, 2008a). With regard to gastro-intestinal disorders or infections, the essential oils and extracts of *E. africanus* and *E. punctulatus* showed activity against *Escherichia coli, Klebsiella pneumoniae* and *Candida albicans* respectively (Table 2). The study also confirms the use of *E punctulatus, E. africanus* and *E. racemosus* for the treatment of respiratory, skin and stomach problems (Van Wyk, 2008a). It should also be noted that nearly all of the essential oils and most of the leaf extracts were active against the yeast *Cryptococcus neoformans* and *Bacillus cereus* (Table 2). This forms a basis for an alternative source of remedies for the treatment of fungal and bacterial infections.

Table 2. The antimicrobial activities of essential oils and leaf extracts of *Eriocephalus*. The activities were determined by disc assay (DD) measured in mm from the disc edge and minimum inhibitory concentrations (MIC) in mg/ml

Species	Extract	Antimicrobial activity													
		CN		CA		BC		BS		SA		KP		EC	
		DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC
E. africanus	EO	5	4	2	4	6	8	2	*	2	32	1	*	R	16
	AE	3	*	R	*	1	*	2	*	R	*	<1	*	R	*
	EO	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	AE	R	*	R	*	R	*	R	*	2.5	3.1	R	*	<1	*
E. aromaticus B	EO	R	*	R	*	3	*	1	*	<1	*	R	*	R	*
	AE	5	1.6	R	*	7.3	0.8	6	*	6	0.4	R	*	R	*
E. brevifolius	EO	R	*	2	8	5.3	8	4	*	5	16	2	>32	2	*
	AE	3	*	R	*	R	*	4	*	<1	*	R	*	R	*
E. capitellatus A	EO	4	4	R	*	3.5	16	*	*	1	*	<1	*	R	*
	AE	<1	*	R	*	R	*	<1	*	<1	*	R	*	R	*
E. decussatus	EO	5.2	16	2	32	3.5	8	1.5	*	2.5	4	<1	*	<1	*
	AE	1.5	2.4	R	*	1	0.9	<1	*	2.5	1.6	R	*	<1	3.1
E. dinteri	EO	6.6	32	1.5	32	3.6	16	1.2	*	3.2	4	1.5	8	1	8
	AE	2.8	6.3	R	*	1.7	0.4	R	*	3	3.1	R	*	<1	*
E. ericoides subsp. *ericoides*	EO	3.2	16	2	16	4	8	1.5	*	2	4	1	16	1	*
	AE	1.5	1.6	R	*	1	1.6	R	*	2.5	1.6	R	*	R	*
E. eximius	EO	4.5	*	1.5	*	4.7	*	1.5	*	3	*	R	*	R	*
	AE	1	*	R	*	1	*	<1	*	1.5	*	R	*	1	*
E. grandiflorus A	EO	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	AE	R	*	R	*	1	*	2	*	R	*	R	*	R	*
	EO	6.2	32	2	32	2.8	8	1.2	*	2.6	4	2.4	8	1	16
	AE	<1	*	R	*	1.8	0.9	<1	*	1.5	0.8	R	*	<1	*
E. luederitzianus A	EO	2.8	*	1.5	*	2.1	*	1.2	*	3.8	*	<1	*	R	*
	AE	R	*	R	*	R	*	R	*	1.5	0.8	R	*	R	*
E. merxmuelleri	EO	6	16	1.5	16	3.5	8	2	*	1.5	2	1.5	8	R	*
	AE	2.4	3.1	R	*	1	0.4	R	*	1.5	0.4	R	*	<1	*
E. microphyllus	EO	5	*	1	*	2	*	1	*	<1	*	2	8	1	16
	AE	2	*	1	*	<1	*	1	*	1	*	R	*	R	*
E. namaquensis A	EO	10	*	1	*	3.5	*	3	*	1.5	*	<1	*	<1	*
	AE	4	*	R	*	2	*	1	*	<1	*	<1	*	<1	*
E. pauperrimus A	EO	6	*	3	*	4	*	2	*	2	*	R	*	R	*
	AE	1	*	R	*	R	*	2	*	R	1.6	<1	*	R	*
E. pinnatus	EO	3.8	16	1.5	16	5	8	<1	*	2.5	8	<1	*	R	*
	AE	3.3	6.3	R	*	1.3	0.2	1	*	1.5	0.4	R	*	<1	*
E. punctulatus A	EO	2	*	<1	*	3	*	1	*	1	*	1	*	1.5	16
	AE	6	0.8	1	*	3	0.8	2	*	R	*	<1	*	R	*
E. purpureus	EO	5	*	R	*	2	*	1.5	*	<1	*	1	*	1	*
	AE	4	*	2	1.6	4	*	1	*	1.5	0.8	R	*	<1	*
E. racemosus	EO	2	2	1	*	4	16	1	*	1	*	<1	16	R	*
	AE	R	*	R	*	2.5	*	1.5	*	1	*	R	*	R	*
E. scariosus	EO	3.5	8	1.5	>32	1.3	12	1	*	1	4	1.5	8	1	8
	AE	2	0.5	R	*	1.5	0.5	R	*	2	1.6	R	*	<1	*
E. spinescens B	EO	2	*	R	*	2	*	<1	*	<1	*	R	*	R	*
	AE	2	*	R	*	2	*	1.5	*	1	*	R	*	R	*
Conventional antimicrobial control		3.5^{a}	$1x10^{-3c}$	8.0^{a}	$1x10^{-3c}$	8.5	$6x10^{-4d}$	6.0^{b}	$6x10^{-4d}$	5.0^{b}	$1x10^{-3d}$	2.0^{b}	$3x10^{-3d}$	5.0^{b}	$3x10^{-3d}$

CN-*Cryptococcus neoformans* ATCC 90115; CA-*Candida albicans* ATCC 10231; BC-*Bacillus cereus* ATCC 11778 ; BS-*Bacillus subtilis* ATCC 6051; SA-*Staphylococcus aureus* ATCC 5923 ; KP-*Klebsiella pneumoniae* NCTC 9633; EC-*Escherichia coli* ATCC 8739; Controls= [a]Nystatin, [b]Neomycin, [c]Amphotericin B, [d]Ciprofloxacin; *Not determined due to insufficient sample or lack of activity. EO- essential oils; AE-acetone extracts.

The antioxidative ability of the leaf extracts of the genus *Eriocephalus* was more pronounced at higher concentrations 30-80µg/ml (Table 3). Species of *Eriocephalus* have various classes of flavonoids in the leaf extracts which are known to have strong antioxidant properties. The activity noted in most of the species could be attributed to the presence of flavones, isoflavones and flavanones that were abundant in the leaf extracts (Zdero *et al*., 1987). The essential oils did not show activity probably due to the fact that the DPPH assay is reported as being effective for essential oils with polar sub fractions and it is most likely that the sub fractions present in *Eriocephalus* oils were apolar hence lack of activity. The other probable reason may be the nature and type of the chemical compounds present in the oils and the quantities which may affect the antioxidant properties of the species under screening (Kulevanova and Panovska, 2001; Amiri, 2011).

Only essential oils were screened for anti-inflammatory activity since most of the uses reported for some of the commercially and traditionally used species are attributed to the essential oil components with anti-inflammatory properties (Van Wyk *et al*., 1997; Dyson, 1998; Van Wyk and Gericke, 2000; Van Wyk, 2008a; Van Wyk, 2008b). Activity ranged from IC_{50} of 21.8-58.8 µg/ml (Table 3). The results show that there are species of *Eriocephalus* which are not known for any traditional use, but have inhibitory ability against the enzyme. These include. *E. dinteri* (35 µg/ml), *E. brevifolius* (30 µg/ml), *E. eximius* (37 µg/ml) and *E. decussatus* (39 µg/ml) (Table 3).
These results support the use of members of *Eriocephalus* in the treatment of inflammatory diseases mediated by 5-lipoxygenase products, i.e. leukotrienes in traditional remedies.

Table 3 Antioxidant and Anti-inflammatory activity of some selected species of *Eriocephalus* against 5-lipoxygenase. The IC_{50} values are given with (n=1).

Species	**Source/locality**	**DPPH IC_{50} (µg/ml)** Leaf extracts**	**5-LOX IC_{50} (µg/ml)** Essential oils**
E. africanus	Malmesbury	47.2 ± 7.2	32.8
E. ambiguus	Schakalsberge	32. 9 ± 2.8	-
E. aromaticus	Ladismith B	45.3 ± 4.8	-
E. brevifolius	Oudtshoorn	49.7 ± 7.2	30.2
E. capitellatus	Swartberg Pass A	40.5 ± 3.2	43.1
E. decussatus	Kamiesberg	44.1 ± 4.3	39.6
E. dinteri	Near Aus	34.9 ± 2.7	35.4
E. ericoides subsp. *ericoides*	Windhoek Namibia	45.1 ± 5.0	43.1
E. eximius	Kamiesberg	39.3 ± 3.6	37.9
E. grandiflorus	Laingsburg A	45.6 ± 5.1	-

Species	Source/locality	DPPH IC_{50} (μg/ml)** Leaf extracts	5-LOX IC_{50} (μg/ml)** Essential oils
E. klinghardtensis	Neiaab Mountain	28.1 ± 1.8	59.7
E. luederitzianus	Windhoek A	48.1 ± 5.9	40.5
E. merxmuelleri	Buschmanberge	39.9 ± 4.5	44.5
E. microphyllus	Kamiesberg	47.0± 6.7	69.4
E. namaquensis	Clanwilliam A	45.3 ± 6.5	-
E. pauperrimus	Nieuwoudtville A	46.6 ± 5.8	69.9
E. pinnatus	Brandberg	53.0 ± 4.4	58.7
E. punctulatus	Nieuwoudtville A	43.2 ± 3.5	63.0
E. purpureus	Kamiesberg	41.5 ± 6.0	98.9
E. racemosus var *racemosus*	Velddrif C	58.8 ± 1.7(01)	-
E. scariosus	Aus-Namibia	35.4 ± 3.8	>100
E. spinescens	Sutherland B	45.3 ± 3.9	-
Control		Vitamin C 2.9 ± 0.01	NDGA 5=+ 0.5

**-Values are means + SE of three replicates NDGA-nordihydroguaiaretic acid.

The essential oils of seventeen species of *Eriocephalus* screened for the presence of acetylcholinesterase inhibitors resulted in several white spots on the TLC plate (Figure 1) an indication of the presence of the enzyme inhibitors. The acetone leaf extracts did not show any activity probably implying absence of the inhibitors or their presence in trace amounts however, the essential oils appear to have more of the inhibiting compounds, as evidenced by the numerous spots on the TLC plate (Amiri, 2011). Perhaps when lower dilutions of the essential oils are used for the screening it would be possible to tell at what concentrations the inhibiting compounds would still inhibit the enzyme.

The results of the qualitative screening give a new dimension to the biological activities of the members of the genus. Firstly, they support the use of the species in traditional remedies as mind-improving plants and the treatment of stress related ailments (Van Wyk *et al*., 1997; Dyson, 1998; Van Wyk and Gericke, 2000; Van Wyk, 2008b).

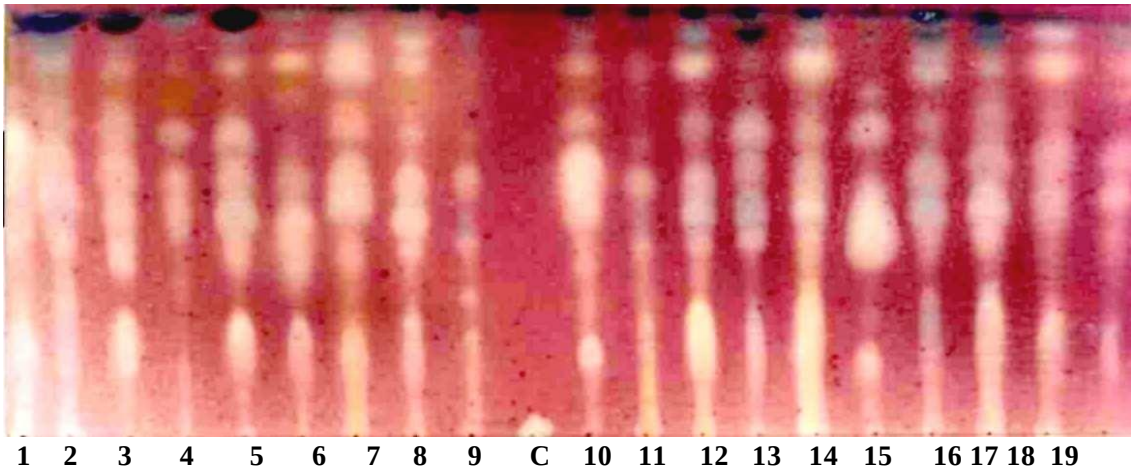

Figure 1. A TLC bioautographic profile of essential oils of 17 species of *Eriocephalus* tested for the presence of inhibitors of acetylcholinesterase enzyme. The white spots against the purple background indicate presence of inhibitory compounds. C is a control (Galathamine). **1** and **10**-*E. punctulatus*; **2** and **19**-*E. purpureus*; **3** and **16**-***E****. africanus****;*** **4**-*E. racemosus****;*** **5** and **18**-***E.*** *ericoides****;*** **6**-***E.*** *capitellatus*; **7**-***E.*** *brevifolius*; **8**-***E.*** *aromaticus****;*** **9**-***E***. *spinescens*; **11**-***E.*** *decussatus*; **12**-***E***. *grandiflorus*; **13**-***E.*** *eximius*; **14**-*E. microphyllus*; **15**-*E. pauperrimus*; **17**-*E. namaquensis*.

Secondly, their ability to inhibit acetylcholinesterase adds to the list of plants that can be explored for the treatment of Alzheimer's disease. This is due to the fact that most of the conventional drugs used for the management of the disease have become less effective while continued use of others poses danger to the user. The factors in Alzheimer's disease include acetylcholine deficiency, free radicals, and inflammation of the brain tissue. Many of the current drugs taken to treat the disease, such as, donepezil, have unpleasant side effects and doctors are keen to find alternatives. There is no cure for Alzheimer's disease, but drugs designed to slow disease progression are available (Singhal *et al.,* 2012). Some herbs may help to improve brain function, but scientific evidence to prove that they can treat Alzheimer's disease, is limited, hence the preliminary results from the screening of *Eriocephalus* are promising and can be explored further (Singhal *et al.,* 2012)
The results of this study also support the use of the plants in the fumigation of houses after death, although it is more of a psychotropic ritual than actual therapy (Dyson, 1998; Van Wyk and Gericke, 2000).

Conclusions

This study provides an *in vitro* scientific validation for the use of some of the members of the genus in the treatment of respiratory ailments, dermal infections and the various gastro-intestinal disorders in traditional herbal practices. The anti- inflammatory and the antioxidant properties reported here indicate the great potential that exists in the genus for exploitation in traditional herbal therapy. The anti-inflammatory properties also confer credibility to the uses of the essential oils of some species in fragrances and cosmetics. The preliminary results in the screening for the presence of acetyl cholinesterase also support the use of the plants in fumigation of houses after death.

Acknowledgements

The authors acknowledge the former Third World Organization for Women in Science (TWOWS now OWSDW) for the generous study fellowship. The National Research Foundation (NRF) of South Africa and Medical Faculty Research Endowment Fund (Faculty of Health Sciences, University of the Witwatersrand) are hereby acknowledged for the research funding.

References

Adamson, R.S. and Salter, T.M. (1950). Flora of the Cape Peninsula. Juta, Cape Town.

Brantner, A. and Grein, E. (1994). Antibacterial activity of plant extracts used externally in traditional medicine. Journal of Ethnopharmacology 44: 35–40.

Amiri, H. (2011).Essential Oils Composition and Antioxidant Properties of Three *Thymus* Species Evidence Based Complementary and Alternative Medicine. DOI: 10.1155/2012/728065:PMCID: PMC3163135.

Cuendet, M., Hostettmann, K., Potterat, O. (1997). Iridoid glucosides with free radical scavenging properties from .*Fagraea blumei*. Helvetica Chimica Acta 80: 1144–1152.

Dyson A. (1998).Discovering Indigenous Healing Plants of Herb and Fragrance Gardens at Kirstenbosch National Botanical Garden. National Botanical Institute, Cape Town, South Africa. pp 33-34.

Eloff, J.N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica, 64:711-713.

Evans, A.T. (1987).Actions of cannabis constituents on enzymes of arachidonate metabolism: anti-inflammatory potential. Biochem. Pharmacol., 36**:** 2035-2037

Goldblatt, P. and Manning, J. (2000). Cape Plants. A Conspectus of the Flora of South Africa. NBI, South Africa and Missouri Botanical Garden, U.S.A.

Hudzicki, J. (2009). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. Protocols. American Society for Microbiology, Washington, DC. Http://www.microbelibrary.org. Accessed 14th October 2012.

Kulevanova, S. and Panovska,T.K. (2001). Antioxidant activity of essential oils of different wild *Thymus* L. Species. Bulletin of the Chemists and Technologists of Macedonia 20: 61-66.

Lourens, A.C.U., Reddy, D., Başer, K.H.C., Viljoen, A.M., Van Vuuren, S.F. (2004). In vitro biological and essential oil composition of four indigenous South African. *Helichrysum* species. Journal of Ethnopharmacology 95: 253–258.

Mambro, V.M., Azzolini, A.E.C.S., Valim, Y.M.L., Fonseca, M.J.V. (2003). Comparison of anti-oxidant activities of tocopherols alone and in pharmaceutical formulations. International Journal of Pharmaceutics 262: 93–99.

Marston, A., Kissling, J. and Hostettmann, K. (2002). A rapid TLC bioautographic method for the detection of acetylcholine esterase and butyrylcholinesterase inhibitors in plants. Phytochemical Analysis: 13:51– 54.

Mehta, M., Adem, A. and Sabbagh M. (2012). New Acetylcholinesterase inhibitors for Alzheimer's disease. International Journal of Alzheimer's Disease Vol. 2012. doi:10.1155/2012/728983.

Merle, H., Verdeguer, M., Blazquez, M.A. and Boira, H. (2007). Chemical composition of the essential oils from *Eriocephalus africanus* L. var. *africanus* populations growing in Spain. Flavour and Fragrance journal 22:461-464.

Müller, M.A.N., Herman, P.P.J. and Kolberg, H.H. (2001). Fascicle 1: *Eriocephalus* and *Lasiospermum*. Flora of Southern Africa 33: 1-63.

Njenga, E.W.(2005).The Chemotaxonomy, Phylogeny and Biological Activity of the genus Eriocephalus L (Asteraceae). PhD thesis, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

Njenga, E.W., van Vuuren S.F. and S.F., Viljoen, A. M. (2005). Antimicrobial activity of *Eriocephalus* L. species. South African Journal of Botany, 71:81-87.

Perry, E.K., Pickering, A.T., Wang, W.W., Houghton, P.J. and Perry, N.S.L. (1999).

Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy Journal of Pharmacy and Pharmacology 51: 527–534.

Sandasi, M., Kamatou, G.P.P. and Viljoen, A.M. (2011). Chemotaxonomic evidence suggests that *Eriocephalus tenuifolius* is the source of Cape chamomile and not *Eriocephalus punctulatus.* Biochemical Systematics and Ecology 39: 328-338.

Singhal, A.K., Naithani, V. and Bangar, O.P. (2012). Medicinal plants with a potential to treat Alzheimer and associated symptoms. International Journal of Nutrition, Pharmacology, Neurological Diseases 2:84-91.

Sircar, J.C. Shwender, C.J. and Johnson, E.A. (1983). Soybean lipoxygenase inhibition by nonsteroidal anti-inflammatory drugs. Prostaglandins. 25: 393-396.

Van Wyk, B-E (2008a). A broad review of commercially important southern African medicinal plants. Journal of Ethnopharmacology 119: 342-355.

Van Wyk, B-E (2008b). Antimicrobial activity of South African medicinal plants. Journal of Ethnopharmacology 119: 462-472.

Van Wyk, B-E. and Gericke, N. (2000). People's Plants. A Useful Guide to Useful Plants of Southern Africa. Briza Publications, South Africa.

Van Wyk, B-E., Van Oudtshoorn, B. and Gericke, N. (1997). Medicinal Plants of South Africa. Briza Publications. South Africa.

Viljoen, A.M., Njenga, EW.., Van Vuuren, S.F.., Bicchi, C., Rubiolo, P and Sgorbini, B. (2006). Essential oil composition and in vitro Biological Activity of seven Namibian species Eriocephalus L (Asteraceae). Journal of Essential Oil Research, 18:124-128.

Watt, J.M. and Breyer-Brandwijk, M.G. (1962).The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd Edn., E. and S. Livingstone, London

Wollenweber, E. and Mann, K. (1989). Exudate flavonoids in three essential oil plants from the Ciskei (South Africa). *Fitoterapia* Volume LX No. 3.

Zdero, C., Bohlmann F. and Müller, M. (1987). Sesquiterpene lactones and other constituents from *Eriocephalus* species. Phytochemistry 26**:** 2763–2775.