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In vitro biological activity and essential oil composition of four indigenous South African *Helichrysum* species

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Abstract

Helichrysum species are used widely to treat various medical conditions. In this study, the anti-microbial, anti-oxidant (DPPH assay) and anti-inflammatory activity (5-lipoxygenase assay) of *Helichrysum dasyanthum*, *Helichrysum felinum*, *Helichrysum excisum* and *Helichrysum petiolare* were investigated. The essential oil compositions of these species were determined. The acetone and methanol extracts as well as the essential oils exhibited activity against Gram-positive bacteria, while both the methanol and acetone extracts of all four species were active in the anti-oxidant assay. The essential oils, on the other hand, displayed activity in the 5-lipoxygenase assay, which was used as an indication of anti-inflammatory activity. Two extracts exhibited promising activity in the anti-microbial assay, the acetone extract of *Helichrysum dasyanthum* with a MIC value of 15.63 μg/ml and the methanol extract of *Helichrysum excisum* with a MIC value of 62.5 μg/ml. The acetone extract of *Helichrysum dasyanthum* was the most active free radical scavenger in the DPPH assay (IC_{50} of 9.53 μg/ml) while values for the anti-inflammatory activity of the essential oils ranged between 25 and 32 μg/ml. The essential oil compositions of three species (*Helichrysum dasyanthum*, *Helichrysum excisum* and *Helichrysum petiolare*) were dominated by the presence of monoterpenes such as α-pinene, 1,8-cineole and *p*-cymene. In the oil of *Helichrysum felinum*, monoterpenes were largely absent. Its profile consisted of a variety of sesquiterpenes in low concentrations with β-caryophyllene dominating.

Keywords: *Helichrysum dasyanthum*; *Helichrysum excisum*; *Helichrysum felinum*; *Helichrysum petiolare*; Anti-oxidant; Anti-inflammatory; Anti-microbial; 5-Lipoxygenase; DPPH

1. Introduction

Species from the genus *Helichrysum* (Asteraceae) are used in Europe (Czinner et al., 2000) and Africa (Hutchings et al., 1996; Hutchings and Van Staden, 1994; Van Wyk et al., 1997; Watt and Breyer-Brandwijk, 1962) in the treatment of various medical conditions. It is a very large genus consisting of approximately 500 species of which 245 are indigenous to South Africa (Hilliard, 1983). *Helichrysum* species are traditionally used in the treatment of wounds, infections and respiratory conditions (Hutchings et al., 1996; Van Wyk et al., 1997; Watt and Breyer-Brandwijk, 1962). Infection often leads to inflammation, and free radicals are released from phagocyte cells during the inflammatory process (Folkerts et al., 2001). Since many respiratory conditions and skin ailments such as atopic dermatitis (Tsukahara et al., 2003) are associated with inflammation (and hence release of free radicals) as well as infection, the presence of anti-inflammatory, anti-oxidant and anti-microbial agents could explain the effectiveness of plants from this genus in the treatment of these conditions.

The extracts as well as the essential oils from *Helichrysum* species have exhibited promising biological activities

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in many in vitro assays which include anti-oxidant (Czinner et al., 2000), anti-microbial (Meyer and Afolayan, 1995) and anti-inflammatory activity (Jäger et al., 1996). This genus has furthermore been a source of many fascinating (and bioactive) compounds (Bremner and Meyer, 2000; Dekker et al., 1983; Mathekga et al., 2000; Tomás-Barberán et al., 1990; Tomás-Lorente et al., 1989). Even though this large genus has been extensively investigated, the biological activity and/or chemical composition for many species still remain unrecorded. This is especially true for the essential oil composition of indigenous South African species. Furthermore, since *Helichrysum* species are distributed widely throughout South Africa, their medicinal use often depends on local availability rather than preference for a particular species (Van Wyk et al., 1997). In this study, the biological activity of four indigenous *Helichrysum* species were investigated. This was done with regard to their anti-microbial, anti-oxidant and anti-inflammatory activity as well as their essential oil composition as part of a continuing project to explore the phytochemistry and medicinal potential of South African flora.

2. Materials and methods

2.1. Plant material

Plant material of the three species *Helichrysum dasyanthum* (Willd.) Sweet, *Helichrysum excisum* (Thunb.) Less, and *Helichrysum felinum* Less were collected on Robinson's Pass in the Southern Cape. The sample of *Helichrysum petiolare* Hilliard and Burtt was obtained from the National Botanical Institute in Pretoria. The material was air-dried and ground. Voucher specimens were prepared and deposited at the Department of Pharmacy and Pharmacology, University of the Witwatersrand.

2.2. Preparation of extracts

Extracts were prepared using cold extraction over a period of 9 h (three extractions of 3 h each). Essential oils were obtained through hydrodistillation using a Clevenger apparatus (3 h).

2.3. Determination of anti-microbial activity

2.3.1. Disc diffusion

Extracts and essential oils were first screened for anti-microbial activity in a comparative disc diffusion assay to identify samples with activity. Samples were screened against *Staphylococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 11775), *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6051), *Yersinia enterocolytica* (ATCC 23715), *Klebsiella pneumoniae* (ATCC 13883), *Cryptococcus neoformans* (ATCC 90112) and *Candida albicans* (ATCC 10231). Paper discs saturated with a 128 mg/ml solution of plant extract were placed onto Tryptone Soya agar (Oxoid) which was inoculated with the pathogens. The plates were refrigerated for 1 h to allow pre-diffusion of the extracts from the disc into the seeded agar layer and then incubated at 37 °C for 24 h. The only exception was *Candida albicans*, which was incubated for 48 h. Zones of inhibition were measured in millimetre from the circumference of the discs to the circumference of the inhibition zone. Neomycin (30 μg/disc, Oxoid) and Nystatin (100 IU/disc, Oxoid) were used as positive controls for anti-bacterial and anti-fungal activities, respectively.

2.3.2. Determination of minimum inhibitory concentration (MIC)

MIC values were determined by a micro-titre plate dilution method (Eloff, 1998) for extracts that exhibited activity in the disc diffusion assay. Stock solutions were obtained by dissolving extracts in acetone. Sterile water (100 μl) was pipetted into all wells of the micro-titre plate before transferring 100 μl of stock solution to the plate. Serial dilutions were made (starting from the first well) to obtain concentrations ranging from 16 to 0.125 mg/ml. A fixed bacterial culture (100 μl) of approximate inoculum size of 1×10^6 cfu/ml was added to all wells and incubated at 37 °C for 24 h. After incubation, a 0.2 mg/ml *p*-iodo-nitrotetrazolium violet (INT) solution (Sigma) (40 μl) was added to each well. Plates were examined after 6 h to observe the colour change for the different concentrations. The concentration that inhibited bacterial growth completely (the first clear well) was taken as the MIC value. A starting concentration of 0.01 mg/ml Ciprofloxacin (Merck) was used as a positive bacterial control. MIC values were at least determined in duplicate.

2.4. Determination of anti-oxidant activity

A modified version of the DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Mambro et al., 2003) was employed in the determination of anti-oxidant activity. DPPH (Fluka), a stable free radical, was dissolved in analytical grade methanol (Ultrafine limited) to obtain a 96.2 μM solution. Extracts were dissolved in DMSO (Saarchem) and serial dilutions were made in order to obtain a 100 μg/ml solution in the first well of the micro-titre plate. Fifty microlitres of extract solution and DPPH (200 μl) or methanol (200 μl; to compensate for coloured extracts) was then added to alternating columns on the plate. Plates were shaken on an automated micro-titre plate reader (Labsystems Multiskan RC) for 2 min. The absorbance was read at 550 nm after 30 min and the percentage decolourisation calculated (Eq. (1)) as an indication of the anti-oxidant activity of a sample. Each experiment was done at least in duplicate and IC_{50} values were calculated using Enzfitter® software. Ascorbic acid was used as a positive control.

$$\text{Decolourisation}\,(\%) = \frac{(\text{Av controls} - (\text{Av sample}_{\text{DPPH}} - \text{Av sample}_{\text{methanol}})) \times 100}{\text{Av controls}} \quad (1)$$

Table 1
Anti-microbial, anti-oxidant and anti-inflammatory activity of selected *Helichrysum* species

Species	Anti-microbial activity				Anti-oxidant activity	Anti-inflammatory activity
	Disc diffusion assay (mm)[a]		MIC (μg/ml)[b]		DPPH IC_{50} (μg/ml)	5-Lipoxygenase IC_{50} (μg/ml)
	Bacillus cereus (ATCC 11778)	*Staphylococcus aureus* (ATCC 12600)	*Bacillus cereus* (ATCC 11778)	*Staphylococcus aureus* (ATCC 12600)		
Helichrysum dasyanthum						
Acetone extract[g]	14	9	312.5	15.63	9.53 (0.22)[c]	na[i]
Methanol extract	10	5	312.5	312.5	12.33 (2.28)	nd[j]
Essential oil	2	<1	4000	nd[d]	na[e]	31.24 (1.31)[f]
Helichrysum excisum						
Acetone extract	9	8	312.5	312.5	15.83 (0.00)	35.09 (1.12)
Methanol extract	8	4	62.5	2000	13.67 (0.23)	na[i]
Essential oil	15	0	2000	nd[d]	na[e]	27.62 (0.43)
Helichrysum felinum						
Acetone extract	8.5	7	156.25	156.250	17.68 (0.15)	38.77 (2.94)
Methanol extract	4	5	500	250	20.71 (5.42)	na[i]
Essential oil	1	0	nd[d]	nd[d]	na[e]	22.89 (7.59)
Helichrysum petiolare						
Acetone extract	4	2.5	500	312.5	44.28 (0.40)	na[i]
Methanol extract	9	7	312.5	625	28.70 (4.61)	na[i]
Essential oil[h]	0	<1	nd[d]	8000	na[e]	25.03 (0.57)
Control	6	6	0.08	0.31	2.47 (0.31)	5 (0.50)

[a] Since most extracts exhibited activity only against *Staphylococcus aureus* and *Bacillus cereus*, only these disc diffusion values are supplied.
[b] Each MIC value was at least determined in duplicate.
[c] Standard deviations supplied in brackets for the DPPH assay ($n = 2$, at least).
[d] Not done since almost none or no activity was observed in the disc diffusion assay.
[e] No activity observed at 100 μg/ml.
[f] Value in parentheses indicate the standard error calculated when data points were fitted to obtain a curve.
[g] The acetone extract of *Helichrysum dasyanthum* was the only extract that exhibited activity against other micro-organisms in the disc diffusion assay. (*Cryptococcus neoformans*, 1 mm; *Candida albicans*, 3 mm; and *Alternaria alternata*, 2 mm).
[h] The essential oil of *Helichrysum petiolare* was the only essential oil that exhibited activity against any other micro-organism in the disc diffusion assay (*Candida albicans*, 2 mm).
[i] Not active (IC_{50} value larger than 100 μg/ml).
[j] Not determined, insufficient extract.

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 $sample_{methanol}$ = average absorbance of sample wells with methanol.

2.5. Determination of 5-lipoxygenase activity

The 5-lipoxygenase assay was used as an indication of anti-inflammatory activity of the essential oils. Linoleic acid (Fluka) was used as substitute substrate for the 5-lipoxygenase enzyme (Cayman). Starting with approximately 100 μg/ml, three 1:1 dilutions in a mixture of DMSO (Saarchem) and Tween® 20 (Merck) were made for each essential oil. Absorbance was measured at 234 nm over a period of 10 min using a single beam spectrophotometer (Specord 40-analytikjena). Linoleic acid is enzymatically converted to a conjugated diene, which results in a continuous increase in absorbance at 234 nm (Schimizu et al., 1990). The standard assay mixture in the 3 ml cuvette consisted of 10 μl of dissolved plant extract, 0.1 M potassium phosphate buffer (pH 6.3), 100 μM linoleic acid and 100 U enzyme. Absorbance was plotted against concentration and the slopes of the straight-line portions of the sample and control curves were used to determine the percentage activity of the enzyme. A mixture of DMSO and Tween served as control (no enzyme inhibition) while the reported value (Baylac and Racine, 2003) for nordihydroguaiaretic acid (NDGA) was used as a positive control. The IC_{50} values (concentration that inhibited enzyme activity by 50%) were calculated using Enzfitter® software.

2.6. Determination of essential oil composition

The oils were analyzed by GC/MS. A quadrupole Hewlett-Packard GCD system with electron impact ionisation of 70 eV was used to obtain mass spectra. The system was equipped with an Innowax FSC column (60 mm × 0.25 mm) and helium used as carrier gas. Oven temperature for GC analysis was held isothermal at 60 °C for 10 min and programmed

to increase to 220 °C at a rate of 4 °C/min. Split flow was adjusted at 50 ml/min. Both injector and detector temperatures were at 250 °C and the mass range was from *m*/*z* 35 to 425. Percentage amounts were calculated by the computer from total ion chromatogrammes. Component identifications were made by comparing their mass spectra and retention indices using commercial libraries and Baser library of essential oil constituents. The results are presented in Table 2.

3. Results and discussion

Since anti-microbial activity was observed almost solely against Gram-positive bacteria, correlating with results from other studies (Mathekga et al., 2000; Meyer and Afolayan, 1995), only these results (Table 1) are supplied. The results indicate that high activity in the disc diffusion assay does not necessarily correlate to low MIC values in the micro-titre plate assay. The disc diffusion assay was only used as an indication of anti-microbial activity since the amount of extract or oil that adhered to the disc was not quantitatively determined.

The most promising results were obtained from the acetone extract of *Helichrysum dasyanthum*, which not only had the lowest MIC value (15.63 μg/ml against *Staphylococcus aureus*), but also inhibited the growth of some other micro-organisms. Flavonoids, kaurenoic acid derivatives, cadinenes and guaianolides (Jakupovic et al., 1989) were previously isolated from *Helichrysum dasyanthum*. Some of these molecules contain phenolic hydroxyl groups and since the presence of these groups is associated with anti-bacterial activity (Tomás-Barberán et al., 1990), this may in part account for the observed activity. However, although very similar phenolic hydroxyl containing compounds were previously isolated from *Helichrysum felinum* and *Helichrysum petiolare* (Jakupovic et al., 1989) extracts from these plants were much less active. In a previous study on *Helichrysum kraussii* (Bremner and Meyer, 2000), two compounds; prenyl-butyrylphloroglucinol and kaurenoic acids were isolated. Kaurenoic acid exhibited much higher activity than the phloroglucinol (which also contains phenolic hydroxyl groups) in the anti-microbial assay. The higher anti-microbial activity of *Helichrysum dasyanthum* might thus be explained by the presence of the kaurenoic acid derivatives, which had not been isolated from either *Helichrysum felinum* or *Helichrysum petiolare*. Further investigation is needed to ascertain whether the kaurenoic acid derivatives are in fact responsible for the promising anti-bacterial activity exhibited by *Helichrysum dasyanthum*. To the best of our knowledge, *Helichrysum excisum* has not yet been investigated, either chemically or biologically. Contrary to results previously reported for other species of *Helichrysum* (Roussis et al., 2000), the essential oils of these species showed very little activity in the anti-microbial assay.

The presence of flavonoids in the solvent extracts of the selected species would also account for the high radical

Table 2
Main components (%) identified in the hydrodistilled essential oil of the four *Helichrysum* species

RRI	Compound	Hd	He	Hf	Hp
1032	α-Pinene	16.6	2.8	–	6.8
1072	α-Fenchene	tr	–	–	0.7
1076	Camphene	0.3	tr	–	0.4
1118	β-Pinene	6.2	1.1	–	0.4
1132	Sabinene	tr	0.2	–	–
1174	Myrcene	1.0	0.7	–	0.5
1188	α-Terpinene	3.5	–	–	0.1
1203	Limonene	6.1	2.2	–	3.1
1213	1,8-Cineole	20.6	34.0	–	22.4
1246	(*Z*)-β-Ocimene	0.9	–	–	0.1
1255	γ-Terpinene	1.1	–	–	1.0
1266	(*E*)-β-Ocimene	0.2	–	–	tr
1280	*p*-Cymene	5.5	6.5	–	9.8
1290	Terpinolene	0.4	–	–	0.3
1286	2-Methyl butyl-3-methyl butyrate	–	0.7	–	–
1348	6-Methyl-5-hepten-2-one	tr	0.1	–	tr
1385	Heptyl acetate	0.1	–	–	–
1406	α-Fenchone	–	–	–	0.1
1452	α,*p*-Dimethylstyrene	0.1	–	–	0.1
1452	1-Octen-3-ol	0.1	–	0.1	0.1
1482	Fenchyl acetate	0.5	–	–	–
1492	Cyclosativene	–	0.1	–	–
1493	α-Ylangene	–	–	0.4	–
1495	α-Amorphene	–	1.3	1.0	–
1497	α-Copaene	0.1	2.5	4.0	1.3
1532	Camphor	tr	tr	–	0.1
1544	α-Gurjunene	–	0.1	1.0	0.1
1553	Linalool	–	–	0.2	–
1557	Italicene	–	–	–	1.0
1566	α-Santalene	–	0.8	–	–
1573	Iso-italicene	–	–	–	0.1
1586	Pinocarvone	–	0.1	–	–
1591	Fenchyl alcohol	0.5	0.3	–	1.5
1594	*Trans*-β-bergamotene	–	0.1	0.9	–
1596	α-Guaiene	0.8	–	–	–
1604	Thymol methyl ether	–	–	–	0.6
1612	β-Caryophyllene	13.3	5.7	27.6	14.0
1628	Aromadendrene	–	–	2.2	0.3
1632	Selina-5,11-diene	–	–	0.7	–
1638	*Cis*-*p*-menth-2-en-1-ol	–	0.1	–	–
1648	Myrtenal	–	0.1	–	–
1661	Alloaromadendrene	–	0.7	7.3	–
1664	*Trans*-pinocarveol	0.2	–	–	0.4
1674	γ-Gurjunene	0.1	0.1	–	0.1
1682	δ-Terpineol	0.2	–	–	tr
1687	α-Humulene	0.6	0.2	9.4	2.0
1683	*Trans*-verbenol	–	0.7	–	
1694	Limonene-4-ol	–	0.1	–	
1695	(*E*)-β-Farnesene	–	–	0.1	
1698	Myrtenyl acetate	–	–	–	0.8
1704	γ-Muurolene	–	–	1.8	2.2
1706	α-Terpineol	0.5	0.8	–	5.1
1719	Borneol	0.3	1.2	–	3.3
1729	*Cis*-1,2-epoxy-terpin-4-ol	–	0.1	–	0.4
1740	α-Muurolene	–	–	–	2.2
1740	Valencene	–	–	2.2	–
1741	δ-Guaiene	1.5	–	–	–
1744	α-Selinene	–	–	1.8	–
1758	*Cis*-piperitol	–	0.1	–	–
1765	Geranyl acetate	–	–	–	0.1
1773	δ-Cadinene	0.2	0.3	2.2	0.7

Table 2 (*Continued*)

RRI	Compound	Hd	He	Hf	Hp
1776	γ-Cadinene	–	0.1	2.0	–
1786	Ar-curcumene	–	–	–	0.6
1798	α-Dihydroagarofuran	3.4	–	–	–
1796	Selina-3,7-(11)-diene	–	–	–	0.6
1799	Cadina-1,4-diene	–	–	0.1	–
1804	Myrtenol	–	0.1	–	–
1838	2-Phenyl ethyl acetate	0.1	–	–	–
1845	*Trans*-carveol	0.1	–	–	0.1
1810	3,7-Guaiadiene	–	–	0.2	0.1
1853	*Cis*-calamenene	tr	0.1	0.8	0.3
1864	*p*-Cymen-8-ol	0.1	0.2	–	0.3
1868	(*E*)-Geranyl acetone	–	0.3	0.1	0.1
1889	Ascaridol	0.1	–	–	–
1918	β-Calacorene	–	–	tr	–
1933	Neryl valerate	–	–	–	0.3
1941	α-Calacorene	0.5	0.1	0.5	0.1
1953	Palustrol	0.2	–	–	0.3
1984	γ-Calacorene	–	–	tr	–
2001	Isocaryophyllene oxide	0.2	0.4	0.2	0.6
2008	Caryophyllene oxide	1.3	2.1	6.9	2.5
2029	Perilla alcohol	–	–	0.2	–
2033	Epiglobulol	–	–	0.6	–
2045	Humulene epoxide I	–	–	0.3	0.1
2050	(*E*)-Nerolidol	0.2	0.8	–	0.1
2057	Ledol	0.3	–	0.6	0.2
2088	6-Epi-cubenol	–	1.2	–	–
2071	Humulene epoxide II	–	–	1.7	0.7
2073	β-Caryophyllene alcohol	0.1	–	1.4	1.9
2080	Cubenol	–	0.1	1.0	–
2098	Globulol	–	–	1.4	0.2
2104	Viridiflorol	–	18.2	0.7	0.9
2144	Rosifoliol	–	–	0.4	–
2144	Spathulenol	–	–	0.6	0.2
2170	β-Bisabolol	–	–	–	0.8
2184	*Cis*-*p*-menth-3-en-1,2-diol	0.3	–	–	–
2187	T-cadinol	–	0.1	0.6	0.2
2193	β-Betulenal	0.5	–	–	–
2203	Copaborneol	–	3.4	2.2	–
2238	Clovenol	0.1	–	0.9	0.1
2239	Carvacrol	–	0.2	–	0.1
2240	Pogostol	0.2	–	–	–
2250	α-Eudesmol	–	–	0.3	–
2255	α-Cadinol	–	0.1	1.0	0.2
2256	Cadalene	0.1	–	–	–
2257	β-Eudesmol	–	0.1	–	–
2262	Intermedeol	–	1.8	–	–
2273	Selin-1-en-4α-ol	–	–	0.2	–
2310	14-Acetoxy-β-caryophyllene	0.2	–	–	–
2316	Caryophylladienol I	–	–	0.2	–
2324	Caryophylladienol II	0.5	0.4	1.5	0.4
2346	Kaur-15-ene	–	–	–	0.3
2363	10-Hydroxy-β-caryophyllene	5.2	–	–	–
2389	Caryophyllenol I	–	0.2	0.8	0.2
2390	10-Hydroxy calamenene	–	0.2	0.1	–
2392	Caryophyllenol II	0.2	–	1.0	0.3
2438	Kaur-16-ene	0.7	–	–	1.3
2498	1-Heptadecanol	–	–	0.2	–
2518	*Cis*-lanceol	–	0.4	–	–
	Total	96.1	94.4	91.6	96.5

Abbreviations: RRI = relative retention indices, Hd = *Helichrysum dasyanthum*, He = *Helichrysum excisum*, Hf = *Helichrysum felinum*, Hp = *Helichrysum petiolare*, and tr = less than 1%.

scavenger activity observed. However, flavonoids are also associated with anti-inflammatory activity (Sala et al., 2003). Disappointingly, only two of the extracts exhibited promising activity (IC_{50} values less than 100 μg/ml) in the 5-lipoxygenase assay. Inflammation is a very complex process and these extracts may exhibit better activity if another assay is employed (e.g., COX-1 and COX-2 inhibition). The essential oils, on the other hand, were more active than the extracts in the 5-lipoxygenase assay, but were not active at concentrations of 100 μg/ml in the anti-oxidant assay.

The GC/MS analyses of the oils led to the identification of the major components with their percentages as shown in Table 2. The compositions of three species (*Helichrysum dasyanthum, Helichrysum excisum* and *Helichrysum petiolare*) were dominated by the presence of monoterpenes such as 1,8-cineole (20–34%), α-pinene (3–17%), and *p*-cymene (6–10%). This correlates with results previously obtained from other Southern African species (Lwande et al., 1993; Ramanoelina et al., 1991). For all three species, 1,8-cineole was the major constituent with viridiflorol (18.2%) (sesquiterpene) also present in high concentration in *Helichrysum excisum*. The profile of *Helichrysum felinum* was totally different from that of any of the other species, with the monoterpenes largely absent. Its profile consisted of a variety of sesquiterpenes in low concentrations with β-caryophyllene (27.6%) dominating together with α-humulene (9.4%), alloaromadendrene (7.3%) and caryophyllene oxide (6.9%) as main constituents. This correlates with data obtained for Greek species where sesquiterpenes also dominated (Roussis et al., 2000). It has been reported that 1,8-cineole possesses anti-inflammatory activity (Juergens et al., 1998) and terpenes such as α-pinene and sesquiterpenes such as β-caryophyllene exhibited activity in the in vitro 5-lipoxygenase assay (Baylac and Racine, 2003). The presence of these components may contribute to the activity of these essential oils as observed in the 5-lipoxygenase assay. Seasonal and population variability was however not taken into account, and further investigation is necessary to obtain a more complete picture of the chemical composition of these oils.

Extracts and/or essential oils of the four selected species thus exhibited biological activity in all of the selected assays. The extracts generally exhibited better activity than the essential oils in the anti-microbial and anti-oxidant assays, while the essential oils were more active than the extracts in the 5-lipoxygenase assay. With regard to the complex chemical composition of the essential oils, it is quite interesting to note that some chemical compounds present in certain members of the genus are totally absent in others. This variation in chemical composition is however not mirrored in the biological results, since all the essential oils exhibited remarkably similar activities in all of the assays. Combinations of different chemical entities may thus result in similar biological activity. These in vitro results provide some scientific validation for the widespread use of plants from this genus in traditional medicine.

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