Chemical Composition and *in vitro* Antimicrobial Activity of the Essential Oils of Two *Helichrysum* Species from Tanzania

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The chemical composition of the essential oils obtained from the aerial parts of *Helichrysum cymosum* and *H. fulgidum*, from Tanzania, were analyzed by GC and GC/MS. A total of sixty-five compounds, representing 92.4% and 88.2% of the two oils, respectively, were identified. *trans*-Caryophyllene, caryophyllene oxide, β-pinene, *p*-cymene, spathulenol and β-bourbonene were found to be the main components. Furthermore, the oils were tested against six gram (±) bacteria and three pathogenic fungi. It was found that the oil of *H. fulgidum* exhibited significant antimicrobial activity, while the oil of *H. cymosum* was not active at all.

Key words: Helichrysum cymosum and *fulgidum*, Volatiles, Antimicrobial Activity

Introduction

The genus *Helichrysum* (Asteraceae) consists of about 500 species. The 245 *Helichrysum* taxa found in Southern Africa, are divided in 30 groups (Hilliard, 1983). *Helichrysum cymosum* and *H. fulgidum* are aromatic perennial herbs with yellow flowers and characteristic odors, which are widespread in southern tropical Africa. Of all *Helichrysum* species occurring in Southern Africa, *H. cymosum*, *H. odoratissimum*, *H. petiolare* and *H. nudifolium* are among the best known and commonly used plants. The smoke of many *Helichrysum* species is used as ritual incense, called "inphepho". There are several different ways of administering these traditional medicines. For coughs and colds, a tea is prepared or the leaves are boiled in milk. For pain relief, leaves are burned and the smoke is inhaled. Leaves are widely used on wounds to prevent infection. Proven antimicrobial activity of these plants will provide scientific evidence for traditional use in wound dressing.

Recent studies on essential oils from African *Helichrysum* species include those on oils of *H. bracteiferum*, *H. cordifolium*, *H. faradifani*, *H. gymnocephalum*, *H. hypnoides*, *H. kraussii*, *H. odoratissimum*, *H. rugulosum*, *H. rusillonii*, *H. serlaginifolium*, and *H. splendidum* (Ramanoelina *et al.*, 1992; De Medici et al., 1992; Gundidza and Zwaving, 1993; Lwande *et al.*, 1993; Theron et al., 1994; Möllenbeck *et al.*, 1997; Chagonda *et al.*, 1999; Kuiate *et al.*, 1999; Cavalli *et al.*, 2001; Baser *et al.*, 2002; Bougatsos *et al.*, 2003).

As a part of a systematic research on the chemical composition of *Helichrysum* species (Chinou *et al.*, 1996, 1997; Roussis *et al.*, 1998, 1999, 2000, 2002), we report in this study the chemical constituents and antimicrobial activity of the essential oils obtained from the aerial parts of *Helichrysum cymosum* and *H. fulgidum* which to the best of our knowledge have never been studied before.

Materials and Methods

Plant materials

The aerial parts of the studied plants were collected in Rungwe district, Mbeya region, Tanzania, during their flowering period in March 2000. *Helichrysum cymosum* (L.) Less. was collected from Kasanga-Mwakaleli village, while *H. fulgidum* (L.) Willd. was collected from Isongole village. The plants were authenticated by comparison with Herbarium specimens by the staff of the Department of Botany, University of Dar es Salaam. Voucher specimens have been deposited in the

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Herbarium of the Department of Pharmacognosy, School of Pharmacy, Muhimbili University College of Health Sciences. The plant materials were air-dried indoors, prior to isolation of the essential oils.

Isolation of essential oils

Plant material (200 g) of each plant was subjected to steam distillation for 3 h (Hellenic Pharmacopeia, 1989), in a Clevenger modified apparatus with a water-cooled oil receiver to reduce overheating artifacts. The essential oils were collected over water, separated, then dried over anhydrous sodium sulfate and stored at 4–6 °C until they were analyzed. The two oils were light yellow with distinct sharp odors and yielded 0.21% v/w and 0.24% v/w, respectively.

GC/MS analysis

The GC/MS analysis of the essential oils was performed using a Hewlett Packard 6890 series II gas chromatograph equipped with an HP-5 capillary column (30 m x 0.25 mm, 0.25 μm film thickness) and a mass spectrometer 5973 of the same company, which was operated on EI mode. Helium was the carrier gas at a flow rate of 1 ml/min. The injector was operated at 200 °C and the oven temperature was programmed as follows: 60 °C for 5 min, then gradually increased to 280 °C at a rate of 3 °C/min. The same program was used for GC analyses.

The identification of components was based on comparison of their mass spectra with those of Wiley and NBS Libraries (Massada, 1976) and those described by Adams (2001), as well as on comparison of their retention indices (Van den Dool and Kratz, 1963) with literature values (Adams, 2001).

Antimicrobial assay

The antimicrobial activities of the essential oils were determined using the dilution technique. Minimum inhibitory concentrations (MICs) were determined for the oils (Janssen *et al.*, 1987) against the two gram positive bacteria *Staphylococcus aureus* (ATCC 25923) and *S. epidermidis* (ATCC 12228), the four gram negative bacteria *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883) and *Pseudomonas aeruginosa* (ATCC 227853), as well as against the pathogenic fungi

Table I. Main components (%) of *Helichrysum cymosum* and *H. fulgidum*.

Compound[a]	*H. cymosum*	*H. fulgidum*	KI[b]
α-Terpinene	2.82	–	905
α-Pinene	0.75	1.69	935
Camphene	–	0.59	950
α-Fenchene	6.25	–	951
β-Pinene	–	8.72	976
1-Octen-3-ol	0.80	–	980
Myrcene	0.37	–	990
Δ-3-Carene	6.84	–	1022
p-Cymene	7.55	–	1030
1,8-Cineole	–	1.14	1032
cis-β-Ocimene	0.25	–	1039
trans-β-Ocimene	0.22	–	1049
γ-Terpinene	0.09	–	1058
trans-Linalool oxide	0.11	0.48	1071
α-Terpinolene	0.27	–	1086
cis-Linalool oxide	–	0.62	1088
Linalool	1.62	3.91	1099
α-Campholenal	–	0.36	1125
endo-Fenchol	0.20	–	1127
(+)-Nopinone	–	0.97	1137
1-Terpineol	0.04	–	1138
trans-Pinocarveol	–	3.18	1140
Camphor	0.04	5.35	1142
endo-Isocamphane	0.06	–	1148
Phellandral	0.09	–	1154
Pinocarvone	–	1.45	1162
Borneol	0.22	1.82	1165
Terpinene-4-ol	0.37	–	1174
p-Cymen-8-ol	0.24	–	1182
β-Fenchyl alcohol	0.88	1.31	1190
Myrtenal	–	5.28	1192
Safranal	0.04	–	1195
α-Phellandrene epoxide	0.24	–	1198
Hexyl-2-methylbutanoate	–	1.97	1208
Nerol	0.36	–	1222
Furfuryl alcohol	0.18	–	1335
Neryl acetate	0.66	–	1364
α-Ylangene	0.45	–	1368
α-Copaene	1.83	0.28	1373
β-Bourbonene	0.18	7.11	1381
β-Elemene	–	2.23	1389
trans-(+)-Carveol	0.09		1396
trans-Caryophyllene	27.02	–	1425
(+)-Aromadendrene	1.42	–	1440
Germacrene-D	–	2.31	1471
γ-Muurolene	–	2.99	1473
α-Amorphene	3.65	1.84	1477
Valencene	1.89	–	1492
α-Muurolene	1.51	1.28	1495
Bicyclogermacrene	0.85	–	1498
γ-Cadinene	1.95	1.34	1509
E,e-α-Farnesene	1.60	–	1516
δ-Cadinene	4.95	–	1520
β-Calacorene	0.41	–	1559
α-Caryophyllene alcohol	1.20	–	1568
Caryophyllenyl alcohol	1.10	–	1570
(+)-Spathulenol	–	7.88	1572

Table I. (cont.).

Compound[a]	*H. cymosum*	*H. fulgidum*	KI[b]
Caryophyllene oxide	7.65	12.45	1579
Viridiflorol	0.70	–	1599
α-Cadinol	0.72	–	1632
δ-Cadinol	0.26	–	1646
t-Muurolol	1.20	7.31	1650
Vulgarol-β	0.13	–	1712
Aromadendrene epoxide	0.08	–	1749
δ-Fenchane	–	2.34	1776
Total	*92.44*	*88.20*	

[a] Compounds listed in order of elution from a HP-5 MS column.
[b] Kovats Indices (KI) on HP-5 MS capillary column.

Candida albicans (ATCC 10231), *C. tropicalis* (ATCC 13801) and *C. glabrata* (ATCC 28838). Standard antibiotics (netilmicin and amphotericin B) were used in order to control the sensitivity of the tested bacteria and fungi, respectively. Technical data have been described previously (Magiatis *et al.*, 2002). MIC values were also determined for standard pure compounds under identical conditions for comparison purposes. The standards included: β-caryophyllene, caryophyllene oxide, β-pinene, spathulenol, linalool and camphor.

Results and Discussion

Sixty-five phytochemicals were identified by the GC and GC/MS analyses as constituents of the essential oils. The main components with their percentages and retention indices are listed in Table I, while the results of the antibacterial and antifungal activities of the essential oils and their main components are presented in Table II.

Fifty components were identified and quantified from the essential oil of *H. cymosum*, 92.44% of the total oil. The major components were: *trans*-caryophyllene (27.02%), caryophyllene oxide (7.65%), *p*-cymene (7.55%), Δ-3-carene (6.84%) and α-fenchene (6.25%).

Twenty-eight constituents were identified and quantified in the oil of *H. fulgidum*, representing 88.22% of the total oil. The major constituents of *H. fulgidum* were caryophyllene oxide (12.45%), β-pinene (8.72%), spathulenol (7.88%), *t*-muurolol (7.31%), β-bourbonene (7.11%) and camphor (5.35%).

The chemical compositions of the two oils do not show much similarity, although the plant species belong to the same genus. Of all the identified components, only caryophyllene oxide occurs in appreciable amounts in both species.

In the antimicrobial screening, the oil of *H. fulgidum* exhibited significant antibacterial and anti-

Table II. Antimicrobial activities (MIC in mg/ml and in mM) of the two studied *Helichrysum* essential oils and their main components.

Essential oil	*S. aureus*	*S. epidermidis*	*P. aeruginosa*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*C. albicans*	*C. tropicalis*	*C. glabrata*
H. cymosum	–	–	–	–	–	–	–	–	–
H. fulgidum	0.37	0.35	0.75	1.25	1.25	2.50	2.50	1.75	1.50
β-Caryophyllene	> 20 (9.8×10^{-2})	> 20 (9.8×10^{-2})	> 20 (9.8×10^{-2})	> 20 (9.8×10^{-2})	> 20 (9.8×10^{-2})	> 20 (9.8×10^{-2})	> 20 (9.8×10^{-2})	> 20 (9.8×10^{-2})	> 20 (9.8×10^{-2})
Caryophyllene oxide	0.073 (3.3×10^{-4})	0.90 (4.1×10^{-3})	0.87 (3.91×10^{-3})	1.23 (5.6×10^{-3})	2.43 (1.1×10^{-2})	> 6.40 (2.9×10^{-2})	–	–	–
β-Pinene	12 (8.8×10^{-2})	16 (0.147)	> 20 (0.147)	> 20 (0.147)	> 20 (0.147)	9.75 (7.2×10^{-2})			
Spathulenol	1.35 (6.1×10^{-3})	1.50 (6.8×10^{-3})	> 20 (9.1×10^{-2})	> 20 (9.1×10^{-2})	> 20 (9.1×10^{-2})	8.5 (3.9×10^{-2})			
Linalool	0.25 (1.6×10^{-3})	0.25 (1.6×10^{-3})	> 20 (0.13)	1.75 (1.1×10^{-2})	> 20 (0.13)	1.25 (8×10^{-3})			
Camphor	2.7 (1.8×10^{-2})	1.95 (1.3×10^{-2})	2.80 (1.8×10^{-2})	2.75 (1.8×10^{-2})	1.33 (9×10^{-2})	1.33 (9×10^{-2})	4.85 (3.2×10^{-2})	3.76 (2.5×10^{-2})	3.56 (2.3×10^{-2})
Amphotericin B							1×10^{-3} (1×10^{-6})	0.5×10^{-3} (0.5×10^{-6})	0.4×10^{-3} (0.4×10^{-6})
Netilmicin	4×10^{-3} (8.9×10^{-6})	4×10^{-3} (8.9×10^{-6})	8.8×10^{-3} (1.9×10^{-5})	8×10^{-3} (17.8×10^{-5})	8×10^{-3} (1.18×10^{-5})	10×10^{-3} (2.21×10^{-5})	–	–	–

fungal activities (MIC values 0.35–2.50 mg/ml), while that of *H. cymosum* was completely inactive. Extracts of *H. cymosum* were also found to have no appreciable antimicrobial activity in a previous study by Sindabiwe *et al.* (1999). It is remarkable, that the oil of *H. fulgidum* exhibited a more specific activity mostly against the gram positive bacteria *S. aureus* and *S. epidermidis*. This antimicrobial activity is suspected to be associated with the high percentage of caryophyllene oxide, spathulenol, β-pinene, camphor and linalool, which exhibited also moderate to strong activities against the assayed microorganisms. Besides, the antibacterial properties of caryophyllene oxide have been reported previously (Magiatis *et. al.*, 2002) while among the main compounds of the oil, which have not been assayed, *t*-muurolol is known for its antifungal activity against *Coriolus versicolor* and *Laetiporus sulphurous* (Chang *et al.*, 2000), and myrtenal inhibits the growth of plant pathogenic fungi (Saito *et al.*, 1996).

The complete phytochemical profile of the essential oils of the two studied *Helichrysum* species as identified by GC/MS analysis and their retention indices, are available to interested readers on request from the authors.

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