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Short Communication

Chemical analysis and antimicrobial activity of essential oil extracted from *Helichrysum aureonitens*

VV Yani[1], OA Oyedeji[2], DS Grierson[1] and AJ Afolayan[1]*

[1] *Department of Botany, University of Fort Hare, Alice 5700, South Africa*
[2] *Department of Chemistry, Lagos State University, PMB 1087, Apapa, Lagos, Nigeria*
* *Corresponding author, e-mail: Jide@eastcape.net*

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Helichrysum **species are well distributed throughout South Africa and are commonly used in ethnomedicine. In the Eastern Cape, *H. aureonitens* is often used by traditional healers to treat wounds. Chemical analysis of the essential oil obtained through hydrodistillation of the aerial part of the plant revealed pinene, limonene, 1,8-cineole and α-terpineol as the prominent compounds of the oil. Eight compounds were identified from the essential oil of the herb, for the first time. The oil had a significant inhibitory effect on Gram-positive and Gram-negative bacteria. Based on its high content of 1,8-cineol oil it was classified as a medicinal essential oil.**

Helichrysum aureonitens, commonly known as impepho-emhlophe or golden everlasting, is commonly used in traditional medicine in the Eastern Cape Province of South Africa. It is a perennial aromatic plant found in mountain areas, rocky outcrops and in open grasslands (Pooley 1998). The shoots, and sometimes the roots, are used for the treatment of many ailments including coughs, colds, fever, wounds, headaches and menstrual pain (Watt and Breyer-Brandwijk 1962, Smith 1966, Hutchings and Van Staden 1994, Hutchings *et al.* 1996). The people of the KwaZulu-Natal Province have been using extracts from the herb topically for many generations, against skin infections (Meyer *et al.* 1997). The herb was listed among the medicinal plants sold by wholesalers in South Africa (National Department of Agriculture 1995).

The dichloromethane and methanol extracts of *H. aureonitens* shoots showed significant inhibition against the Gram-positive bacteria *Bacillus cereus*, *B. pumilus*, *B. subtilis*, *Micrococcus kristinae* and *Staphylococcus aureus* (Meyer and Afolayan 1995). A flavone, 3,5,7-trihydroxyflavone (galangin), isolated from the acetone extract of the herb (Afolayan and Meyer 1997) showed a broad-spectrum antimicrobial activity against bacteria, fungi and viruses (Meyer *et al.* 1997, Afolayan and Meyer 1997). Despite the apparent extensive available information on the antimicrobial activity of extracts from the plant and the demand for the herb on the international market, there is no literature on the chemical profile and antimicrobial potential of its essential oil. This study was therefore undertaken to investigate the composition and antimicrobial property of the essential oil of *H. aureonitens*.

Fresh plant material was collected from the Hogsback mountains in the Eastern Cape and air-dried at room temperature for five days. Essential oil was extracted from the plant (600g) by steam distillation for three hours, using a Clevenger apparatus (British Pharmacopoeia 1980).

GC-MS analysis of the oil was performed on a Hewlett Packard HP-6890 gas chromatography system interfaced with an HP-5973 mass spectrometer. Electron ionisation was at 70eV with an ion source temperature of 240°C. The column was an HB-5 (30m x 1.25mm, id), similar to DB-5; film thickness was 0.25µm, while helium was the carrier gas. The oven temperature was increased from 70°C to 240°C with a rise of 5°C per min^{-1}. The essential oil was diluted two-fold with hexane and 0.2µl of the diluted oil was injected into the GC-MS. n-Alkane was run at the same conditions for retention indices determination. Constituents of the oil were identified by comparison of their mass spectra and Kovat indices with literature (Adams 1989, Joulain and Koenig 1998, ESO 1999).

Three Gram-positive (*Bacillus cereus*, *Micrococcus kristinae* and *Staphylococcus epidermidis*) and three Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Serratia marcescens*) laboratory-cultured bacterial strains were obtained from the Department of Microbiology at Rhodes University.

The minimum inhibitory concentration (MIC) values of the essential oil against the bacteria were determined with a microplate dilution method using 96-well microtiter plates (Eloff 1998). Acetone (40µl) was added to the essential oil in 40µl of hexane. Each organism was maintained in a nutrient agar slant (Biolab) and was recovered for testing

Table 1: Chemical constituents of the essential oil of *H. aureonitens*

KI[1]	Compound	MS peak[2]	Peak % area
928	α-pinene	93[a],77,41,136[b]	7.7
942	α-fenchene	93[a],121,79,136[b]	0.2
944	camphene	93[a],79,121,136[b]	0.9
974	β-pinene	93[a],41,69,136[b]	0.7
978	6-methyl-5-hepten-2-one	43[a],56,61,127[b]	3.8
983	β-myrcene	41[a],93,69,136[b]	1.5
1 006	n-hexyl acetate	43[a],56,61,144[b]	0.6
1 022	*para*-cymene	119[a],134[b],91,41	1.9
1 026	limonene	67[a],93,79,136[b]	8.0
1 030	1,8-cineole	43[a],81,55,154[b]	21.5
1 042	*trans*-β-ocimene	91[a],79,67,136[b]	0.5
1 056	γ-terpinene	93[a],77,136[b],121	1.0
1 068	*cis*-pinene hydrate*	43[a],79,69,154[b]	2.0
1 071	*cis*-linalool oxide	43[a],59,93,170[b]	1.1
1 089	α-terpinolene	93[a],121,136[b],79	1.1
1 096	linalool	43[a],71,55,154[b]	1.5
1 108	1-octen-3-yl acetate*	43[a],99,54,172[b]	4.9
1 115	fenchol*	81[a],43,67,154[b]	1.3
1 120	3-octanyl acetate*	43[a],83,55,172[b]	0.4
1 153	hexyl isobutyrate*	43[a],89,71,172[b]	0.4
1 169	borneol	95[a],41,67,154[b]	3.3
1 180	terpinen-4-ol	43[a],71,93,154[b]	2.5
1 193	α-terpineol	59[a],43,93,154[b]	6.1
1 289	bornyl acetate*	43[a],95,121,196[b]	0.5
1 345	*trans*-geraniol	41[a],69,81,154[b]	0.3
1 365	neryl acetate	43[a],69,107,194[b]	1.3
1 382	α-copaene	105[a],119,161,204[b]	0.6
1 461	α-humulene	93[a],80,67,204[b]	1.0
1 466	allo-aromadendrene	41[a],91,79,204[b]	0.8
1 482	α-amorphene*	161[a],93,119,204[b]	0.4
1 506	α-muurolene	105[a],41,91,204[b]	0.6
1 529	δ-cadinene	119[a],161,105,204[b]	1.2
1 648	α-cadinol	43[a],95,121,204[b]	0.6

* Compounds identified for the first time in the essential oil of *Helichrysum* species

[1] KI = Kovat indices

[2] MS peak, major fragmentation with peak intensity in a decreasing order, where a = base peak; b = molecular ion peak (M^+)

by growth in nutrient broth No. 2 (Biolab) for 24hr. Before use, each culture was diluted 100-fold with fresh, sterile nutrient broth. The microtiter plates were prepared using serial dilution starting with 5mg per ml^{-1} and incubated for 24–48h at 37°C. As an indicator of bacterial growth, 40µl of 0.2mg per ml^{-1} p-Iodonitrotetrazolium (p-INT) solution was added to each well and incubated at 37°C for 30min. The colourless tetrazolium salt was reduced to a red-coloured product by biological activity of the organisms, thereby making the inhibition of the bacterial growth visible as clear wells. MIC values were recorded as the lowest concentration resulting in complete inhibition of bacterial growth. Each treatment was replicated three times. Streptomycin and chloramphenicol (5mg per ml^{-1} each) as well as pure solvents and sample-free solution were used as controls.

Steam distillation of *H. aureonitens* yielded a light yellow oil with 0.4% yield (w/w). A total number of 42 compounds were detected by the GC-MS analysis of the oil, out of which 32 compounds (80%) were identified. The oil was dominated by monoterpenoids (74.8%) with oxygenated monoterpenes accounting for 49.5%. Total hydrocarbon content was 30.17%. The percentage sesquiterpenoid constituent of the oil was very low (5.2%) with only one oxygenated sesquiterpene (0.56%) identified (Table 1).

The main components of the essential oil (Table 1) were identified as 1.8-cineole (21.5%), limonene (8.0%), *a*-pinene (7.7%), a-terpineol (6.1%), 1-octen-3-yl acetate (4.9%), 6-methyl-5-hept-2one (3.8%) and borneol (3.3%). Seven minor compounds (0.4–4.9%) – *cis*-pinene hydrate, 1-octen-3-yl acetate, 3-octanyl acetate, hexyl isobutyrate, fenchol, bornyl acetate and amorphene – were identified for the first time as constituents of essential oil from *Helichrysum* species.

The essential oil of *H. aureonitens* exhibited significant antimicrobial activity against Gram-positive and Gram-negative bacteria with MICs in the range of 2.500–0.039mg per ml^{-1}. It was interesting to note that the oil inhibited the growth of *S. marcescens* at 1.25mg per ml^{-1} whereas streptomycin and chloramphenicol did not exhibit such growth inhibition, even at concentrations of 1.25mg per ml^{-1} (Table 2).

According to ITC Market Study (1986) and Sticher (1977), this oil can be classified as medicinal because of its high 1,8-cineole content (21.5%). This probably provides some pharmaceutical rationale for the popular use of this plant in traditional herbal medicine.

Table 2: MIC values in mg per ml^{-1} of the essential oil from *H. aureonitens*, two positive control antibiotics and the negative solvent control acetone

Bacterial species	Essential oil	Streptomycin	Chloramphenicol	Acetone
Gram +ve				
Bacillus cereus	>1.250	>1.250	0.039	>200
Micrococcus kristinae	0.039	0.039	0.039	>200
Staphylococcus epidermidis	0.039	0.039	>1.250	>200
Gram -ve				
Escherichia coli	0.039	0.039	0.039	>200
Pseudomonas aeruginosa	>1.250	0.039	0.039	>200
Serratia marcescens	1.250	>1.250	>1.250	>200

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