South African Journal of Botany 2002, 68: 100–105
Printed in South Africa — All rights reserved

SOUTH AFRICAN JOURNAL OF BOTANY
ISSN 0254–6299

The composition and antimicrobial activity of the essential oil of the resurrection plant *Myrothamnus flabellifolius*

AM Viljoen[1]*, ME Klepser[2], EJ Ernst[3], D Keele[3], E Roling[3], S van Vuuren[1], B Demirci[4], KHC Baser[4] and B-E van Wyk[5]

[1] *Department of Pharmacy and Pharmacology, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa*
[2] *College of Pharmacy, Ferris State University, Borgess Medical Center Department of Pharmacy, Kalamazoo, Michigan 49001, USA*
[3] *College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242, USA*
[4] *Medicinal and Aromatic Plant and Drug Research Center (TBAM), Anadolu University, 26470 Eskisehir, Turkey*
[5] *Department of Botany, Rand Afrikaans University, PO Box 524, Auckland Park 2006, Johannesburg, South Africa*
* *Corresponding author, e-mail: viljoena@chiron.wits.ac.za*

Received 30 July 2001, accepted in revised form 6 November 2001

An investigation of the antibacterial and antifungal activity of *Myrothamnus flabellifolius* against eleven different pathogens showed inhibition of all micro-organisms except for *Salmonella typhimurium* and *Alternaria alternata*. A preliminary screening was done by the disc diffusion method and three susceptible organisms were selected to demonstrate the rapid onset of antimicrobial activity using time-kill methodology. Essential oil concentrations, ranging from 0.0625% to 2%, exhibited strong fungicidal activity against *Candida albicans* and were found to be bacteriostatic against *Staphylococcus aureus* with microbiocidal effects increasing in a concentration-dependant manner. Essential oil rapidly reduced viable counts of *Pseudomonas aeruginosa* but regrowth was noted after 24 hours. The results have been generated in duplicate in two separate microbiology laboratories using different time-kill methods and the results are in agreement. Eighty-five compounds were identified by GC-MS in the hydro-distilled essential oils which contained pinocarvone and *trans*-pinocarveol as the major terpenoids. The antimicrobial properties of the essential oil are presented as a possible rationale for the traditional use of the resurrection bush, *Myrothamnus flabellifolius* in African herbal medicinal preparations.

Introduction

Myrothamnus flabellifolius (Myrothamnaceae) is a woody shrub about 0.4m in height with a wide distribution in southern Africa. The plants have an affinity for rocky areas and are usually wedged into crevices of large boulders. The small aromatic leaves are fan-shaped and conspicuously toothed on the upper leaf margin. The most remarkable feature of the plant is that the leaves fold up when desiccated. In the rainy season the seemingly dead plants are revived from the dry state. This unique feature is reflected in the English (resurrection bush) and Zulu (uvukwabafile) vernacular names and is used as a symbol of hope in traditional African psychological treatment against severe depression (Credo Mutwa, pers. comm. to B-EVW).

The leaves and twigs are used in many medicinal preparations and some of the traditional uses are summarised from Van Wyk *et al*. (1997) and Hutchings *et al*. (1996). Infusions are drunk for colds and respiratory ailments, nosebleeds and fainting. Decoctions are taken orally to alleviate backache, kidney problems, haemorrhoids and menstrual pains. Externally the plant may be used to treat abrasions and the dried powdered leaves are used in dressings for burns and wounds. The plants may be burnt and the smoke inhaled to treat chest pains and asthma. The smoke may also be directed into the vagina to treat infections and pains in the uterus. The Pedi smoke the leaves in pipes to alleviate chest pains and the Karanga chew the aromatic leaves for mouth complaints. In central Africa the plant is used as a tonic and to treat breast complaints. Shona healers have used the plant to treat epilepsy, madness and coughs. Numerous other uses, mostly related to colds, flu, chest complaints and wound healing are recorded by Neuwinger (2000) and Von Koenen (2001).

The major compounds in the essential oil of *Myrothamnus flabellifolious* have been reported by Da Cunha (1974), noting carvone and perillic acid as the major constituents. Gibbs (1974) recorded the presence of 1,8-cineole and diosphenol. The essential oil composition was recently published by Chagonda *et al*. (1999) who identified 43 compounds with *trans*-pinocarveol (28.7–28.8%), pinocarvone (13.4–21.3 %), α-pinene (tr-23.0 %) and β-selinene (5–9.9

%) being major constituents. Due to the conflicting reports on the composition of the essential oils distilled from *Myrothamnus flabellifolious*, we decided to re-examine the oil composition using GC-MS as well as the antimicrobial properties by disc diffusion and time-kill methods.

Material and Methods

Essential oil analysis

The aerial parts of three separate plants were collected in the growing season (February 2001) from plants growing near Klipriviersberg, Johannesburg, South Africa. The plant material was hydro-distilled for three hours in a Clevenger apparatus and analytical GC analysis was performed on a Shimadzu 17A gas chromatograph using the following parameters: Column: J&W-DB1 (30m x 0.25mm id., 0.25µm film thickness), Temperatures: injection port 230°C, column 60°C for 1min, 5°C min^{-1} to 180°C, 180°C for 2min, (total = 25min). These results indicated no qualitative and negligible quantitative variation within a population. Due to this chemical invariability and the large amounts of oil required for the antimicrobial testing (especially time-kill studies) the oil obtained from each of the three plants were pooled and analysed with GC-MS operating under the following conditions; Column: HP-Innowax (60m x 0.25mm id., 0.25µm film thickness), Temperatures: injection port 250°C, column 60°C for 10min, 4°C min^{-1} to 220°C, 220°C for 10min, 1°C min^{-1} to 240°C (total = 80min).

Antimicrobial testing

Disc diffusion assays were performed on the hydrodistilled oils. Reference bacterial strains included in the study were *Pseudomonas aeruginosa* (ATCC 33584), *Escherichia coli* (ATCC 22922), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Serratia odorifera* (ATCC 33132), *Proteus vulgaris, Salmonella typhimuium* (ATCC 14028). Reference fungal strains *Candida albicans* (ATCC 10231), *Cryptococcus neoformans* (ATCC 90112), *Aspergillus niger* (clinical strain) and *Alternaria alternata* (clinical strain) were tested. Base layers of Mueller Hinton (Oxoid) agar were prepared for bacterial studies and Sabouroud's Dextrose Soya (Oxoid) agar were prepared for fungal cultures. Spore suspensions yielding an inoculum size of 1 X 10^6 thoroughly mixed into the overlaying agar surface. With aseptic manipulation, 6mm discs were saturated with the pure oil (*ca*. 6.5mg) and placed on the set agar. Neomycin discs (30µg, Oxoid) were used for positive bacterial controls. Nystatin discs (100 iµ, Oxoid) were used for positive fungal controls. All plates were incubated at 37°C for 48hr with the exception of the two moulds *A. niger* and *A. alternata* which were incubated at 25°C for seven days.

Death kinetic assays were performed on *S. aureus* (3 strains), *C. albicans* (2 strains) and *P. aeruginosa* (1 strain). For *S. aureus* (ATCC 25923) and *C. albicans* (ATCC 10231) the time-kill study was carried out in two separate laboratories using different time-kill methodologies. At the University of the Witwatersrand (hereafter referred to as the UW method), cultures were grown in Tryptone Soya (Oxoid) broth and centrifuged for 10min at 5 000rpm. The supernatant was discarded and the pellets resuspended in 10mL of a 0.9% NaCl solution. Oil concentrations of 0.125, 0.25, 0.5, 1 and 2% (v/v) were incorporated into 50ml Tryptone soya (Oxoid) broth with 0.5% Tween and a final inoculum of approximately 1 x 10^6CFU ml^{-1}. The different concentrations were incubated at 37°C in a shaking water bath. At time intervals ranging from 0min–24hrs, aliquots of 1ml were transferred to 9ml inactivation broth consisting of 0.1% peptone (Oxoid), 5% lecithin (Merck) and yeast extract (Oxoid). Five serial dilutions were performed in 0.9% NaCl solution, from the inactivation broth 100µl was plated onto Tryptone soya (Oxoid) agar for each oil concentration. The plates were incubated at 37°C for 48hr and colony forming units (CFU's) counted and death kinetics expressed in log_{10} reduction time-kill plots. Controls were included in the study having the same broth formulation but without the oil. The assay was performed in triplicate.

At the University of Iowa (hereafter referred to as the UI method), time-kill procedures were conducted as described previously by Klepser *et al*. (1997). Fungi and bacteria were obtained from stored samples and subcultured twice on Potato Dextrose agar or Meuller Hinton agar (Remel) prior to testing. Suspensions containing test organisms were prepared in sterile water for fungi and normal saline for bacteria by touching three to five colonies from a 24h old culture plate and adjusting the resulting suspension to approximately 1 x 10^6 to 5 x 10^6CFU ml^{-1} using spectrophotometric methods. One milliliter of the suspension containing the test organism, along with 0.05ml Tween 80 was added to 9ml of media, RPMI 1640 buffered with MOPS to pH 7.0 for fungi and Mueller Hinton broth for bacteria with or without drug, providing the starting inoculum of approximately 5 x 10^5CFU/ml. The range of concentrations tested was 0.0625, 0.125, 0.25, 0.5 and 1% (v/v) oil. The culture vials were incubated with agitation at 35°C. At predetermined time points (0, 2, 4, 6, 8, and 24h following the addition of oil), a 0.1ml sample was removed from each culture vial, serially diluted 1:10 in sterile water or saline and a 30ml aliquot was plated on Potato Dextrose or Mueller Hinton agar (Remel). Colony counts were determined after incubation of the plates at 35°C for 24 to 48h. When colony counts were suspected to be less than 1 000CFU/ml, 30ml samples were removed and plated without dilution. The limit of quantification by these methods is 100CFU/ml (Klepser *et al*. 1998a). The carryover effect of the oil was evaluated over the range of concentrations used in this study as previously described (Klepser *et al.* 1998a). All time-kill curve experiments were conducted in duplicate.

Results and Discussion

Table 1 lists the zones (mm) showing the antimicrobial activity for all the test organisms screened. With the exception of *S. typhimurium* and *A. alternata* the essential oil exhibited microbial activity against all the pathogens tested.

The death kinetics of *S. aureus, C. albicans* and *P. aeruginosa* are represented as time-kill curves (log_{10} reduction values) in Figure 1A–1H . Figure 1A–1C displays the cidal effect of the essential oil on two strains of *C. albicans* (ATCC 90028 and ATCC 10231). The cidal activity was exhibited for

Table 1: Results of the disc diffusion assay of *Myrothamnus flabellifolious* essential oils. The diameter of the zone of inhibition is expressed in mm including the disc (6mm). Neomycin and nystatin served as controls for bacteria and fungi respectively

Test Organism	Zone	Control	
		Neomycin	Nystatin
Pseudomonas aeruginosa	8	8	–
Escherichia coli	12	16	–
Staphylococcus aureus	12	20	–
Enterococcus faecalis	10	14	–
Proteus vulgaris	10	18	–
Serratia odorifera	12	18	–
Salmonella typhimurium	6	14	–
Candida albicans	16	–	20
Cryptococcus neoformans	22	–	26
Aspergillus niger	26	–	26
Alternaria alternata	6	–	10

concentrations ranging between 0.0625% and 0.25% with the strain ATCC 10231 being less sensitive. The candidacidal activity is reflected in both methods used in this study (UW and UI). In the UI method the minimum percentage of inhibition (MPI) >0.125% (Figure 1B) whereas viable counts were considerably lower at the same concentration in the UW assay. This deviation could probably be ascribed to a lower initial inoculum as observed at 0min for the culture used in the UW assay (Figure 1C) compared to the UI method (Figures 1A and 1B). At higher concentrations (0.5% and 1%) the death kinetics show a decrease in the number of surviving *P. aeruginosa* cells within 60min but the CFU's increased over time (Figure 1D). This suggests that the oil produces an initial bacteriostatic effect but regrowth of the organism occurs with time. For *S. aureus* the essential oil generally follows a concentration-dependent antibacterial activity (Figure 1E–1H). The methicillin sensitive strain of *S. aureus* (Figure 1E and 1F) proved to be more sensitive on exposure to the essential oils compared to the methicillin resistant strains MRSA 1616 and MRSA 1639, as illustrated in Figures 1G and 1H respectively.

Comparison of the time-kill plots for the three organisms studied showed that the killing rate was the greatest for *C. albicans* and *S. aureus* with initial activity followed by subsequent regrowth for *P. aeruginosa*. These results correlate directly with the disc diffusion results which as an initial screening process indicated inhibition of *C. albicans* > *S. aureus* > *P. aeruginosa*.

Eighty-five compounds were identified in the hydro-distilled essential oil representing 87.79% of the total composition (Table 2). The major compounds identified in the essential are pinocarvone (11.13%), *trans*-pinocarveol (19.57%), limonene (6.09%), *trans*-*p*-menth-1-(7)-8-diene-2-ol (7.43%) and *cis*-*p*-menth-1-(7)-8-diene-2-ol (6.76%). This is the first comprehensive report on the essential oil chemistry of this important medicinal plant and these results could not corroborate the oil composition reported by Da Cunha (1974) and Gibbs (1974). The results however correspond to the analysis reported by Chagonda *et al*. (1999) who also recorded *trans*-pinocarveol and pinocarvone as major constituents. Although no variation was recorded within a population, more plant populations representing the wide geographical distribution range of this plant need to be analysed to determine if various chemotypes are involved.

With reference to terpinen-4-ol Chalchat *et al*. (1997) expresses caution at directly correlating antimicrobial activity to the action of the major essential oil compounds. It is, however, interesting to tentatively correlate the observed antimicrobial activity to essential oil composition as activity has been reported for essential oils accumulating the same terpenoids found in *M. flabellifolius*. Pinocarvone is one of the major constituents of Hyssop oil (*Hyssopus officinalis*) which is a popular home-remedy with bactericidal properties (Kerrola *et al*. 1994). Sagrero-Nieves and Bartley (1995) reported *trans*-pinocarveol as a major constituent (27%) in the essential oil of *Chenopodium ambrosioides*. Kishore *et al*. (1993) found the essential oil of the latter species to be fungitoxic against several dermatophytes while Lall and Meyer (1999) reported the acetone extract of *Chenopodium ambrosioides* to be active against resistant strains of *Mycobacterium tuberculosis.* Structure-activity relationships are continually being investigated for essential oil compounds (Hinou *et al*. 1989, Griffin *et al*. 1999). The latter study illustrates the antimicrobial activity for bicyclic terpenoids (e.g. myrtenol) mentioning that the ketone is less active than the corresponding alcohol. In an attempt at relating structure to the recorded antimicrobial activity, it is of interest that *M. flabellifolius* accumulates two oxygenated bicyclic terpenoids with the alcohol *trans*-pinocarveol being present in higher quantities than the corresponding ketone, pinocarvone. Hinou *et al.* (1989) also reports that the *trans*-isomers are more active than the corresponding *cis*-isomers.

Myrothamnus flabellifolius has been used traditionally to treat various ailments, which may be caused by bacterial or fungal infections (for a summary of the recorded uses in Africa see Neuwinger (2000)). As the leaves are mostly used in these herbal preparations it is possible that the essential oils could be responsible for the medicinal properties ascribed to *M. flabellifolius*. The essential oil yield was calculated at 0.04% wet wt. It is noteworthy that the essential oil exhibited antimicrobial properties as concentrations <0.04% (v/v). The volatile constituents (mono- and sesquiterpenoids) will also be released and absorbed during rituals in which the smoke from the burnt leaves is inhaled. Cryptococcosis is a chronic infection involving mainly the lungs and meninges and *A. niger* is implicated in bronchopulmonary lung infections (Boyd and Hoerl 1981). Both *C. neoformans* and *A. niger* show marked antimicrobial activity as indicated in Table 1. It is interesting to note that *trans*-pinocarveol, the major essential oil component in *M. flabellifolius*, is used in pharmaceutical preparations such as Ozopulmin™ to treat respiratory tract disorders including asthma. *Pseudomonas aeruginosa* is an opportunistic pathogen, which may be responsible for a vast number of nosocomial infections, in particular burn patients. *Myrothamnus flabellifolius* is traditionally used as a dressing for burns and wounds. The oil was not found to be active *in vitro* against the specific strain of *P. aeruginosa. Candida albicans* was selected for an in-depth fungal time-kill investigation due to its moderate antimicrobial susceptibility as presented in the disc diffusion assay (Table 1) but more due

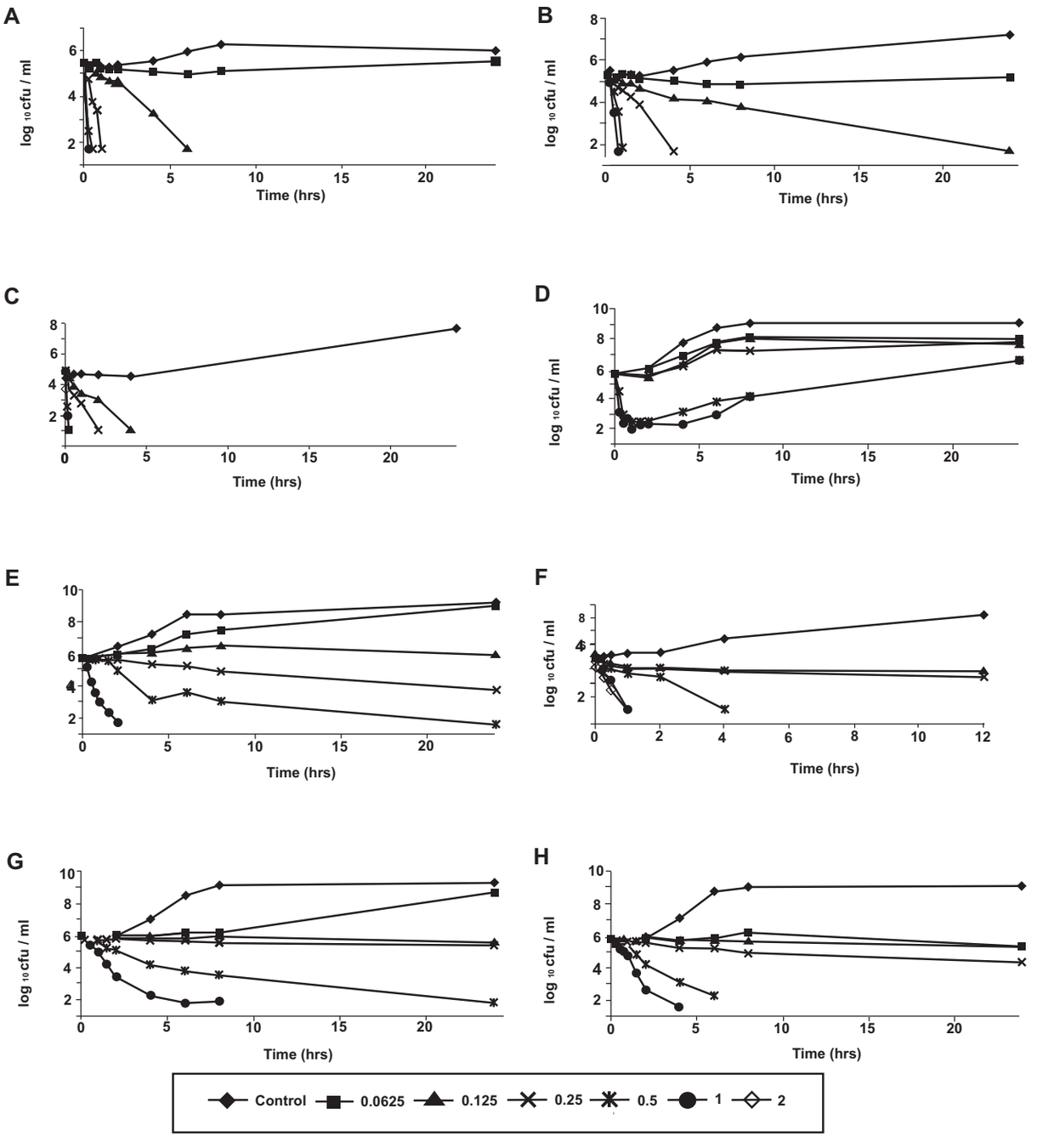

Figure 1: Plots of mean values for $\log_{10}$ of the numbers of CFU ml^{-1} versus time for *Myrothamnus flabellifolius* essential oil (oil concentrations expressed as a % v/v) tested on *Candida albicans* UI ATCC 90028 (A) UI ATCC 10231 (B) UW ATCC 10231 (C) *Pseudomonas aeruginosa* UI ATCC 27853 (D) *Staphylococcus aureus* UI ATCC 25923 (E) UW ATCC 25923 (F) UW MRSA 1616 (G) UI MRSA 1639 (H)

to the prevalent pathogenesis. More than 80% of all fungal bloodstream infections are a direct result from the *Candida* species and since the eighties, there has been an explosive rise in the rate of candidial infections (Klepser *et al.* 1998b). The candidacidal activity reported here could possibly provide scientific support for the traditional use of *M. flabellifolius* for *Candida*-related infections (e.g. mouth and vaginal infections). The activity of *M. flabellifolius* essential oil against *S. aureus* was investigated because of its association with primary infections of the skin. The traditional use of *M. flabellifolius* as a dressing to treat wounds and abrasions could also be related to the antibacterial properties illustrat-

Table 2: Essential oil composition of *Myrothamnus flabellifolius*

RI	Compound name	%[1]	RI	Compound name	%[1]
1032	α-pinene	1.61	1697	carvotanacetate	0.03
1076	camphene	0.12	1704	γ-muurolene	0.023
1118	β-pinene	0.16	1706	α-terpineol	0.25
1132	sabinene	0.03	1719	borneol	0.04
1174	myrcene	0.16	1726	germacrene D	1.13
1203	limonene	6.09	1740	α-muurolene	0.86
1213	1,8-cineole	0.15	1747	*trans* carvyl acetate	0.39
1218	β-phellandrene	0.02	1751	carvone	1.12
1220	*cis*-anhydrolinalool oxide	0.01	1758	*cis*-piperitol	3.68
1224	*o*-mentha-1(17)5,8-triene	0.18	1773	δ-cadinene	0.75
1280	*p*-cymene	0.17	1776	γ-cadinene	0.21
1384	α-pinene oxide	0.01	1797	*p*-menthyl acetophenone	0.13
1408	1,3,8-*p*-menthtriene	0.08	1802	cuminaldehyde	0.08
1435	γ-campholene aldehyde	0.04	1804	myrtenol	1.07
1452	α-*p*-dimethylstyrene	0.18	1807	perilla aldehyde	0.10
1466	α-cubebene	0.03	1811	*trans*-*p*-menth-1-(7)-8-diene-2-ol	7.43
1468	*trans*-1,2-limonene epoxide	0.03	1845	*trans*-carveol	1.27
1482	fenchyl acetate	0.05	1853	*cis*-calamenene	0.09
1497	α-copaene	0.83	1865	isopiperitenone	0.27
1528	α-bourbonene	0.02	1882	*cis*-carveol	0.20
1535	β-bourbonene	0.20	1896	*cis*-*p*-menth-1-(7)-8-diene-2-ol	6.96
1536	pinocamphone	0.10	1941	α-calacorene I	0.07
1541	benzaldehyde	0.01	1945	1,5-epoxy-salvial-4(14)-ene	0.04
1549	β-cubebene	0.09	1953	palustrol	0.07
1553	linalool	1.80	1956	*p*-isopropyl benzaldehyde	0.06
1562	isopinocamphone	0.15	1984	α-calacorene II	0.06
1565	linalyl acetate	0.23	2008	caryophyllene oxide	0.08
1571	*trans*-*p*-menth-2-en-1-ol	0.02	2037	salvial-4(14)-en-1-one	0.07
1586	pinocarvone	11.13	2057	ledol	0.29
1597	bornyl acetate	1.78	2069	germacrene D-4β-ol	0.07
1600	β-elemene	0.47	2080	cubenol	0.26
1611	terpinen-4-ol	0.40	2088	1-epi-cubenol	0.43
1616	hotrienol	0.33	2104	viridiflorol	0.17
1624	*cis*-dihydrocarvone	0.08	2113	cumin alcohol	0.06
1639	*trans*-*p*-mentha-2,8-diene-1-ol	2.70	2144	spathulenol	0.81
1648	myrtenal	0.94	2187	T-cadinol	0.85
1658	sabinyl acetate	0.09	2209	T-muurolol	1.47
1661	alloaromadendrene	0.05	2219	δ-cadinol	0.55
1661	*trans*-pinocarvyl acetate	0.02	2255	α-cadinol	3.00
1664	*trans*-pinocarveol	19.57	2264	intermedeol	0.22
1678	*cis*-*p*-menth-2,8-dien-1-ol	2.30	2273	selin-11-en-4α-ol	0.43
1687	α-humulene	0.12	2324	caryophylladienol II (=*caryophylla-2(12), 6(13)-dien-5α-ol)*	0.10
TOTAL					87,79

[1] area percent of total integration

ed for *S. aureus*. The antimicrobial properties, with speculation on possible structure activity relationships reported here for *Myrothamnus flabellifolius* essential oil, may provide some pharmaceutical rationale for the popular traditional use of this plant in African medicinal herbal preparations.

Acknowledgements — The Research Committee and the Faculty of Health Sciences Research Endowment Fund of the University of the Witwatersrand and the National Research Foundation are thanked for financial support.

References

Boyd RF, Hoerl BG (1981) Basic Medical Microbiology 2nd Edn. Little Brown and Company (Inc), USA. ISBN 0316104337

Chalchat JC, Garry RP, Menut C, Lematy G, Malhuret R, Chopineau J (1997) Correlation between chemical composition and antimicrobial activity. VI. Activity of some African essential oils. ***Journal of Essential Oil Research* 9**: 67–75

Chagonda LS, Makanda C, Chalchat JC (1999) Essential oils of four wild and semi-wild plants from Zimbabwe: *Colophospermum mopane* (Kirk ex Benth.) Kirk ex Leonard, *Helichrysum splendidum* (Thunb.) Less, *Myrothamnus flabellifolia* (Welw.) and *Tagetes minuta* L. ***Journal of Essential Oil Research* 11**: 573–578

Da Cunha P (1974) Identification and determination of principle constituents of essential oil of *Myrothamnus flabellifolius* collected in Angola. Bol. Fac. Farm. ***Univ. Coimbra Ed Cient*** **34**: 49–61

Gibbs RD (1974) Chemotaxonomoy of Flowering Plants. Vol. 3. McGill-Queens University Press

Griffin SG, Wyllie SG, Markham JL, Leach DN (1999) The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. ***Flavor and Fragrance Journal*** **14**: 322–332

Hinou JB, Harvala CE, Hinou EB (1989) Antimicrobial activity screening of 32 common constituents of essential oil. ***Pharmazie*** **44**: 302–303

Hutchings A, Scott AH, Lewis G, Cunningham AB (1996) Zulu Medicinal Plants — an inventory. University of Natal Press, Pietermaritzburg, South Africa. ISBN 0869809237

Kerrola K, Galambosi B, Kallio H (1994) Volatile components and odor intensity of four phenotypes of hyssop *(Hyssopus officinalis* L.). ***Journal of Agricultural and Food Chemistry*** **42**: 776–781

Kishore N, Mishra AK, Chansouria JP (1993) Fungitoxicicity of essential oils against dermatophytes. ***Mycoses*** **36**: 211–215

Klepser ME, Ernst EJ, Lewis RE, Ernst ME, Pfaller MA (1998a) Influence of test conditions on antifungal time-kill curve results: proposal for standardized methods. ***Antimicrobial Agents and Chemotherapy*** **42**: 1207–1212

Klepser E, Lewis RE, Pfaller MA (1998b) Therapy of *Candida* infections: susceptibility testing, resistance and therapeutic options. ***The Annals of Pharmacotherapy*** **32**: 1353–1361

Klepser ME, Wolfe EJ, Jones RN, Nightingale CH, Pfaller MA (1997) Antifungal pharmacodynamic characteristics of fluconazole and amphotericin B tested against *Candida albicans* ***Antimicrobial Agents and Chemotherapy*** **41**: 1392–5

Lall N, Meyer JJ (1999) *In vitro* inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. ***Journal of Ethnopharmacology*** **66**: 347–354

Neuwinger HD (2000) African Traditional Medicine. Medpharm, Stuttgart, Germany. ISBN 3887630866

Sagrero-Nieves L, Bartley JP (1995) Volatile constituents from the leaves of *Chenopodium ambrosioides* L. ***Journal of Essential Oil Research*** **7**: 221–223

Van Wyk B-E, Van Oudtshoorn B, Gericke N (1997) Medicinal Plants of South Africa. Briza, Pretoria, South Africa. ISBN 1875093095

Von Koenen E (2001) Medicinal, Poisonous, and Edible Plants in Namibia. Klaus Hess, Windhoek, Namibia. ISBN 9991674748

Edited by AK Jäger