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The Geographical Variation and Antimicrobial Activity of African Wormwood (*Artemisia afra* Jacq.) Essential Oil

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Abstract

The aerial parts of 16 individual *Artemisia afra* plants from four natural populations were hydrodistilled and the essential oil analyzed by GC/MS. The oil composition varied quantitatively and qualitatively within and between natural populations and showed no correlation to the geographical distribution. The antimicrobial activity was demonstrated by means of time-kill methodology using the respiratory pathogens *C. neoformans* and *K. pneumoniae*. Antimicrobial activity was most prominent within 10 min at concentration 0.75% for *K. pneumoniae* and within 60 min at concentration 1% for *C. neoformans.* Investigation of the four major compounds most abundant in the *A. afra* oil (artemisia ketone, 1,8-cineole, α- and β-thujone) indicated minimal antimicrobial activity when investigated independently and in various combinations against *K. pneumoniae*.

Key Word Index

Artemisia afra, Asteraceae, essential oil composition, 1,8-cineole, artemisia ketone, α-thujone, β-thujone, artemisia alcohol, camphor, antimicrobial activity, death kinetics.

Introduction

Artemisia is a large and widespread genus housing approximately 350 species (1). In South Africa *Artemisia afra* Jacq. extends from the mountainous regions of the south western Cape, along the eastern coast to the Limpopo Province. This plant is a multi-stemmed aromatic perennial shrub, growing up to two meters in height. Glaucous featherlike leaves with a pungent aromatic smell characterize *A. afra.* Members of the genus are frequently used to treat diseases such as malaria, diabetes, hepatitis and microbial infections. In South Africa *A. afra* is commonly known as African wormwood, "umhlonyane" (Xhosa, Zulu), "lengana" (Sotho, Tswana) and "wildeals" (Afrikaans) (2). African wormwood is primarily used for colds, coughs and influenza as it is said to clear the respiratory and bronchial passages (3,4). The leaves are prepared as an infusion or decoction and used to treat digestive complaints (2) and skin ulcerations (3). The aerial parts of *A. afra* are rich in essential oils and often used as an inhalant (5), which prompted us to investigate the composition and antimicrobial activity as assessed by death kinetic assay against the respiratory pathogens *K. pneumoniae* and *C. neoformans* of the essential oils.

Studies have indicated chemotypic variation in the oil composition for *A. afra* (6) and this impacts on selecting favorable chemotypes for commercial development as identification of superior clones from a chemical perspective becomes problematic. Further variabilitys were noted (7) when comparing the samples in this study with that collected from Fort Hare (Eastern Cape). Analysis of the oil *A. afra* (8), also from Fort Hare indicated higher α-thujone (78.7 %) than what was noted in this report. The aim of this study was to produce chemical profiles for various clones of *A. afra* and to determine if the major chemical constituents play a role in the antimicrobial activity of the *A. afra* oil using the respiratory pathogen *K. pneumoniae*.

Experimental

Oil isolation and analysis: Four natural plant populations were selected; Setibeng (Lesotho), Giant's Castle (KwaZulu

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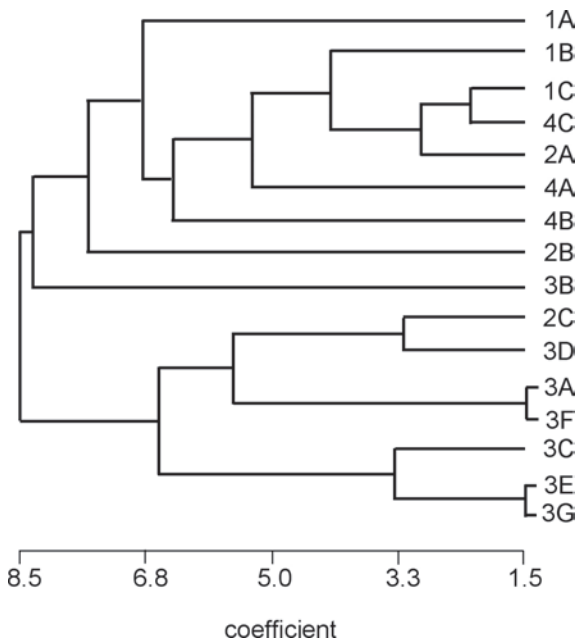
Natal), Qwa-qwa (Free State) and Klipriversberg (Gauteng). Three individual plants were selected in each population except from Klipriversberg, where seven plants were collected. The oils were obtained through hydrodistillation in a Clevenger-type apparatus and analyzed by GC/MS using a Hewlett-Packard GCD system. Innowax FSC column (60 m x 0.25 mm) was used with helium as the carrier gas. The GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min. Split flow was adjusted to 50 mL/min. The injector and detector temperatures were at 250°C and MS were taken at 70 eV. Mass range was from m/z 35-425. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatogram. Library searches were carried out using Wiley and Adams GC/MS Libraries and Başer Library of Essential Oil constituents. The oil compositions of all 16 individual plants and the combined sample from Klipriversberg used in the time-kill methodology (3H) are given in Table I.

Antimicrobial evaluation: Death kinetic assays (9) were performed on *K. pneumoniae* (NCTC 9633) and *C. neoformans* (ATCC 90112), both respiratory pathogens associated with lung infections, to validate the traditional use of *A. afra* to treat disorders associated with the respiratory tract. Due to the larger volumes of oil required for these assays, a collective sample from Klipriversberg was used to obtain the oil needed for the time-kill assay. To determine the possible interactive role of the major compounds, time-kill studies were undertaken for various combinations of the major compounds: 1,8-cineole and artemisia ketone; α- and β-thujone; α-thujone and 1,8-cineole; α- and β-thujone and 1,8-cineole; artemisia ketone and α-thujone; artemisia ketone and α- and β-thujone; artemisia ketone, α-thujone and 1,8-cineole as well as artemisia ketone, α- and β-thujone and 1,8-cineole. Each compound and various combinations were studied at the original concentration to that found in the collective sample from Klipriversberg (1,8-cineole at 17.8%, artemisia ketone at 10.1%, α-thujone at 18.8% and β-thujone at 12.5%). The number of colony forming units (CFU) were determined at 0 min, 15 min, 240 min, 8 h and 24 h when exposed to *K. pneumoniae.*

Data analysis: The percentage composition of the oil samples was used to determine a relationship between the different samples by cluster analysis using the NTSYS software (10). Correlation was selected as a measure of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition.

Results and Discussion

The geographical variations for some major oil constituents have been documented (6). For instance, the major component reported from Ethiopian oils was artemisyl acetate (24.4 -32.1%), 1,8-cineole (63.4%) in the Kenyan oil, α- and β-thujone (52%) in the Zimbabwean oil and α-thujone (52.5-54.2%) in the South African oil. The authors also reported 1,8-cineole as the major component in cultivated South African clones. Other studies have shown that the main chemical constituents found in the oil of *A. afra* were α-thujone, β-thujone, camphor, borneol and 1,8-cineole (2). Analysis of the oil composition of all 16 individual plants (Table I) in this study, also showed inter-population and geographical composition variation. Major oil constituents between populations varied considerably. Figure 1 graphically summarizes the oil data (Table I). Individuals from the same population are not coherently clustered but scattered throughout the dendrogram. Considering the major compounds for example it is noted that 1,8-cineole present in the oils of plant F (Klipriversberg) at 50% was only present at 2% in the oils of plant C of the Setibeng population. Similarly, α-thujone (78%) was detected in plant C oil of the Setibeng population and it was not present in the oils of plant A of Setibeng, plant C oil of Giant's Castle and the oils from plants C, D, E, F and G of Klipriversberg. Other observations include the oils of plant A and B from the Setibeng population which were similar as both had approximately the same levels of 1,8-cineole (11-12%), santolinyl acetate (20-24%) and camphor (3-4%). The oils of the same plants had two interesting components, santolina alcohol (9% and 3%) and santolinyl acetate (24% and 20%) that were not present in the oils of Giant's Castle samples, neither in most of the Klipriversberg oil samples and only selectively in the oil of the Qwa-qwa population. Santolina alcohol was also found in the Zimbabwean *A. afra* oil (11). In the Setibeng oil samples α- and β-thujone were not detected in the oil from plant A, but were present in higher quantities in the oil of plant C (α-thujone, 78% and β-thujone, 17%). Artemisia ketone (15%), yomogi alcohol (4%) and artemisia alcohol (9%) were only present in the oils of plant A from the Giant's Castle and not detected in the oils of plant B and C from that same population. On the contrary, β-thujone was higher in the oil of plant B (58%) than the oil of plant A (8%). Camphor was only observed in the oils of plants B (19%) and



1 = Setibeng; 2 = Giants Castle; 3 = Klipriversberg; 4 = Qwa-qwa; A-G represent individual plants sampled in the specific population

Figure 1. Dendrogram constructed on the essential oil data matrix in Table I

Table I. The percentage composition of the oils of four natural populations of *Artemisia afra*

		Setibeng			Giant's Castle			Klipriversberg								Qwa-qwa		
RRI	Compounds	1A	1B	1C	2A	2B	2C	3A	3B	3C	3D	3E	3F	3G	3H	4A	4B	4C
	Yields (%)	1.0	0.6	1.5	0.8	1.7	1.9	0.3	0.7	0.5	0.6	0.4	0.6	0.5	0.2	0.4	0.7	0.4
1014	tricyclene	-	-	-	-	-	0.2	-	0.1	-	0.2	-	0.2	-	-	-	-	-
1032	α-pinene	0.6	1.3	-	-	0.2	0.3	1.0	0.1	0.9	1.4	1.2	1.3	0.8	0.2	0.1	0.9	0.4
1035	α-thujene	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-
1043	santolinatriene	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-
1076	camphene	0.6	0.5	-	-	1.6	6.5	2.2	0.1	1.5	4.7	2.1	3.4	2.2	1.0	0.2	-	-
1118	β-pinene	-	-	-	-	-	0.4	0.8	-	0.3	1.1	0.3	0.9	0.4	0.1	-	-	-
1132	sabinene	-	0.2	-	0.2	1.0	0.1	0.3	0.1	0.1	0.2	0.2	0.3	0.1	1.0	0.6	-	0.2
1174	myrcene	-	-	-	0.3	0.4	0.7	0.7	0.1	0.2	1.0	0.2	0.5	0.6	0.1	0.7	0.2	0.1
1188	α-terpinene	-	-	-	-	0.2	0.4	0.8	0.1	0.4	0.7	0.4	0.9	0.2	-	0.3	0.4	-
1195	dehydro-1,8-cineole	-	-	-	-	-	0.1	0.1	-	-	0.1	0.1	0.4	-	-	0.2	0.1	-
1203	limonene	-	-	-	-	0.1	0.7	0.1	-	0.1	0.1	0.2	-	0.2	-	-	-	-
1213	1,8-cineole	12.0	10.5	2.3	12.7	4.8	14.8	43.3	6.6	20.6	24.4	11.4	50.1	8.9	17.8	12.6	22.5	2.6
1246	(Z)-β-ocimene	-	-	-	0.4	0.5	0.4	1.5	0.4	0.4	2.4	0.7	0.8	0.9	-	1.2	1.0	1.0
1255	γ-terpinene	-	-	-	0.3	0.2	0.5	1.8	0.2	0.7	1.2	0.4	1.7	0.4	-	0.6	0.7	0.2
1266	(E)-β-ocimene	-	-	-	-	-	-	0.3	0.1	0.1	0.4	0.1	0.1	0.2	-	0.2	0.2	0.1
1280	p-cymene	0.6	0.6	0.3	0.4	0.9	1.0	0.3	0.2	0.3	0.6	0.2	0.7	0.2	2.2	0.5	1.1	-
1285	isoamyl isovalerate	0.2	-	-	-	-	-	0.3	-	-	-	-	0.1	-	-	-	-	-
1290	terpinolene	-	-	-	-	-	0.1	0.4	-	0.2	0.2	0.1	0.4	0.1	-	0.1	-	-
1299	2-methyl butyl isovalerate	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1358	artemisia ketone	-	-	-	14.5	-	-	-	15.6	26.7	-	28.0	-	24.0	10.1	-	-	-
1403	yomogi alcohol	-	-	-	3.5	-	-	-	-	3.1	-	-	-	-	4.9	0.6	-	-
1405	santolina alcohol	8.9	3.2	-	-	-	-	-	10.3	-	-	2.0	-	2.4	2.0	-	9.3	4.1
1409	santolinyl acetate	24.3	20.2	-	-	-	-	-	-	-	-	-	-	-	0.2	0.8	8.0	-
1437	α-thujone	-	23.8	77.7	38.6	0.9	-	0.1	5.6	-	-	-	-	-	18.8	27.5	15.0	55.6
1448	artemisyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	1.8	4.0	-	-
1450	*trans*-linalool oxide furanoid form	-	-	-	-	-	-	0.1	-	-	-	-	-	0.1	-	-	-	-
1451	β-thujone	-	5.6	16.8	7.9	57.7	-	-	1.4	-	-	-	-	-	12.5	29.9	4.3	11.6
1452	1-octen-3-ol	0.2	-	-	-	-	-	0.5	-	0.8	0.9	0.5	0.7	0.6	-	-	-	-
1474	*trans*-sabinene hydrate	0.2	-	0.2	0.4	0.3	1.1	3.4	0.4	1.1	1.4	0.9	2.8	0.7	0.3	0.1	0.2	-
1495	bicyloelemene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	0.2
1497	α-copaene	-	-	-	0.2	-	0.2	0.2	0.2	-	0.4	-	0.1	0.4	0.1	-	-	-
1510	artemisia alcohol	0.6	0.6	-	9.3	-	-	-	27.8	12.1	-	10.1	-	9.6	5.5	0.2	-	-
1532	camphor	2.8	3.9	-	-	19.4	49.0	15.6	-	17.6	31.5	27.2	16.8	29.5	-	1.0	0.6	-
1540	chrysanthenone	-	-	-	-	-	-	-	4.7	-	-	-	-	-	0.4	-	-	-
1553	linalool	-	-	-	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-
1556	*cis*-sabinene hydrate	0.2	-	0.2	0.3	0.3	0.7	1.4	0.3	0.7	0.7	0.6	1.8	0.1	0.3	-	0.2	-
1571	*trans*-p-menth-2-en-1-ol	-	1.1	-	0.1	0.1	2.3	0.4	1.2	0.2	3.0	0.1	0.4	-	1.0	0.2	0.3	-
1582	*cis*-chrysanthenyl acetate	15.2	-	-	-	-	0.4	-	-	-	-	-	-	-	-	6.1	-	-
1586	pinocarvone	-	0.3	0.2	-	0.8	-	0.6	0.3	0.5	0.6	0.3	0.6	0.6	0.2	-	0.3	0.1
1597	bornyl acetate	4.5	0.4	-	-	-	0.3	1.8	-	0.1	0.3	0.5	0.3	0.2	0.2	-	-	-
1611	terpinen-4-ol	0.7	0.2	0.4	1.4	0.7	2.0	5.2	0.8	2.6	3.0	1.5	4.9	1.5	2.1	1.3	2.2	0.5
1612	β-caryophyllene	-	-	-	-	0.6	0.3	0.4	0.2	0.1	0.7	-	0.4	0.9	-	0.5	1.9	0.3
1617	lavanduyl acetate	0.7	0.2	-	-	-	-	-	0.8	-	-	-	-	-	-	-	-	-
1638	*cis*-p-menth-2-en-1-ol	-	-	-	-	-	1.8	0.2	0.8	0.9	2.1	0.1	-	0.4	0.7	0.1	0.2	-
1639	*trans*-p-menth-2,8-dien-1ol	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-
1643	dehydrosabinaketone	-	0.2	0.2	-	1.6	-	-	-	-	-	-	-	-	0.1	0.1	-	0.1
1648	myrtenal	-	-	-	-	-	0.1	0.3	-	-	0.4	0.2	0.5	0.3		0.4	0.3	-
1651	sabinaketone	-	-	-	-	0.4	-	-	-	-	-	-	-	-		-	-	0.1
1658	sabinyl acetate*	-	-	0.2	-	-	-	-	-	-	-	-	-	-	0.1	0.2	-	-
1663	*cis-v*erbenol	-	-	-	-	-	0.1	-	-	-	-	-	-	-		-	-	-
1664	*trans*-pinocarveol	0.6	-	0.3	-	1.2	-	0.4	0.1	0.5	0.6	-	0.7	0.5	0.1	0.6	0.7	-
1688	(Z)-β-farnesene	-	-	-	0.3	-	0.2	-	-	-	-	-	-	-		0.2	0.7	0.5
1682	δ-terpineol	-	-	-	0.2	-	0.3	0.6	-	0.4	0.4	0.3	0.6	0.3	0.1	-	-	-
1684	dehydro carvyl acetate	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1686	lavandulol	0.3	-	-	-	-	-	-	0.4	-	-	-	-	-	-	-	0.5	0.2
1687	α-humulene	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	0.1	-	-
1689	*trans-p*iperitol	-	-	-	-	-	1.1	0.1	0.6		0.7	0.2	0.1	0.3		-	-	-
1706	α-terpineol	-	-	0.2	-	0.6	0.5	1.8	0.1	0.3	0.4	0.5	0.7	0.2	0.2	0.2	0.4	-
1719	borneol	8.2	2.0	-	-	1.0	1.9	5.6	-	0.3	2.9	1.1	1.8	0.9	2.7	-	0.2	-

Table I. continued

		Setibeng			Giant's Castle			Klipriversberg								Qwa-qwa		
RRI	**Compounds**	**1A**	**1B**	**1C**	**2A**	**2B**	**2C**	**3A**	**3B**	**3C**	**3D**	**3E**	**3F**	**3G**	**3H**	**4A**	**4B**	**4C**
1720	*trans-s*abinol	-	-	0.1	0.3	-	-	-	-	-	-	-	-	-	-	0.7	-	-
1726	germacrene D	-	-	-	1.6	0.7	0.7	0.7	-	-	1.6	-	1.0	1.4	-	1.4	3.5	1.2
1729	*cis*-1,2-epoxy-terpinen-4-ol	0.2	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1742	β-selinene	0.3	0.4	-	-	0.2	0.4	-	-	0.5	0.4	0.2	-	0.5	0.2	-	-	-
1748	pipertone	-	-	-	-	-	1.0	-	1.5	-	1.6	-	-	-	0.7	-	-	-
1754	*trans-p*iperitone oxide	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-
1755	bicyclogermacrene	-	-	0.2	0.4	1.1	-	1.2	-	0.5	-	0.1	0.1	0.6	-	1.7	3.9	1.3
1758	*cis*-piperitol	-	-	-	-	-	2.9	-	1.4	-	2.7	-	0.1	0.5	0.8	-	-	-
1764	*cis*-chrysanthenol	1.7	-	-	0.6	-	1.8	-	-	-	-	-	-	-	-	2.2	-	-
1773	δ-cadinene	0.3	-	0.2	-	-	-	0.1	0.2	-	0.3	0.2	-	0.3	-	0.2	0.5	0.2
1786	*ar-c*urcumene	0.2	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3
1802	cuminaldehyde	-	-	0.2	-	0.2	-	-	-	-	-	-	-	-	0.1	-	-	-
1804	myrtenol	-	-	-	-	-	-	0.2	-	0.1	-	-	0.4	0.1	-	0.1	0.2	-
1811	*trans*-p-menth-1(7),8-dien-2-ol	-	-	-	-	-	0.2	-	-	-	0.3	-	-	-	-	-	-	-
1864	p-cymen-8-ol	-	-	-	-	-	0.1	-	-	-	0.1	-	-	-	0.1	-	-	-
1889	ascaridole	-	-	-	-	0.2	0.3	-	0.1	-	0.3	-	-	-		-	0.2	-
1889	isopiperitone	-	-	-	-	-		-		-	-	-	-	-	0.3	-	-	-
1896	*cis*-p-mentha-1-(7),8-dien-2-ol	-	-	-	-	-	-	-	-	-	-	0.2	-	0.2	-	-	-	-
1957	epi-cubebol	-	-	-	-	-	-	0.2	0.2	-	-	-	0.1	0.3	0.1	-	0.2	0.3
1957	cubebol	-	-	-	-	-	0.2	0.3	0.3	-	0.3	0.3	0.2	0.4	-	-	0.1	-
1969	(Z)-jasmone	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-
1977	4β-hydroxy achipendol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-
2008	caryophyllene oxide	-	-	-	0.3	-	0.2	-	0.3	-	-	0.3	0.1	0.3	0.3	-	0.2	0.1
2008	p-mentha-1,8-dien-10-ol	-	-	-	-	-	-	0.3	-	0.3	0.2	-	-	-	-	-	-	-
2033	4α-hydroxy achipendol	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	0.9	0.5
2050	(E)-nerolidol	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	0.2	0.1
2073	p-mentha-1,4-dien-7-ol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.0	-	-
2074	caryophylla-2(12),6(13)-dien-5-one	-	-	-	-	-	-	0.1	-	0.1	-	-	-	-	-	-	-	-
2098	globulol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-
2113	cumin alcohol	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-
2126	4-hydroxy-4-methylcyclohex-2-enone	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-
2130	1-epi-cubenol	-	-	-	-	-	-	0.1	0.1	-	-	0.1	-	0.1	0.1	-	0.1	-
2144	spathulenol	0.2	0.4	-	0.2	0.1	0.4	0.4	-	0.1	0.1	-	-	-	0.5	0.2	1.0	-
2202	germacrene D-4-ol	-	-	-	-	-	-	-	-	-	0.1	-	-	0.1	-	-	-	-
2209	T-muurolol	-	-	-	0.9	-	0.9	1.3	1.2	0.1	1.2	1.4	0.6	1.7	0.5	0.1	0.7	-
2232	α-bisabolol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2241	p-isopropyl phenol	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-
2247	*trans*-α-bergamotol	-	-	-	-	-	0.1	0.1	-	-	-	-	-	-	-	-	0.3	-
2255	α-muurolol	-	-	-	-	-	-	0.1	-	-	0.1	-	-	0.1	-	-	0.1	-
2264	intermediol	-	-	-	-	-	0.5	1.0	1.5	0.7	1.0	1.5	0.4	1.0	0.2	-	-	-
2324	caryophylladienol-II	-	-	-	-	-	-	0.2	0.1	0.1	0.1	0.1	-	0.1	-	-	0.2	-
2606	β-costol	-	0.6	-	-	-	-	-	-	0.1	-	-	-	0.1	-	-	-	-
2607	octadecanol	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Total %	84. 6	77.9	100.0	95.3	98.6	98.2	99.3	86.6	96.4	97.5	96.1	98.1	96.6	91.0	99.0	85.1	81.0

RRI = retention indices calculated against n-alkanes; % calculated from TIC data; *correct isomer not identified

C (49%) within this population. The major compounds accumulated in the oils of plants collected from the Qwa-qwa population were 1,8-cineole (plant A and B), santolina alcohol (plant B), α-thujone (plants A, B and C) and β-thujone (plants B and C). Most of the oils had some level of α- and/or β-thujone with the highest being α-thujone at 78% reported from that of plant C in Setibeng. It was interesting to note that the oil of plant B from Klipriversberg had β-thujone (1%) while the oils of plants A and B only had α-thujone (0.1% and 6%, respectively). The oils of the three plants from Giant's Castle showed within population variation. The oil of plant A accumulated artemisia ketone (15%) and artemisia alcohol (9%) while oils of the other two plants were devoid of these compounds. These two compounds were also present in the oils of plant B,

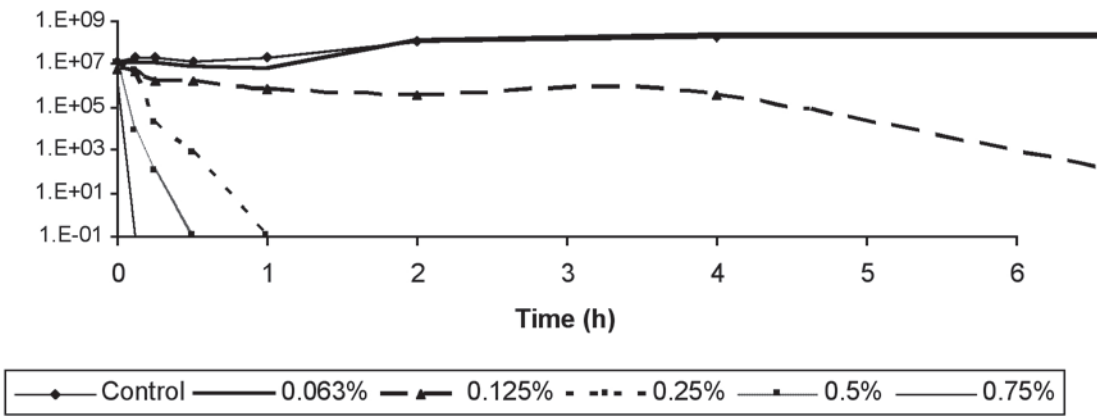


Figure 2. Death kinetics of *K. pneumoniae* exposed to *A. afra* oil at concentrations 0.063-0.75% over the first 6 h of a 24 h incubation period

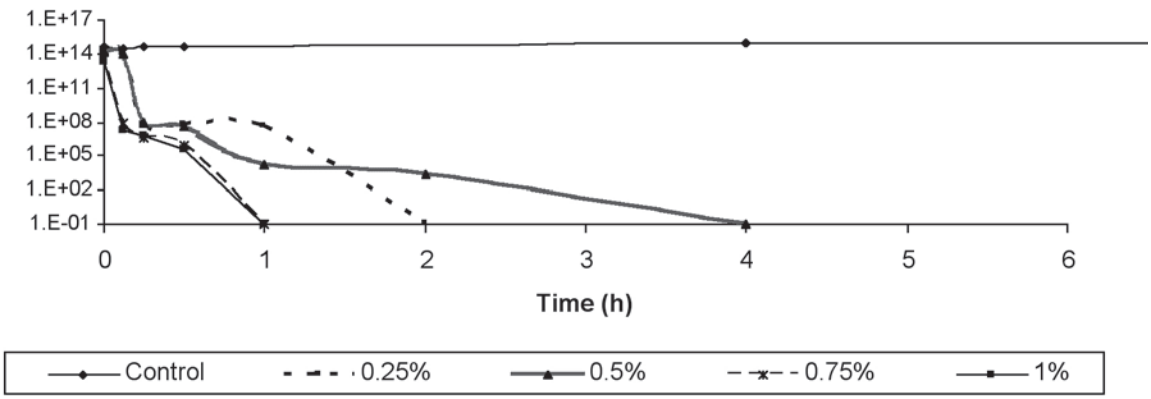


Figure 3. Death kinetics of *C. neoformans* exposed to *A. afra* oil at concentrations 0.25-1% over the first 6 h of a 24 h incubation period

C, E and G from Klipriversberg. From these results displayed here, chemical variation is evident in the major and minor oil compounds, both within and between natural populations and not correlating to geographical distribution.

The antimicrobial activity for African wormwood oil has been previously reported (7,11,12), hence for this report only time-kill analysis is presented to validate the traditional use as a treatment regimen for respiratory disorders. The time-kill plots for *K. pneumoniae* (Figure 2) demonstrates the concentration dependent antimicrobial activity where the bactericidal efficacy is greatest at 0.75% within 10 min, followed by 0.5% after 30 min and 0.25% after 60 min. No bactericidal effect was noted at 0.125% concentration, however reduced colony forming units were observed when comparatively evaluating against the control having no oil. Very little antimicrobial activity was initially noted at concentration 0.063% with regrowth of *K. pneumoniae* after 60 min. Time-kill plots for *C. neoformans* (Figure 3) show a bactericidal efficacy for all concentrations tested with concentrations 0.25% to 1% having a cidal effect within 4 h. The greatest death kinetic is seen at 1% within 60 min. The use of *A. afra* oil to treat respiratory ailments is validated by the rapid bactericidal rate over time for *K. pneumoniae* and *C. neoformans.*

Figure 4 illustrates the logarithmic results obtained for the chemical constituents independently and in combination over 24 h. Within 2 h exposure to the 0.5% *A. afra* oil, *K. pneumoniae* showed a bactericidal effect. This is more clearly noted in Figure 2 which gives a narrower time scale. None of the compounds independently or in combination showed any bactericidal effect when investigated over 24 h. Single compounds 1,8-cineole; artemisia ketone and α-thujone as well as the compounds in combination (artemisia ketone with 1,8-cineole; artemisia ketone with α-thujone) indicated no antimicrobial activity in the time-kill analysis and plots are approximately equivalent to that of the control which displays microbial growth without any test substance. The combination of compounds; α- and β-thu-

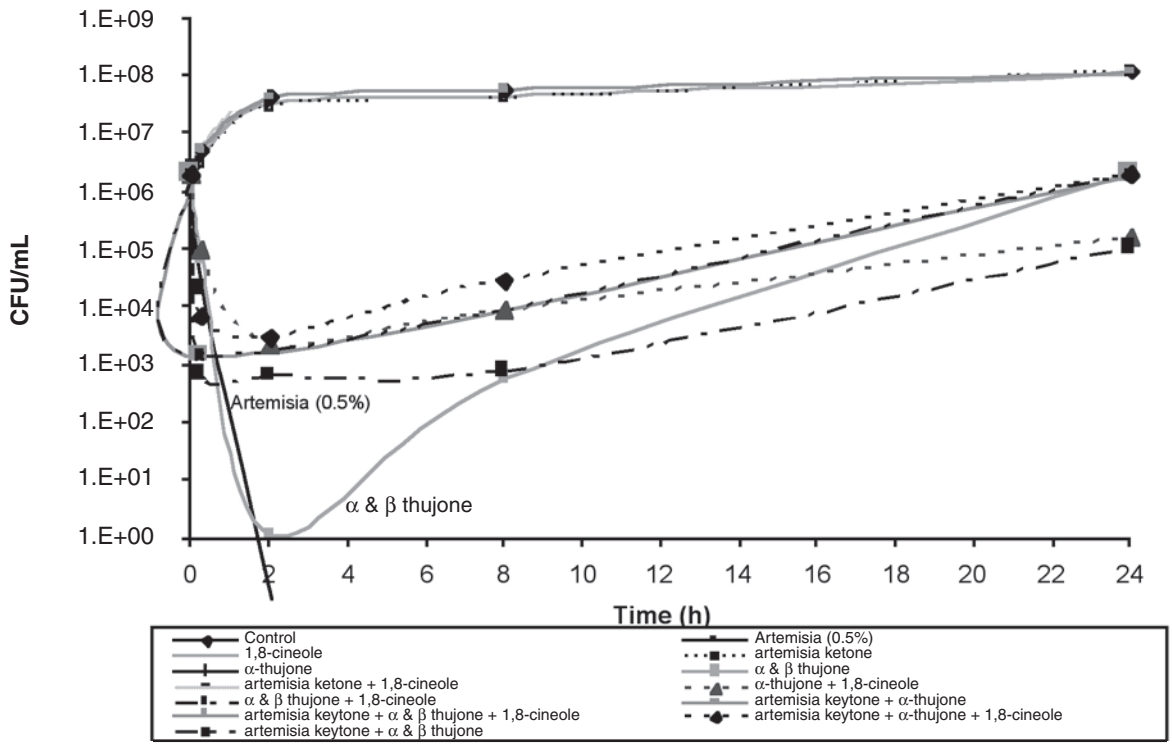


Figure 4. The death kinetics of *K. pneumoniae* exposed to *A. afra* oil at a 0.5% concentration with combinations of α- and β-thujone, artemisia ketone and 1,8-cineole as indicated in the legend

jone with 1,8-cineole; artemisia ketone, α- and β-thujone with 1,8-cineole; artemisia ketone with α- and β-thujone; artemisia ketone and α-thujone in combination with 1,8-cineole and α–thujone with 1,8-cineole showed a reduction in colony forming units but regrowth emerging after 2 h. The combination α- and β-thujone indicated the highest reduction in colony forming units of all the compounds studied however regrowth followed. For *A. afra* oil at 0.5% oil concentration, bactericidal efficacy was obtained within 2 h and maintained for the full 24 h test period suggesting that the minor compounds or combination thereof were possibly responsible for complete cidal efficacy within the oil of *A. afra* (Figure 4).

Commercial preparations need to be standardized as *A. afra* oil showed immense chemical variation. The problems associated with standardization is a very complex matter with herbal products as the activity may not be ascribed to a single chemical entity, but to a combination of constituents, some of which have not yet been identified. At the present time, most herbal products are standardized on the basis of the concentration of a single active or marker compound in a concentrated extract. If the active or marker compound is present in appropriate quantity, it is assumed that all the other necessary components are also presented and uniform activity is assumed. In this study the oil of plant C from Giant's Castle population proved to be the favorable plant to be cloned for cultivation. This plant accumulated as major constituents; camphene (7%), 1,8-cineole (15%), high content of camphor (49%) and no thujone. If thujone is administered in high doses it may cause confusion, convulsions and coma (3). Therefore caution has to be taken with respect to most of the plant samples, if they are to be used in preparation of commercial tinctures as six out of the 16 plant samples had β-thujone and seven samples had α-thujone. Standardization may be achieved by the selection of a favorable chemotype based on efficacy (e.g. broad spectrum of antimicrobial activity) and safety (e.g. low thujone content) for cloning and cultivation.

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