Chapter 3 – Results and Discussion

3.1 Essential oil and non-volatile extract yields

The relative percentage of essential oil and solvent extract yield (on a dry weight basis) is given in Table 3.1.

Table 3.1 Yields of essential oil and non-volatile extracts of *Mentha longifolia* subspecies *polyadena* analysed.

Sample Number	Locality	Yield* (%) Essential oil	Solvent extract
1	Potchefstroom (North-West Province)	1.09	10.73
2	Lydenburg (Mpumulanga Province)	0.88	8.94
3	Dullstroom (Mpumalanga Province)	0.72	8.50
4	Komukwane (Botswana)	0.61	10.28
5	Prins Albert (Eastern Cape Province)	0.36	11.82
6	Wakkerstroom (Mpumulanga Province)	0.59	10.17
7	Clocolan (Free State Province)	1.46	9.03
8	Pretoria (Gauteng Province)	1.01	7.37

* Yield expressed on a dry weight basis

The essential oil yield ranged from 0.36% (Prins Albert) to 1.46% (Clocolan).

Monfared *et al.*, (2002), reported the total oil yield of *M. longifolia* growing in Iran ranged from 0.80% to 2.10%, whereas Kokkini and Papageorgiou (1998), reported the oil yield of *M. longifolia* growing wild in Greece ranged from 0.90% to 1.00%.

The same variation was observed for the solvent extracts where the yield ranged from 7.37% (Pretoria) to 11.82% (Eastern Cape population).

3.2 Gas chromatography coupled to mass spectroscopy (GC/MS)

Table 3.2 confirms that compounds such as piperitenone, piperitenone oxide, and *cis*-piperitone epoxide were found to be present only in the Prins Albert and Komukwane populations. *Cis*-piperitone epoxide was reported for the first time and hence constitutes a new chemotype. Pulegone was found mainly in the Lydenburg population with much smaller quantities detected in the Potchefstroom, Dullstroom, Komukwane and Clocolan populations. The Lydenburg population also contains the highest level of menthone relative to the other populations. The Komukwane population contains the highest level of piperitenone, *cis*-piperitone epoxide, β-caryophyllene, linalool, germacrene-D and myrcene relative to the other populations. The Prins Albert population has the highest level of piperitenone oxide and borneol relative to the other populations. The bar graph (Figure 3.1) depicts the differences in content of the major compounds in the eight different populations. Menthofuran was the major compound in most populations, except the Komukwane and Prins Albert populations. The Potchefstroom and Clocolan populations contain the highest level of limonene, whereas the Dullstroom population has the highest level of 1,8-cineole. It is thus evident that *Mentha longifolia* subsp. *polyadena* exhibits both qualitative and quantitative variation between natural populations.

Table 3.2 Essential oil compositions of the eight populations of *Mentha longifolia* subspecies *polyadena* as determined by GC/MS.

RI[a]	Compound	Po	L	D	K	PA	W	C	Pr
924	α-Thujene	0.2	0.1	t	0.1	t	0.1	t	t
930	α-Pinene	1.8	0.5	1.2	2.0	t	1.1	0.9	1.4
938	Camphene	1.6	0.4	0.7	0.9	t	0.1	t	0.5
958	Sabinene	0.8	0.3	0.7	0.9	t	0.7	0.9	0.6
961	1-Octen-3-ol	-	t	-	-	t	-	-	-
963	β-Pinene	2.1	0.8	1.6	2.0	t	1.8	1.7	1.7
974	3-Octanol	-	-	-	T	0.1	-	-	-
975	Myrcene	1.2	0.6	0.3	2.3	t	0.8	0.2	0.4
1002	Benzene acetaldehyde	t	-	-	t	t	t	-	-
1002	α-Terpinene	0.1	0.2	t	0.1	t	t	t	t
1003	*p*-Cymene	0.2	0.2	t	0.1	t	t	t	t
1005	1,8-Cineole	7.2	1.4	8.9	4.5	0.3	4.1	6.3	3.3
1009	Limonene	7.2	1.4	3.8	4.5	0.3	4.1	6.3	3.3
1017	γ-Terpinene	-	-	-	-	-	-	-	-
1027	*cis*-β-Ocimene	-	-	-	-	-	-	-	t
1035	*trans*-β-Ocimene	0.2	0.3	0.1	0.1	t	t	t	t
1037	*trans*-Sabinene hydrate	0.2	0.8	0.2	0.1	t	t	-	t
1064	Terpinolene	0.1	0.1	t	0.1	t	t	-	t
1066	*cis*-Sabinene hydrate	-	0.1	-	-	-	-	-	-
1074	Linalool	0.2	1.5	0.3	1.8	0.7	0.1	t	t
1074	*trans*-*p*-2-Menthen-1-ol	-	0.1	-	-	-	-	-	-
1120	Menthone	0.4	5.0	0.3	0.3	-	0.6	1.7	t
1126	Isomenthone	0.1	0.2	t	0.9	-	t	0.5	t
1134	Menthofuran	55.3	51.4	58.0	0.9	-	59.7	61.6	52.8
1134	Borneol	2.0	0.8	t	1.5	5.1	t	-	-
1148	*p*-Cymen-8-ol	-	-	-	-	0.2	-	-	-
1148	Terpinen-4-ol	0.4	1.1	0.2	0.2		0.3	0.1	t
1153	Myrtenal	-	0.1	-	-	t	-	-	-
1159	α-Terpineol	0.1	0.4	0.1	0.2	0.8	0.1	t	t
1168	Myrtenol	-	-	-	-	t	-	-	-
1210	Pulegone	0.4	12.0	0.2	0.1		t	0.2	t
1236	*cis*-Piperitone epoxide	-	-	t	35.7	14.7	-	-	-
1265	Bornyl acetate	1.0	0.2	1.0	0.1	0.5	0.2	t	0.4

RI[a]	Compound	Po	L	D	K	PA	W	C	Pr
1269	6-Hydroxy-carvotanacetone*	-	-	-	0.2	-	-	-	-
1275	Thymol	-	-	-	0.3	-	-	-	-
1289	Piperitenone	-	-	-	2.9	1.6	-	-	-
1332	Piperitenone oxide	-	-	-	14.6	65.7	-	-	-
1332	δ-Elemene	-	t	-	-	0.2	-	-	-
1345	α-Cubebene	-	-	-	-	-	-	-	-
1375	α-Copaene	-	-	-	-	-	-	-	-
1379	β-Bourbonene	0.2	0.4	0.4	0.6	0.1	0.1	0.1	0.5
1385	β-Cubebene	-	-	-	-	-	-	-	-
1388	β-Elemene	0.1	0.2	1.3	0.4	0.2	0.3	1.2	0.6
1414	β-Caryophyllene	4.9	5.3	3.9	8.0	2.5	5.3	3.6	5.7
1422	β-Copaene*	0.1	0.1	t	0.2	-	0.2	t	t
1426	β-Gurjunene*	-	-	-	-	-	-	-	-
1447	α-Humulene	0.6	0.7	1.9	1.3	0.2	0.7	0.5	0.8
1456	*allo*-Aromadendrene	-	-	-	-	-	-	-	-
1474	Germacrene-D	1.6	2.4	0.9	4.5	1.2	3.8	1.5	1.2
1487	Bicyclogermacrene	0.7	1.0	1.1	1.0	t	2.6	0.7	0.5
1500	γ-Cadinene	0.3	0.2	3.4	0.4	-	0.6	0.9	0.8
1505	δ-Cadinene	0.5	0.3	t	0.3	-	0.8	0.6	1.8
1549	*trans*-Nerolidol	-	0.3	0.4	-	0.2	-	-	-
1551	Spathulenol	0.3	0.4	0.5	0.3		0.9	0.5	1.3
1561	β-Caryophyllene oxide	1.1	0.2	0.3	0.9	0.2	1.1	1.1	0.4
1616	T-Cadinol	0.2	0.2	0.1	t	t	0.3	-	0.4
1618	δ-Cadinol	t	t	t	t	0.2	0.3	0.2	0.5
1626	α-Cadinol	0.4	0.3	0.1	0.7	t	0.4	0.5	0.9
	Total	**93.8**	**92.0**	**91.9**	**96.0**	**95.0**	**91.2**	**91.8**	**79.8**

Key: RI[a] = Retention index relative to C_8 – C_{17} *n*-alkanes on the DB-1 column, t = trace (<0.05 %), * based on mass spectra only.

Po = Potchefstroom (North-West Province), L = Lydenburg (Mpumalanga Province), D = Dullstroom (Mpumalanga Province), K = Komukwane (Botswana), PA = Prins Albert (Eastern Cape Province), W = Wakkerstroom (Mpumalanga Province), C = Clocolan (Free State Province), Pr = Pretoria (Gauteng Province).

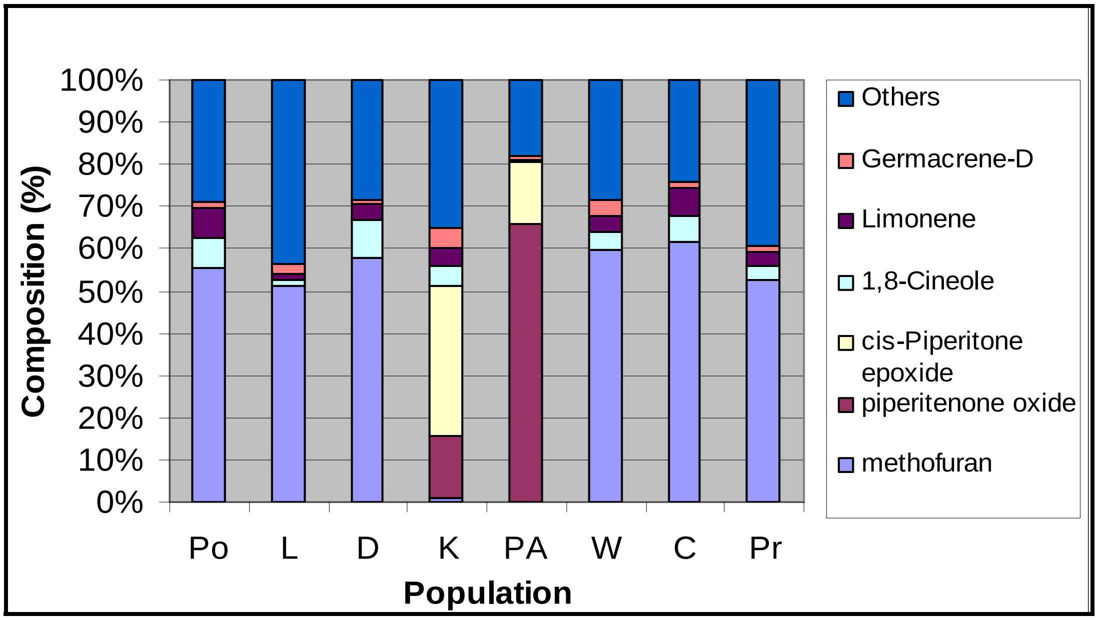

Figure 3.1 Graph showing the relative amounts (%), of selected essential oil compounds from different populations. **Key** : Po - Potchefstroom; L - Lydenburg; D - Dullstroom; K - Komukwane; PA - Prins Albert; W - Wakkerstroom; C - Clocolan; Pr - Pretoria.

3.3 Qualitative cluster analysis of essential oils

Cluster analysis of the essential oil data from Table 3.2 was generated using NTSYSpc-2 and a dendrogram was constructed. The dendrogram in Figure 3.2 shows that the eight populations are divided into two major clusters with respect to the chemical compositon of their volatile oils. The first cluster, A1, consists of 6 populations with the exception of Komukwane and Prins Albert, the latter populations are united in a subcluster (A2). The first cluster, A1, is defined by the high content of menthofuran in the six populations, while the Komukwane and Prins Albert population are united in A2 on the basis of the shared high levels of *cis*-piperitone epoxide and piperitenone oxide. The relatively higher levels of menthone and pulegone in the Lydenburg population distinguishes it from the other populations in A1.

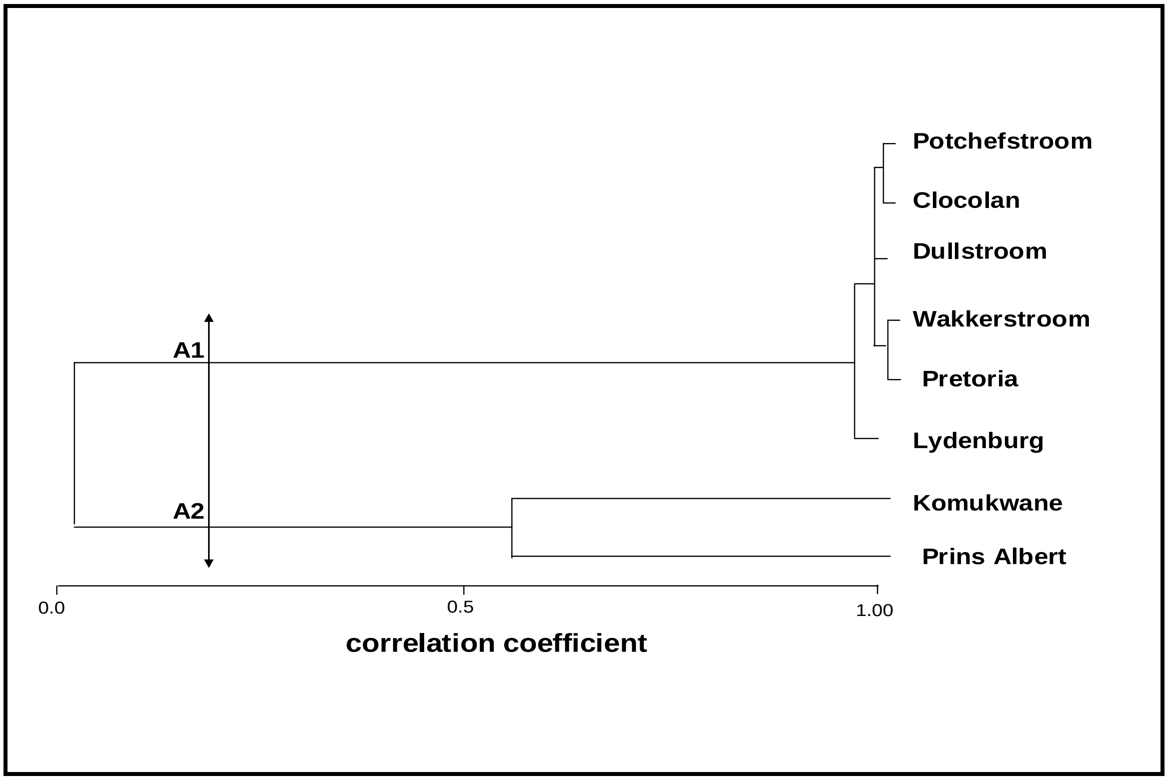

Figure 3.2 Dendrogram obtained from the cluster analysis performed on the essential oil composition (Table 3.2), using the unweighted pair-group method with arithmetic average (UPGMA).

The Potchefstroom and Clocolan populations have a correlation co-effecient very close to 1. This implies that the two populations are very similar in terms of the compounds present within their essential oils. There are some compounds present in the Potchefstroom population that are absent in the Clocolan population. These compounds include *trans*-sabinene hydrate, terpinolene, borneol and t-cadinol (Table 3.2). Furthermore, the same three compounds were shown to be present in all populations except Clocolan. The Wakkerstroom and Pretoria populations also have a correlation coefficient very close to 1. Compounds present in the Wakkerstroom population but absent in the Pretoria population are benzene acetaldehyde and borneol. As these compounds are also present in the Potchefstroom population there is correlation between the Potchefstroom and Wakkerstroom populations.

The Dullstroom population was associated in the dendrogram with the Lydenburg and Prins Albert populations on the basis of shared *trans*-nerolidol. The Dullstroom population also has *cis*-piperitone epoxide in common with the Komukwane and Prins Albert populations. Some compounds such as *cis*-sabinene hydrate, *trans*-p-2-menthen-1-ol and myrtenal were present only in the Lydenburg population. The Lydenburg population also gave the highest yield of compounds such as pulegone (12.0%), menthone (5.0%) and *trans*-sabinene hydrate (0.8%).

Other major constituents present in these populations were 1,8-cineole (ranging from 0.3% in the Prins Albert population to 8.9% in the Dullstroom population); limonene (ranging from 0.3% in the Lydenburg population to 7.2% in the Potchefstroom population); β-caryophyllene (ranging from 2.5% in the Prins Albert population to 8.0% in the Komukwane population); and germacrene-D (ranging from 0.9% in the Dullstroom population to 4.5% in the Komukwane population). The Lydenburg population contained the highest amount of menthone (5.0%), with much lower levels of menthone (between 0.3% and 0.6%) in most of the other populations. The highest levels of borneol was found in the Prins Albert population (5.1%) followed by the Potchefstroom (2.0%) and Komukwane (1.5%) populations.

The oxygen containing monoterpenes were by far the most abundant group of components present in the essential oils of all the samples. The amount ranged from 89.6% in the Prins Albert population to 56.5% in the Pretoria population.

Hence it can be concluded that from the eight samples studied, there are two major chemotypes. In the first chemotype, menthofuran is the major compound (ranging from 51.4% in the Lydenburg population to 61.6% in the Clocolan population). Furthermore, from this major chemotype, the Lydenburg population is distinguished from the other five populations due to the relatively high content of pulegone (12.0%). The second chemotype is primarily composed of *cis*-piperitone epoxide and piperitenone oxide containing plants.

3.4 High performance liquid chromatography analysis

The HPLC profiles (Figure 3.3) are more conservative and less variable compared to the essential oils. The two major peaks (Rt 22.39 and 26.47 min) were present in all 8 samples, although the relative amounts varied. The λ_{max} corresponds with that suggested for flavanones that typically exhibit two major absorption peaks in the region of 250-350 nm (Markham, 1982). Also common to all eight profiles is the detection of another flavanone derivative at 20.70 min.

Figure 3.3 HPLC profiles for the solvent extracts of *Mentha longifolia* subspecies *polyadena* of the eight populations.

The peak with retention time of 31.97 min was present in all the populations except the Prins Albert population. The peak detected at 33.38 min (λ_{max} 243; 278 and 340 nm) (Figure 3.3, Table 3.3) corresponds to one of the major peaks in all population samples except Prins Albert. The λ_{max} data corresponds with that suggested for flavones that typically exhibit two major absorption peaks in the region 240-400 nm (Mabry *et al*., 1970). Other common compounds present in all populations correspond to the retention times of 20.70 min, 21.76 min, 25.83 min and 32.75 min. Furthermore, the peaks corresponding to the retention times of 29.57 and 30.09 min were present in all the populations except Komukwane (Table 3.3). The Wakkerstroom and Pretoria populations had a peak corresponding to a retention time of 34.53, which was not detected in any of the other populations (Table 3.3).

Little is known of the secondary metabolites of South African counterparts of the *M. longifolia* species complex. Microchemical tests have indicated the presence of the following flavonoids: acacetin, hesperiden, as well as luteolin and apigenin glucuronides have been reported from European populations (Bourweig and Pohl, 1973), where eight flavonoid compounds were isolated and identified. These include luteolin-7-glucoside, luteolin-7-rutinoside, lutoelin-7-glucuronide, apigenin-7-glucuronide, acacetin-7-rutinoside, diosmetin-7-rutinoside (which are all representative of flavone derivatives), hesperetin-7-rutinoside and eriodictyol-7-rutinoside (which are both flavanone derivatives).

Table 3.3 Summary of the HPLC data (retention time and UV maxima) for the solvent extracts from the eight different populations of *Mentha longifolia* subspecies *polyadena* used in the study.

R.t. (mins)	U.V. (nm)	T.I.*	Po	L	D	K	PA	W	C	Pr
3.99	287 and 336		+	+	+	+	+	+	+	+
20.70	283 and 323	Flavanone	+	+	+	+	+	+	+	+
21.76	283 and 323	Flavanone	+	+	+	+	+	+	+	+
22.39	205 and 329		+	+	+	+	+	+	+	+
22.80	286 and 328		+	+	+	-	+	+	+	+
23.43	233; 286 and 312		+	+	+	+	+	+	+	+
24.59	289 and 319		+	+	+	+	+	+	+	+
25.83	220; 288 and 326		+	+	+	+	+	+	+	+
26.47	283 and 330	Flavanone	+	+	+	+	+	+	+	+
27.90	234; 284 and 336		+	+	-	+	-	+	+	+
29.57	291 and 332		+	+	+	-	+	+	+	+
30.09	223; 286 and 326		+	+	+	-	+	+	+	+
30.41	265 and 321		-	-	-	+	+	+	-	-
30.85	244; 284 and 341		-	+	-	+	+	+	+	+
31.10	227; 278 and 345		+	+	+	+	+	+	+	+
31.77	285 and 342		+	+	+	+	+	+	+	+
31.97	219; 285 and 334		+	+	+	+	-	+	+	+
32.75	297 and 331		+	+	+	+	+	+	+	+
33.38	243; 278 and 340	Flavone	+	+	+	+	-	+	+	+
34.25	278 and 335	Flavone	+	+	+	+	-	+	+	+
34.53	231; 286 and 327		-	-	-	-	-	+	-	+
35.00	290 and 328		-	-	+	+	-	+	+	+
35.34	269 and 328		-	-	+	+	-	+	+	+
39.17	283 and 327		-	-	-	+	-	-	+	+
40.99	273; 327 and 366		-	+	+	+	-	+	-	+
44.50	271 and 327		-	-	-	+	+	+	+	+

Key : R.t. – retention time; U.V. – ultra-violet light absorption; T.I. – tentative identification; Po – Potchefstroom; L – Lydenburg; D – Dullstroom; K – Komukwane; PA – Prins Albert; W – Wakkerstroom; C – Clocolan; Pr - Pretoria.

* - this study focussed on the tentative identification of only flavanone and flavone derivatives (belonging to the flavonoid group of compounds) in the extracts.

3.5 Antimicrobial activity

Both the essential oil and the extracts were used to investigate antimicrobial activity against selected yeasts, Gram-positive and Gram-negative bacteria based on the traditional uses of this plant.

Candida albicans: The Komukwane and Prins Albert essential oil samples showed moderate to good activity (Table 3.4 - MIC values of 3 mg/ml and 0.5 mg/ml respectively). The other oil samples showed little to no activity. The MIC values ranged from 0.5 to 32 mg/ml against *C. albicans,* showing a 64-fold variation in antimicrobial activity. The solvent extracts of all eight populations showed moderate activity, with MIC values ranging from 2 mg/ml (Komukwane and Lydenburg) to 8 mg/ml (Wakkerstroom).

Cryptococcus neoformans: The Komukwane and Prins Albert essential oil samples showed good activity (MIC values of 0.5 mg/ml and 1.6 mg/ml respectively), whereas the samples from Dullstroom and Clocolan showed poor activity (MIC value of 24 mg/ml). The remaining samples showed moderate activity (MIC values in the range of 6-8 mg/ml). The solvent extracts from all the populations showed moderate to good activity ranging from 0.4 mg/ml (Dullstroom) to 4 mg/ml (Potchefstroom, Lydenburg and Clocolan).

Staphylococcus aureus: The Komukwane and Prins Albert essential oil samples showed the best inhibition (MIC value of 6 mg/ml), whereas the MIC values ranged from 8 mg/ml to 12 mg/ml for the other samples. The extracts showed moderate to good antibacterial activity, with MIC values ranging from 1 mg/ml (Lydenburg and Pretoria) to 4 mg/ml (Prins Albert).

Table 3.4 Microdilution assay results of the essential oils and non-volatile extracts tested on eleven selected pathogens.

Samples	Yeasts				Gram-positive pathogens								Gram-negative pathogens									
	C a		C n		S a		S e		B c		E f		K p		S t		E c		Y e		M c	
	EO	Ex	EO	Ex	EO	Ex	EO	Ex	EO	Ex	EO	Ex	EO	Ex	EO	Ex	EO	Ex	EO	Ex	EO	Ex
Potchefstroom	32	3	8	4	12	2	12	1	8	1.5	12	8	32	4	32	6	32	2	12	0.5	8	2
Lydenburg	24	2	8	4	8	1	4	1	8	1	8	4	6	16	32	3	32	2	6	0.5	8	1
Dullstroom	32	4	24	0.4	8	1.5	1	1	8	1.5	8	4	32	4	24	2	32	2	6	0.5	4	1
Komukwane	3	2	0.5	1	6	2	4	1	8	2	8	4	24	16	8	8	16	3	4	0.5	4	2
Prins Albert	0.5	3	1.6	1.5	6	4	*	1.5	*	2	*	4	4	16	*	4	*	6	*	0.8	*	2
Wakkerstroom	32	8	8	0.8	8	2	4	1	12	1.5	8	4	8	16	32	6	32	4	8	1	4	2
Clocolan	32	4	24	4	12	2	2	1	6	1	12	4	8	16	32	3	32	4	8	0.5	4	1
Pretoria	24	4	6	2	8	1	3	0.5	4	1.5	16	4	8	1	*	2	*	6	4	0.5	*	1
+ Control Amphotericin B	3.13**		6.25**		N/A		N/A		N/A		N/A		N/A		N/A		N/A		N/A		N/A	
Ciprofloxacin	N/A		N/A		3.13**		0.78**		0.39**		6.25**		25**		0.39**		12.5**		1.56**		1.5**	
- Control	NG		NG		NG		NG		NG		NG		NG		NG		NG		NG		NG	

Key: Ca – *Candida albicans* (ATCC 10231); Cn - *Cryptococcus neoformans* (ATCC 90112); Sa - *Staphylococcus aureus* (ATCC 12600); Se - *Staphylococcus epidermidis* (ATCC 2223); Bc - *Bacillus cereus* (ATCC 11778); Ef - *Enterococcus faecalis* (ATCC 29212); Kp - *Klebsiella pneumoniae* (NCTC 9633); St - *Salmonella typhimurium* (ATCC 14028); Ec - *Escherichia coli* (ATCC 8739); Ye - *Yersinia enterocolitica* (ATCC 23715); Mc - *Moraxella catarrhalis* (clinical strain).

* not tested due to insufficient sample; ** - all MIC values ("M") for the + controls are recorded as M x 10^{-4}; N/A – Not Applicable; NG – no growth.

Staphylococcus epidermidis: Most of the essential oil samples showed moderate to good activity in particular the Dullstroom and Clocolan samples (MIC values of 1 mg/ml and 2 mg/ml respectively).

Bacillus cereus: There was moderate activity overall, with the Clocolan and Pretoria essential oil samples showing the highest activity (MIC value of 6 mg/ml and 4 mg/ml respectively). The extracts showed good activity against *B. cereus*, with MIC values ranging from 1 mg/ml (Lydenburg and Clocolan) to 2 mg/ml (Komukwane and Prins Albert). There was a three-fold variation (MIC values ranging from 4 mg/ml to 12 mg/ml) in antimicrobial activity shown between the different localities for the essential oils but little variation for the extract samples.

Enterococcus faecalis: There was moderate overall activity with the Lydenburg, Dullstroom, Komukwane and Wakkerstroom essential oil samples showing the highest activity. All the extracts showed the same MIC activity (4 mg/ml) with the lowest MIC activity (8 mg/ml) recorded for the Potchefstroom sample. There was little to no variation in antimicrobial activity exhibited by both the extracts and the essential oils.

Klebsiella pneumoniae: The Lydenburg and Prins Albert essential oil samples showed the highest activity (MIC values of 6 mg/ml and 4 mg/ml respectively), whereas the Pretoria (MIC value of 1 mg/ml), Potchefstroom and Dullstroom (both 4 mg/ml) phenolic extracts showed moderate to good activity. The antimicrobial activity ranged from 4 mg/ml to 32 mg/ml for the essential oils and from 1 mg/ml to 16 mg/ml for the extracts thus showing an eight to sixteen fold variation in the essential oil and phenolic extract samples respectively.

Salmonella typhimurium: The Komukwane essential oil sample was the only population to show moderate activity (MIC value of 8 mg/ml). The extracts showed moderate to good activity with MIC values ranging from 2 mg/ml (Dullstroom and Pretoria) to 8 mg/ml (Komukwane).

Escherichia coli: The Komukwane essential oil sample was also the only sample to exhibit activity (MIC value of 16 mg/ml). The extracts showed good to moderate activity ranging from 2 mg/ml (Potchefstroom, Lydenburg and Dullstroom) to 6 mg/ml (Prins Albert and Pretoria).

Yersinia enterocolitica: There was moderate activity overall, with the Komukwane and Pretoria essential oil samples showing the most promising activity (MIC value of 4 mg/ml). The extracts of all the populations showed good activity with a MIC value of 0.5 mg/ml in all the populations except Prins Albert (0.8 mg/ml) and Wakkerstroom (1 mg/ml).

Moraxella catarrhalis: The essential oil samples from Dullstroom, Komukwane, Wakkerstroom and Clocolan showed moderate activity (MIC value of 4 mg/ml). The extracts again showed good activity with MIC values ranging from 1 mg/ml to 2 mg/ml. There was little variation in antimicrobial activity for both the essential oil and solvent extracts from the different localities.

In general, the essential oil of *M. longifolia* subsp. *polyadena* from the different localities showed poor to moderate activity against *C. neoformans* but no activity against *C. albicans*, except for the Komukwane and Prins Albert populations which showed good activity. This may have been due to the high content of *cis*-piperitone epoxide and piperitenone oxide in both populations.

The Dullstroom and Clocolan populations showed the least activity against *C. neoformans.* This demonstrated the antimicrobial variability between populations in different geographical localities. In general, activity against the Gram-positive pathogens was moderate to good with the Potchefstroom population showing the least activity. The highest sensitivity for the Gram-positive pathogens was against *S. epidermidis*. This confirms the rationale for the traditional use of this plant in the treatment of wounds and skin infections.

Gram-negative activity against *K. pneumoniae* was moderate except for the Potchefstroom, Dullstroom and Komukwane populations where there was no activity. There was no activity against *S. typhimurium* and *E. coli* except for the Komukwane population which showed moderate activity. Activity against *Y. enterolitica* and *M. catarrhalis* was moderate amongst all populations. Mimica-Dukic *et al*., (2003), reported that the essential oil of *M. longifolia* found in Yugoslavia, contained the major compounds piperitone (38.8%) and menthone (11.2%). The oil manifested poor to moderate activity against Gram-negative strains such as *E. coli*, *S. typhmurium* and *Y. enterolitica*. The plant also showed some activity against *C. albicans*. The essential oil profile in the same study, revealed the relatively high content of oxygenated monoterpenes (66.7%).

These results are in agreement with previous studies, which concluded that essential oils in general are more active against Gram-positive than Gram-negative bacteria (Zohri *et al*., 1995; Mimica-Dukic *et al*., 2003). The physical nature of the Gram-negative cell wall is presented as a possible explanation for the lower permeability to hydrophobic (essential oil) molecules.

In a previous study, Dorman and Deans (2000) investigated the antibacterial activity of various volatile oil constituents including borneol and menthone. The study revealed that borneol did not inhibit the growth of *E. faecalis*, *K. pneumoniae, Moraxella* species and *Y. enterocolitica*, but did inhibit the growth of *S. aureus* and *E. coli.* Menthone was reported to inhibit the growth of all the pathogens mentioned except *E. faecalis.* All the populations in the current study, except those from Prins Albert and those from Clocolan and Pretoria contained small quantities of both menthone and borneol respectively.

In a different study investigating the antimicrobial activity of some Egyptian aromatic plants, Ross *et al*. (1980) showed that high quantities of menthone in a specific oil composition resulted in good antimicrobial activity.

The antimicrobial activity of the solvent extracts was generally moderate to good against all pathogens except *K. pneumoniae*, where the extracts showed moderate to no activity. The extract obtained from the Pretoria population displayed good activity against *K. pneumoniae*. All the extracts showed good activity against *S. epidermidis* and *Y. enterocolitica* with MIC values ranging from 0.5 mg/ml to 1.5 mg/ml and 0.5 mg/ml to 1.0 mg/ml respectively, thus providing a possible reason for the extensive use of this plant in traditional medicine for treating skin infections and wounds.

The antimicrobial results of the extracts ranged between good to moderate for most of the pathogens tested and this provides a possible rationale for the widespread use of this plant in traditional medicine for the treatment of skin infections and wounds, gastrointestinal infections, and respiratory tract infections all of which are associated with at least one or more of the selected pathogens. The extracts showed much greater overall antimicrobial activity against the pathogens as compared to the essential oil samples.

3.6 Correlation between essential oil composition and antimicrobial activity

There was relatively good correlation shown between the recorded antimicrobial activity and the chemical composition of the samples being tested as shown in Figure 3.4. The antimicrobial data (Table 3.4) were subjected to a qualitative cluster analysis. Data extracted from GC/MS analysis as presented in Table 3.2 were compared to the dendrogram.

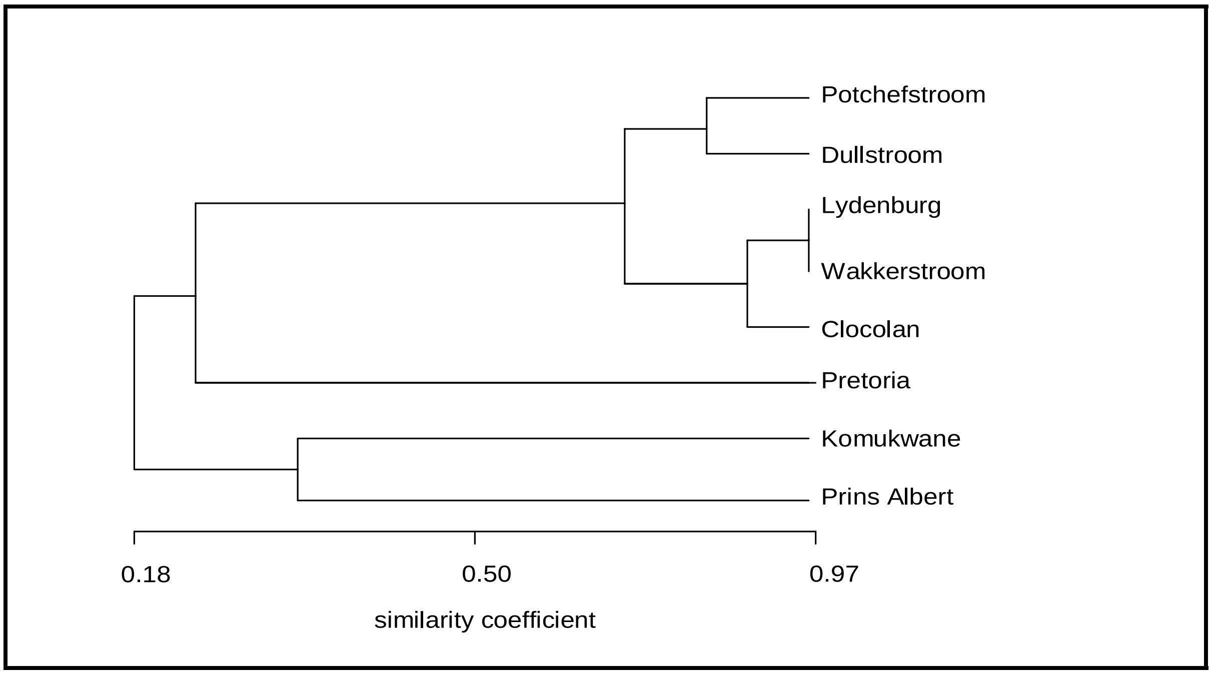

Figure 3.4 Dendrogram obtained from cluster analysis of the microbiological data as presented in Table 3.2 (essential oils only).

The Komukwane and Prins Albert essential oil samples, characterised mainly by high levels of *cis*-piperitone epoxide and piperitenone oxide displayed similar antimicrobial activity, which was different to the remaining six populations that were characterised mainly by high levels of menthofuran. Samples from Lydenburg and Wakkerstroom displayed a similar spectrum of activity (Table 3.4) and are hence closely clustered together in Figure 3.4, thus suggesting that the biological activity of an essential oil is a reflection of its chemical composition. Both essential oil samples showed almost identical activity towards *C. neoformans*, *S. aureus*, *S. epidermidis*, *E. faecalis*, *S. typhimurium* and *E. coli*. Based on the similar antimicrobial properties, one would expect some chemical congruence in terms of the essential oil composition for these two population samples. Figure 3.2 shows the Lydenburg population to be chemically similar to the Wakkerstroom population in subcluster A1, with the exception being in the content of pulegone and menthone, both of which are relatively higher in the Lydenburg population (12% and 5.0% respectively).

One is inclined to expect plants with similar chemical profiles to also display comparable biological activities, in this case, antimicrobial activity. From Figure 3.2 it is observed that samples from Potchefstroom, Clocolan, Dullstroom, Wakkerstroom and Pretoria have a very high correlation co-efficient and from Table 3.2 quantitative and qualitative similarities in essential oil composition are apparent (e.g. menthofuran, limonene, pulegone and β-caryophyllene). The oils from these five populations however show different antimicrobial activities (Table 3.4). Compared to Dullstroom and Clocolan, the Potchefstroom and Wakkerstroom populations exerted more superior activity against the yeast *C. neoformans*. The Wakkerstroom sample however displayed better activity against *S. epidermidis* and *K. pneumoniae* when compared to the Potchefstroom population.

Mangena and Muyima, (1999), suggested that in addition to the antimicrobial action of the major compounds one should also consider the contribution of minor compounds, as it could be possible that the components of the essential oil may exert their antimicrobial effect in a synergistic manner. Onawunmi *et al*. (1985), illustrated synergism of the essential oil compounds in lemon grass, where one of the compounds myrcene, showed no activity on its own but enhanced the activity of α- and β-citral. Dorman and Deans (2000) reported that β-pinene showed antibacterial activity against *S. aureus*, *E. faecalis*, and *Y. enterolitica*. These findings are in agreement with the results obtained in this study owing to the presence of β-pinene in all eight populations.

Pattnaik *et al.* (1997), reported that 1,8-cineole inhibited a large number of bacteria including *S. aureus* at a concentration of 6.7 mg/ml. These findings are in agreement with the results obtained in this study owing to the presence of 1,8-cineole in all eight populations. In contrast, Raman *et al*. (1995) performed a study using tea tree oil and found 1,8-cineole to be inactive against *S. aureus* using a TLC-bioautographic assay. Further tests and chemical analysis would be necessary to investigate this discrepancy.

In some plants, one main constituent may predominate, whereas in other species there is no single component which predominates. Instead, there is a balance of various components. The presence of trace components, even those as yet unidentified, can influence the odour, flavour, and possibly also the biological activity of the oil to a significant extent. Generally, the biological activity of the oils is the combined effect of both their active and inactive compounds which can influence resorptions, rate of reactions and bioavailability of the active compounds. Several active compounds may have synergistic effects. To add to the complexity of volatile oils, there is evidence that the time of harvest influences the oil composition and consequently the potency of their biological activity (Deans and Svaboda, 1988; Lis-Balchin *et al*., 1992; Galambosi *et al*., 1993). Other factors such as genotype, chemotype, geographical origin and environmental conditions, can all influence the composition of the final natural product (Svaboda and Deans, 1992; Collins *et al*., 1994; Svaboda and Deans, 1995; Galambosi *et al*., 1999).

A study in Northern Finland by Clark and Menary (1980) reported low contents of menthofuran in *Mentha piperita* L. Possible reasons for this were reported to be long day treatments with full light intensity, low temperature and rather high moisture levels of air that are typical during the growing period in Northern Finland. As these factors vary in different geographical regions, hence the quantitative levels of the chemical compounds also vary apart from the qualitative differences which are also apparent in this study.

Recent research suggests that the pulegone (d-isomer) is metabolised in the liver to menthofuran, via a highly reactive metabolite which binds irreversibly to the components of liver cells in which metabolism takes place, quickly destroying the liver (Thomassen *et al*., 1990). Pulegone (d-isomer) has in addition been shown to rapidly destroy cytochrome P_{450} in rats (Moorthy, 1991). Therefore, the use of *Mentha longifolia* subsp. *polyadena* should be discouraged in patients with a history of liver disease or those taking cytochrome P_{450} inducing drugs such as certain antiepileptic agents (e.g. phenytoin, cabamazepine and phenobarbitone), progestogens and ethanol. In addition, certain potentially hepatotoxic drugs such as paracetamol, isoniazid, and indomethacin, should not be used concurrently with *Mentha longifolia* preparations, due to the effects of potentially high pulegone levels.

Taking the process one step further, there would be a need to standardise commercial preparations of these essential oils, owing to the large variation between natural populations of this plant. At present however, standardisation techniques do not take into account the effects of minor compounds on the overall antimicrobial activity. The current method of standardisation in many herbal products is based on the concentration of a single (major) compound in an essential oil. The essential oils showed good to moderate activity against *C. neoformans* and *S. epidermidis* from all the pathogens tested. This may provide a rationale for preparing essential oil products from this plant, on a commercial basis to treat such infections.