SUPPLEMENTARY MATERIAL

Chemical composition, cytotoxicity, antimicrobial and antifungal activity of several essential oils

Sara Cannas^a, Donatella Usai^a, Roberta Tardugno^b, Stefania Benvenuti^b, Federica Pellati^b, Stefania Zanetti^a, Paola Molicotti^a

Affiliation

^a*Dipartimento di Scienze Biomediche, Microbiologia Sperimentale e Clinica, Università degli Studi di Sassari viale San Pietro 43/b 07100 Sassari, Italy;* ^b*Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia, Via Campi 183, 41125 Modena Italy.*

Corresponding author: Dr. Sara Cannas Ph.D, Clinical and Experimental Microbiology, Department of Biomedical Sciences, University of Sassari, Viale S. Pietro 43/B, 07100 Sassari, Italy
e-mail: sarac1977@libero.it - Phone:+39079228761

Usai Donatella: dusai08@yahoo.it

Tardugno Roberta: roberta.tardugno@unimore.it

Benvenuti Stefania: stefania.benvenuti@unimore.it

Pellati Federica: federica.pellati@unimore.it

Zanetti Stefania: zanettis@uniss.it

Molicotti Paola: molicott@uniss.it

Abstract

Essential oils (EOs) are known and used for their biological, antibacterial, antifungal and antioxidant properties. Numerous studies have shown that EOs exhibit a large spectrum of biological activities *in vitro*. The incidence of drug-resistant pathogens and the toxicity of antibiotics have drawn attention to the antimicrobial activity of natural products, encouraging the development of alternative treatments. The aim of this study was to analyse the phytochemical and the cytotoxic characteristic of 36 EOs; we then evaluated the antimicrobial activity of the less toxic EOs on Gram positive, Gram negative and fungi strains. The results showed low cytotoxicity in 7 EOs and good activity against Gram negative and *Candida* spp. strains. Based on our results, EOs could be proposed as a novel group of therapeutic agents. Further experiments are necessary to confirm their pharmacological effectiveness, and to determine potential toxic effects and the mechanism of their activity in *in vivo* models.

Key words

essential oil, cytotoxicity, antimicrobial activity, antifungal activity, chemical composition

Abbreviations

GC-MS: gaschromatography/mass spectrometry

GC-FID: gaschromatography/flame ionization detector

MBC: Minimum bactericidal concentration

ATCC: American Type Culture Collection

IC₅₀: half maximal inhibitory concentration

3. Experimental

3.1. Chemicals and Reagents

All reference standards used for GC analysis were of chromatographic grade and were purchased from Sigma-Aldrich (Milan, Italy). Chromatographic grade organic solvents were from Sigma-Aldrich (Milan, Italy).

3.2. Essential Oil Samples

The commercial essential oil samples selected for this study are listed and numbered as follows: *Citrus aurantium* (L.) var. *dulcis* (1), *Eugenia caryophyllata* Thunb. (2), *Eucalyptus globulus* Labill. (3), *Pelargonium asperum* Willd. (4), *Lavandula angustifolia* Mill. (5), *Cymbopogon citratus* (DC.) Stapf (6), *Mentha piperita* L. (7), *Myrtus communis* L. (8), *Origanum vulgare* L. (9), *Rosmarinus officinalis* L. (10), *Salvia sclarea* L. (11), *Melaleuca alternifolia* Cheel (12), *Cedrelopsis grevei* Baill. (13), *Cinnamomum camphora* (L.) J. Presl (14), *Cinnamosma fragrans* Baill. (15), *Eucalyptus citriodora* Hook. (16), *Melaleuca viridiflora* Sol. Ex Gaertn. (17), *Cymbopogon martini* (Roxb.) W. Watson (18), *Melissa officinalis* L. (19), *Thymus vulgaris* L.- white thyme linalol/geraniol bio. (20), *T. vulgaris* L. - white thyme linalol/thymol sel. (21), *T. vulgaris* L. - red thyme carvacrol sel. (22), *T. hyemalis* Lange - red thyme cineole bio. (23), *T. vulgaris* L. - red thyme geraniol bio. (24), *T. vulgaris* L. - red thyme geraniol sel. (25), *T. vulgaris* L. - red thyme bio. France (26), *T. vulgaris* L. - red thyme sel. France (27), *T. vulgaris* L. - red thyme bio. Spain (28), *T. vulgaris* L. - red thyme 4-thujanol (29), *T. vulgaris* L. - sweet thyme (30), *T. vulgaris* L. - red thyme 4-thuvalol sel. (31), *T. vulgaris* L. - red thyme 4-thuvalol bio. (32), *T. vulgaris* L. - red thyme immature plant (33), *T. vulgaris* L. - red thyme thymol bio. (34), *T. vulgaris* L. - citrus thyme (35), *Juniperus communis* L. (36). The samples nominated as “bio” received a specified treatment pre/during and post land, growth and conservation of the plants. The plants and soils were treated with 100% natural fertilizers and/or pesticides. On the other hand, the samples specified as “sel.” were obtained from conventional crops selected in order to obtain certain specific characteristics required by the oil market.

The percentage composition of all the samples analysed, except for genus *Thymus* is shown in Table S1 while the composition of the several thyme samples is shown in Table S2. All the samples were protected from light and humidity until required for chemical analysis.

3.3. GC-MS Analysis

Analyses were performed on a 7890A gas chromatograph (Agilent Technologies, Waldbronn, Germany), coupled with a 5975C Network mass spectrometer (Agilent Technologies). The compounds were separated on an HP-5 MS cross-linked poly-5% diphenyl-95% dimethyl polysiloxane (30 m x 0.25 mm i.d., 1.00 mm film thickness) capillary column (Agilent

Technologies). The column was initially 45 °C, then increased to 100 °C at a rate of 2 °C/min then it was raised to 250 °C at a rate of 5 °C/min and finally it was held for 5 min. The injection volume was 0.1 µL, with a split ratio 1:50. Helium was used as the carrier gas at a flow rate of 0.7 mL/min. The injector temperature was set at 250 °C. MS detection was performed with electron ionization (EI) at 70 eV, operating in the full-scan acquisition mode in the m/z range 40-400. The essential oils were diluted 1:20 (v/v) with *n*-hexane before GC-MS analysis.

3.4. GC-FID Analysis

Analyses were carried out on a 7820 gas chromatograph (Agilent Technologies), with flame ionization detector (FID). The compounds were separated on a HP-5 cross-linked poly-5% diphenyl-95% dimethyl polysiloxane (25 m x 0.2 mm i.d., 0.25 mm film thickness) capillary column (Agilent Technologies). The injection volume and the temperature program were the same as described above. The split ratio was 1:20. Helium was used at a flow rate of 1 mL/min. The injector and detector temperatures were set at 250 °C and 300 °C, respectively. The essential oils and the reference standards were diluted 1:20 (v/v) with *n*-hexane before GC-FID analysis.

Qualitative and semi-quantitative analysis

The compounds were identified by the comparison of their linear retention indices (LRI) relative to C₈-C₄₀ *n*-alkanes (Sigma-Aldrich, Milan-Italy) under the above-mentioned conditions with those provided in the literature (Adams RP, 2007). Furthermore, the identification of the several constituents was carried out by the comparison of their mass spectra with those recorded in the National Institute of Standards and Technology (NIST version 2.0d, 2005) and, when necessary, identification was carried out by co-injection of available reference compounds.

The relative percentage amounts of individual components were expressed as the percentage peak area relative to the total composition of the essential oil obtained by the GC-FID analysis. Semi-quantitative data were acquired as the mean of triplicate analyses for each sample.

3.5. Cytotoxicity

In this study we evaluated the cytotoxicity of the 36 essential oils, using the MTT (3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay. The quantity of formazan (presumably directly proportional to the number of viable cells) is measured by recording changes in absorbance at 570 nm using a plate reading spectrophotometer. Viable cells with active metabolism convert MTT into a purple-colored formazan product with an absorbance maximum near 570 nm. For this purpose three cellular lines were used: human epithelial carcinoma (Hep-2), heterogeneous human epithelial colorectal adenocarcinoma cells (Caco-2) and human conjunctival cells (WKD). The assay was performed following the manufacturer's instructions.

3.6. Strains

A collection of 17 strains were selected for this study: 2 ATCC *Staphylococcus aureus* strains and 5 Gram negative strains: 2 ATCC *Pseudomonas aeruginosa*, 2 ATCC and 1 clinical *Escherichia coli* strains. Finally, 11 clinical isolates belonging to 5 different species of *Candida* spp.: *C. albicans* (3), *C. tropicalis* (2), *C. parapsilosis* (2) *C. glabrata* (2) and *C. krusei* (2).

All the microorganisms were identified using standard methods and stored on Sabouraud Dextrose, Mannitol salt and Mac Conkey agar plates until the studies were performed.

3.7. Determination of Minimum Bactericidal Concentration (MBC)

Yeasts were cultivated at 37 °C on Sabouraud Agar plates (Kima, Padova, Italy) for 24 hours. The inoculum was prepared by a dilution of the colonies in salt solution, at a concentration of 0.5 McFarland standard and confirmed by a spectrophotometric reading at a wavelength of 530 nm. The sensitivity test was carried out in RPMI 1640, using 96-well plates. Essential oil concentrations were prepared by serial one to two dilutions from 16% (v/v) to 0,125% (v/v). After shaking, 100 µL of each oil dilution and 100 µL of yeast suspension were added to each well and then incubated at 35 °C for 24 hours (CLSI Performance Standards for antimicrobial susceptibility testing 2009). In order to determine the MBC values, 10 µL of *Candida* suspension were seeded on Sabouraud-dextrose medium, and the plates were incubated for 24-48 h at the temperature of 37 °C. Bacterial strains were added to the BHI broth and incubated at 35° C until they reached a visible turbidity equivalent to 0.5 on the McFarland scale (approximately 10^8 CFU ml⁻¹). The cultures were diluted to 10^6 CFU ml⁻¹ in Muller–Hinton broth. An inoculum of 100 µL of microbial culture was added to 100 ML of each concentration of essential oils in each well and incubated for 24 h at 37 °C in 96-well plates (Techno Plastic Products AG, Transadingen, Switzerland). Experimental groups that showed no observed visible turbidity were subcultured on the surface of a Plate Count Agar for colony counting. The MBC was considered as the lowest concentration that could inhibit 99% of bacterial growth (Pearson et al., 1980, Clinical and Laboratory standards Institute 2008). The clinical isolates were cultured from specimens of hospitalized patients at the Department of Biomedical Sciences of the University of Sassari. Each test was performed in duplicate.

References

- Adams RP. 2007. Identification of essential oil components by gas chromatography/mass spectrometry. 4th edition. Carol Stream: Allured Publishing Corporation.
- CLSI Performance Standards for Antimicrobial Susceptibility Testing. 2009. 18th Informational Supplement. CLSI M07-A8. Wayne, PA: Clinical and Laboratory Standards Institute. 15-18.
- Pearson RD, Steigbigel RT, Davis HT, Chapman SW. 1980. Method of reliable determination of minimal lethal antibiotic concentrations. Antimicrob Agents Chemother. 18: 699-708.

Clinical and Laboratory Standards Institute. 2008. Performance standards for antimicrobial disk and dilution susceptibility test: M2-A9; 2006. Performance standards for antimicrobial susceptibility testing, 18th informational supplement: M100-S18, Wayne, Pa, USA.

Legends

Table S1: Essential oil qualitative and semi-quantitative analysis

Table S2: Qualitative and semi-quantitative analysis of *Thymus* essential oil samples

Table 1 Essential oil qualitative and semi-quantitative analysis.

Sample n.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	36
Compound ^a	LRI ^b	<i>C. sinensis</i>	<i>E. caryophyllata</i>	<i>E. globulus</i>	<i>P. asperum</i>	<i>L. angustifolia</i>	<i>C. citratus</i>	<i>M. piperita</i>	<i>M. communis</i>	<i>O. vulgare</i>	<i>R. officinalis</i>	<i>S. sclarea</i>	<i>M. alternifolia</i>	<i>C. grevei</i>	<i>C. camphora</i>	<i>C. fragrans</i>	<i>E. citriodora</i>	<i>M. viridiflora</i>	<i>C. martinii</i>	<i>M. officinalis</i>	<i>J. communis</i>
α -Thujene	926					0.06				0.34			0.75		0.80	0.45					1.23
α -Pinene	933	0.53		0.86	0.30	0.10		1.93	30.66	0.95	22.86		2.41	1.07	5.24	4.83	0.39	11.06			40.05
Camphene	947					0.07		0.18		0.17	5.12				0.19	0.10					0.43
Sabinene	973	0.28						0.37					0.09		16.52	7.48					6.28
1-Octen-3-ol	979					0.46														0.39	
β -Pinene	979			0.30				1.71		0.60	7.45		0.65	1.61	3.42	7.09	0.92	3.23		1.40	2.22
Octan-3-one	986					0.46														0.20	
Octan-2-one	988						2.15														
β -Myrcene	992	1.87		0.56	0.12	0.76	14.78	0.18		2.34	1.27	0.38	0.63		1.75	1.91		0.60			8.67
Octan-3-ol	997							1.47													
α -Phellandrene	1004			0.46						0.17			0.35			0.15					
3-Carene	1009													0.32							
α -Terpinene	1017									1.08			7.49		0.46	0.47					0.68
<i>o</i> -Cymene	1021													0.23							
<i>p</i> -Cymene	1025				0.26	0.24		0.32	3.23	12.36	1.64		5.33	0.35		1.48		1.16		1.03	1.83
Limonene	1029	95.41				0.46		5.70		0.54		0.13	1.50	0.44							8.81
1,8-Cineole	1033			95.97		0.40	0.71	5.27	45.17		32.19		2.16		54.92	53.86	3.31	56.61			
<i>cis</i> -Ocimene	1038				0.15	1.47						0.18				0.94					
<i>trans</i> -Ocimene	1048				0.20	3.42	0.46					0.32				0.67			1.26		
γ –Terpinene	1059			0.86		0.10				7.60			18.39		0.87	1.09		0.40			1.15
<i>trans</i> -Sabinene hydrate	1066														0.34						
<i>cis</i> -Linalool oxide	1072				0.36	0.33															
Terpinolene	1088												3.13		0.27	0.38		0.25			1.10
<i>trans</i> -Linalool oxide	1088				0.30	0.36															
<i>cis</i> -Sabinene hydrate	1098																				

Linalool	1103	10.93	35.47	0.88	1.56	0.54	11.57	3.84	2.82	0.76
6-Camphenol	1106				0.41					
<i>cis</i> -Rose oxide	1112	0.85								
Fenchol	1114		0.80					0.15		
<i>trans</i> -Rose oxide	1128	0.28								
<i>trans</i> -Pinocarveol	1140				2.37			0.63		0.27
Camphor	1144		0.38			17.94				
Isopulegol	1148							7.85		
Citronellal	1155							71.70		3.91
Menthone	1157	2.45		20.20						
Isomenthone	1166	4.35								
Borneol	1166		0.95			2.05		0.66		
Neomenthol	1169			14.07						
Lavandulol	1171		1.82							
Terpinen-4-ol	1179		4.29		0.86	0.52	41.93	2.16	2.93	0.93
Menthol	1181			32.29						0.46
<i>p</i> -Cymen-8-ol	1186									2.36
α -Terpineol	1192	1.17	1.47	0.59	1.93	1.30	0.54	2.97	0.27	7.72
Myrtenal	1196				0.73				0.46	2.17
Verbenone	1210									7.28
Nerol	1226						0.26			
Carveol	1231									
Citronellol	1237	25.53							8.03	0.83
Thymol methyl ether	1239									
Pulegone	1245			2.17						
Carvone	1249			0.35						
Neral	1250	0.40		27.33						20.98
Piperitone	1260			1.39						
Linalyl acetate	1264		36.41				70.28			
Geraniol	1265	16.16		5.89						83.27
Geranial	1278	0.65		38.13						1.40
										29.53

Citronellyl formate	1282	5.08																	
Bornyl acetate	1289								0.95									0.29	
Lavandulyl acetate	1294		2.09																
Thymol	1295							5.12											
Menthyl acetate	1297				4.96														
Carvacrol	1305							62.61										0.19	
Geranyl formate	1306		2.93																
Myrtenyl acetate	1331							6.05											
δ -Elemene	1341												0.85						
α -Terpinyl acetate	1354							0.13						2.27					
α -Cubebene	1355		0.33										0.64					0.43	
Citronellyl acetate	1358													1.82					
Neryl acetate	1368			0.46							0.26								
Cyclosativene	1369												0.81						
Eugenol	1373	82.75																	
α -Copaene	1381		0.48								1.69		8.08					0.68	
Geranyl acetate	1387			0.81	2.69		0.82									8.39	2.19		
β -Cubebene	1388										0.48								
β -Bourbonene	1389		1.01			0.30													
β -Elemene	1396										0.45		10.00					1.67	
Cyperene	1403												1.15						
α -Gurjunene	1412											0.47	0.51						
β -Caryophyllene	1425	7.87	0.94	2.04	0.33	0.36	0.28	0.89	2.08	2.85	0.55	0.70	0.67	2.30	1.28	2.16	0.99	18.08	1.66
α -Bergamotene	1441				0.26														
Aromadendrene	1446											1.60							
α -Humulene	1460	0.92							0.31					0.83	0.77			1.32	1.69
β -Farnesene	1461			1.14															
Rotundene	1461												0.45						
Alloaromadendrene	1468																		
Ishwarane	1472												26.64						
γ -Muurolene	1481		0.66										2.88					1.14	
<i>Ar</i> -Curcumene	1487												1.86						

Germacrene D	1488			0.73							6.95			0.26				5.35	1.19	
β-Selinene	1490												1.32							
Valercene	1495										0.27									
α-Selinene	1499													0.39					0.50	
Viridiflorene	1502									1.13	1.48					2.09				
α-Muurolene	1504												3.62						0.92	
γ-Cadinene	1521												1.67					0.24	0.84	
δ-Cadinene	1530			1.52						0.35	1.63				0.71			0.91	3.10	
Calamenene	1530												6.69							
Eugenyl acetate	1535	7.15																		
α-Calacorene	1546												0.41							
Elemol	1551												0.81							
Geranyl butirate	1564			1.13																
trans-Nerolidol	1568															1.28				
Palustrol	1571												0.26							
Spathulenol	1587																			
Phenylethyl tiglate	1592			1.24																
Caryophyllene oxide	1593					0.13	0.59	1.01	0.39							0.46		1.41		
Viridiflorol	1603															6.28				
γ-Eudesmol	1634			5.71																
Cubenol	1641												0.29							
Monoterpene hydrocarbons	98.08	3.04	1.03	6.68	15.23	10.39	33.89	26.16	38.34	1.01	40.72	4.03	29.51	27.03	1.31	16.69	1.26	2.43	72.45	
Monoterpene oxygenated:	89.90	95.97	73.81	86.96	77.77	82.76	59.17	68.58	55.48	82.92	47.06	1.52	65.81	65.07	92.72	64.81	94.47	61.11	3.52	
–Alcohols			54.45	45.95	6.77	48.42	6.27	0.86	4.41	12.37	44.90	1.06	10.88	8.94	15.88	8.21	86.09	4.11	3.23	
–Phenolics	82.75							67.72										0.19		
–Ketones			6.80	0.84	2.15	24.11			17.94									0.20		
–Esters	7.15		10.38	39.77	2.69	4.96	7.00		0.95	70.54				2.27	1.82		8.39	2.19	0.29	
–Aldehydes			1.05		65.46		0.73								71.70			54.42		
–Ethers		95.97	1.13	0.40	0.71	5.27	45.17		32.19		2.16		54.92	53.86	3.31	56.61				
Sesquiterpene hydrocarbons	8.79		5.67	3.18	0.59	0.66	0.28	0.89	2.39	14.17	5.73	68.29	2.16	3.78	1.28	4.26	0.99	25.90	13.82	
Sesquiterpenes oxygenated			5.71			0.13	0.59	1.01	0.39			1.36				8.01		1.41		
Total	98.08	98.69	99.01	86.22	96.82	93.59	93.94	93.93	96.64	96.59	98.09	93.52	75.20	97.49	95.88	95.31	93.78	96.72	90.85	89.79

^a Compounds are listed in order of elution.

^b Linear retention index (LRI) calculated on HP-5 column.

Table S 2 Qualitative and semi-quantitative analysis of *Thymus* essential oil samples

sample n.	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
Compound ^a	LRI ^b	<i>T. vulgaris</i>	<i>T. vulgaris</i>	<i>T. vulgaris</i>	<i>T. hyemalis</i>	<i>T. vulgaris</i>	<i>T. vulgaris</i>	<i>T. vulgaris</i>	<i>T. vulgaris</i>	<i>T. vulgaris</i>	<i>T. vulgaris</i>	<i>T. vulgaris</i>	<i>T. vulgaris</i>	<i>T. vulgaris</i>	<i>T. vulgaris</i>	<i>T. vulgaris</i>	
α-Pinene	933	0.20			4.85						0.24			3.20	0.19	0.64	
Camphene	947				7.41					0.70		0.71	0.91			0.56	
Sabinene	973				1.47												
1-Octen-3-ol	979				0.14												
β-Pinene	979	1.84	0.36		2.97				0.33	0.71	1.11	1.25	1.14		0.78	1.07	
Octan-3-one	986				0.21												
β-Myrcene	992	2.23	1.69		2.07		0.97	0.99									
α-Terpinene	1017								0.67	2.61	1.62	4.29	3.82		1.17		
p-Cymene	1025	3.03	10.11	29.26	2.98	1.00	0.56	2.36	2.26	29.29	2.67	30.55	3.71	2.48	11.71	24.86	5.33
Limonane	1029	2.92	2.83	0.42		0.26	0.50	0.47	0.49	1.18	3.87	1.01	3.22	5.30	1.20	1.03	
1,8-Cineole	1033				32.49		0.16	0.23	0.23	5.49	0.36	2.06	0.50	0.55	1.25	0.43	47.73
trans-Ocimene	1048				0.45												
γ –Terpinene	1059			0.28	0.17	2.71	0.28	0.54	0.60	7.10	6.27	13.98	10.62	8.47	0.29	8.51	0.84
trans-Sabinene hydrate	1066	6.09		2.96	0.65		4.56	1.00	0.98	5.17	1.36	5.44	8.33	2.12	10.92		9.32
Terpinolene	1088	1.81			0.18					0.30	1.29	0.36	1.67	1.71		0.43	

<i>Cis</i> -Sabinene hydrate	1098	7.40	8.60		0.34		0.44			1.05	37.65	0.52	13.99	33.79			0.33
Linalool	1103	43.69	8.07		4.05	42.06	2.62	75.91	75.74								
<i>trans</i> -Pinocarveol	1140		1.60		0.21												
Camphor	1144				15.04	0.47	0.75	0.21	0.21	1.08		0.34			0.38	0.27	11.71
Borneol	1166	3.74	0.25	0.34	5.28	0.26	0.42	0.28	0.26	5.37		0.68	0.24			1.30	6.38
Terpinen-4-ol	1179	13.79	48.20		0.97	0.53	0.82	0.61	0.59	2.37	17.8	1.11	26.51	16.31		1.38	1.56
<i>p</i> -Cymen-8-ol	1186				0.15												
α -Terpineol	1192	2.30	4.79	0.32	2.89	0.44	0.34	0.36	0.35	3.90	3.86	0.31	3.45	3.51	2.58	0.73	3.85
Verbenone	1210				0.36												
Nerol	1226				0.18												
Carveol	1231	0.37	0.97			0.85	2.08	0.72	0.69		4.30		2.47	3.84			
Linalyl acetate	1264				0.90			5.39	5.27								
Geraniol	1265	1.46	0.88			17.91	32.76			2.24		0.35	0.89				0.48
Geranial	1278						1.13										
Bornyl acetate	1289				1.47		0.29										
Thymol	1295	0.58	0.91	38.59	0.27	1.29	0.40	3.33	3.39	21.39		25.19	0.59		63.00	48.65	
Carvacrol	1305	0.40	0.74	27.26				0.20	0.19	3.49		5.60			2.16	3.01	
α -Terpinyl acetate	1354				0.46												
Geranyl acetate	1387					23.61	38.19			0.28		0.28					
β -Caryophyllene	1425	1.88	2.71	0.24	2.22	3.70	4.93	3.43	3.41	1.00	2.96	3.80	3.46	3.98	1.48	1.55	1.52
α -Humulene	1460				0.25		0.23	0.14	0.13								
Alloaromadendrene	1468				0.73												
γ -Cadinene	1521				0.31												
δ -Cadinene	1530				0.68												
Spathulenol	1587				1.14												

Caryophyllene oxide	1593	0.29	1.64	0.18	0.69	0.64	0.88			0.77			0.30				0.39
<i>Monoterpene hydrocarbons</i>		12.03	14.99	29.96	22.54	3.97	1.34	4.34	4.34	38.87	18.12	48.87	25.47	23.83	16.40	36.97	8.44
<i>Monoterpene oxygenated:</i>		79.82	75.01	69.47	66.06	87.42	84.96	88.24	87.90	51.83	65.33	41.88	56.97	60.12	80.29	55.77	81.36
– Alcohols		78.84	73.36	3.62	14.71	62.05	44.04	78.88	78.61	20.10	64.97	8.41	55.88	59.57	13.50	3.41	21.92
– Phenolics		0.98	1.65	65.85	0.42	1.29	0.40	3.53	3.58	24.88		30.79	0.59		65.16	51.66	
– Ketones					15.61	0.47	0.75	0.21	0.21	1.08		0.34			0.38	0.27	11.71
– Esters					2.83	23.61	38.48	5.39	5.27	0.28		0.28					
– Aldehydes								1.13									
– Ethers					32.49		0.16	0.23	0.23	5.49	0.36	2.06	0.50	0.55	1.25	0.43	47.73
<i>Sesquiterpene hydrocarbons</i>		1.88	2.71	0.24	4.19	3.70	5.16	3.57	3.54	1.00	2.96	3.80	3.46	3.98	1.48	1.55	1.52
<i>Sesquiterpenes oxygenated</i>		0.29	1.64	0.18	1.83	0.64	0.88			0.77			0.30				0.39
Total		94.02	94.35	99.85	94.62	95.73	92.34	96.15	95.78	92.47	86.41	94.55	86.20	87.93	98.17	94.29	91.71

^a Compounds are listed in order of elution.

^b Linear retention index (LRI) calculated on HP-5 column.