J. Essent. Oil Res., **9**, 419-426 (Jul/Aug 1997)

RESEARCH REPORT

Uruguayan Essential Oils. Part V. Composition of Bergamot Oil

Eduardo Dellacassa, Daniel Lorenzo and Patrick Moyna
Catedra de Farmacognosia y Productos Naturales, Facultad de Quimica
Universidad de la Republica, Montevideo, Uruguay

Antonella Verzera* and Antonella Cavazza
Dipartimento Farmaco-chimico, Facoltà di Farmacia, Università di Messina, Italy

Abstract

The analysis of industrial and laboratory-prepared bergamot oils produced in Uruguay during the 1995 production season was carried out by GC and GC/MS. The composition of the Uruguayan bergamot oils were compared with those of Italian bergamot oils. They were found to be very similar; however, they could be differentiated from their psoralen and coumarin contents. The enantiomeric ratio of linalool and linalyl acetate was also studied by GC using a β-cyclodextrin column. The results were similar to that found in Italian oils.

Key Word Index

Citrus bergamia, Rutaceae, bergamot oil, essential oil composition, linalool, linalyl acetate, enantioselective GC, coumarins, psoralens.

Introduction

Citrus bergamia is mainly cultivated in Italy and therefore most of the bergamot oil on the market is of Italian origin. Because of the great interest in bergamot oil, which is widely used in the food and cosmetic industries, bergamot cultivation has expanded to the Ivory Coast, Brazil, Argentina, Corsica, Guinea, Turkey and Cameroon. Some information about the composition of these oils is reported in the literature (1-4).

In Uruguay, bergamot has been cultivated in a limited area of about 3 h since 1980 using planting stock of Italian origin. Recently the fruit has been processed by local industries using "birillatrice" machines for the juice and "sfumatrice" machines for the oil. The yield of the oil is about 4%.

This paper reports the results relating to industrial and laboratory-prepared bergamot oils produced in Uruguay during the 1995 production season. The volatile fraction, the nonvolatile residue and the linalool and linalyl acetate enantiomeric ratio were analyzed in order to define the composition and to evaluate the quality of Uruguayan bergamot oil. Its composition was then compared with that of the previously analyzed Italian bergamot oil (5-10).

*Address for correspondence

Received: March 1996
Revised: June 1996

1041-2905/97/0004-0419$04.00/0—©1997 Allured Publishing Corp.

Table I. Chemical composition of industrial Uruguayan and Italian bergamot oils

Peak no.	Compound	Uruguayan oils 1	Uruguayan oils 2	Uruguayan oils 3	Italian oils Min	Italian oils Max	Italian oils $\bar{X}$
1	tricyclene	0.003	-	-	0.002	0.007	0.004
2	α-thujene	0.268	0.268	0.281	0.190	0.491	0.353
3	α-pinene	1.094	1.077	1.137	0.731	1.844	1.346
4	camphene	0.029	0.028	0.030	0.022	0.053	0.038
5	sabinene	{ 6.740	6.552	6.879	0.777	1.778	1.751
6	β-pinene				4.374	11.024	7.481
7	6-methyl-5-hepten-2-one	-	-	-	0.002	0.010	0.005
8	myrcene	1.033	1.016	1.023	0.656	1.575	0.986
9	octanal	0.030	0.024	0.025	0.025	0.056	0.039
10	α-phellandrene	0.046	0.042	0.042	0.025	0.056	0.039
11	δ-3-carene	-	-	-	0.002	0.009	0.004
12	α-terpinene	0.135	0.122	0.132	0.081	0.282	0.167
13	p-cymene	0.250	0.257	0.318	0.060	0.893	0.265
14	limonene + β-phellandrene	38.135	41.173	42.545	25.626	53.187	39.196
15	1,8-cineole	t	t	t	0.010	0.021	0.015
16	(Z)-β-ocimene	0.027	0.028	0.024	0.017	0.068	0.026
17	(E)-β-ocimene	0.193	0.187	0.180	0.104	0.361	0.253
18	γ-terpinene	6.485	6.190	6.349	5.727	11.378	8.249
19	cis-sabinene hydrate	0.020	0.019	0.019	0.024	0.063	0.039
20	octanol	0.003	-	-	0.002	0.021	0.005
21	terpinolene	0.273	0.256	0.260	0.210	0.475	0.347
22	linalool	9.521	8.979	8.908	1.745	20.260	8.090
23	nonanal	0.040	0.045	0.050	0.012	0.076	0.037
24	heptyl acetate	0.011	0.009	0.014	t	0.008	0.004
25	cis-limonene oxide	-	-	-	0.002	0.019	0.007
26	trans-limonene oxide	-	-	-	0.002	0.015	0.006
27	camphor	-	-	-	0.002	0.012	0.004
28	citronellal	0.010	0.009	0.009	0.004	0.030	0.015
29	terpinen-4-ol	0.021	0.018	0.018	0.013	0.042	0.023
30	α-terpineol	0.037	0.028	0.029	0.030	0.098	0.060
31	decanal	0.072	0.065	0.063	0.037	0.086	0.061
32	octyl acetate	0.132	0.120	0.116	0.071	0.185	0.116
33	nerol	0.027	0.024	0.024	0.010	0.111	0.041
34	neral	0.126	0.110	0.109	0.122	0.344	0.229
35	trans-sabinene hydrate acetate	{ 31.835	0.072	{ 28.853	0.057	0.127	0.089
36	linalyl acetate		30.474		15.616	40.375	28.234
37	geraniol	t	t	t	0.004	0.010	0.006
38	geranial + perillaldehyde	0.199	0.171	0.163	0.247	0.494	0.366
39	bornyl acetate	0.026	0.016	0.014	0.010	0.037	0.019
40	undecanal	0.017	0.012	0.011	0.004	0.024	0.008
41	nonyl acetate	0.026	0.022	0.021	0.004	0.053	0.019
42	methyl geranate	0.008	0.004	t	0.002	0.015	0.006
43	linalyl propionate	0.062	0.056	0.047	0.013	0.066	0.035
44	δ-elemene	t	0.002	t	-	t	t
45	α-terpinyl acetate	0.153	0.132	0.124	0.093	0.263	0.171
46	citronellyl acetate	0.031	0.024	0.013	0.012	0.053	0.028
47	neryl acetate	0.334	0.292	0.265	0.145	0.670	0.347
48	geranyl acetate	0.317	0.281	0.261	0.175	0.799	0.375
49	dodecanal	0.030	0.043	0.043	0.002	0.047	0.023
50	decyl acetate	0.027	0.021	0.020	0.013	0.053	0.034
51	cis-α-bergamotene	t	t	t	0.020	0.048	0.030

Table I. Continued

Peak no.	Compound	Uruguayan oils 1	2	3	Italian oils Min	Max	$\bar{X}$
52	β-caryophyllene	0.502	0.360	0.344	0.225	0.524	0.349
53	trans-α-bergamotene	0.387	0.337	0.312	0.211	0.437	0.299
54	α-humulene*	0.047	0.070	0.063	0.017	0.045	0.027
55	(Z)-β-farnesene*	0.060	0.048	0.040	0.038	0.089	0.057
56	cis-β-santalene*	0.020	0.015	0.018	0.005	0.009	0.007
57	germacrene D	0.100	0.070	0.063	0.039	0.106	0.062
58	bicyclogermacrene	0.053	0.040	0.037	0.023	0.076	0.039
59	(E,E)-α-farnesene	t	t	t	-	t	t
60	β-bisabolene	0.556	0.472	0.431	0.298	0.648	0.423
61	(E)-nerolidol	0.026	0.021	0.013	0.012	0.036	0.020
62	tetradecanal	t	t	t	0.004	0.010	0.008
63	2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol	0.023	0.019	0.012	0.007	0.024	0.013
64	campherenol	0.026	0.020	0.020	0.010	0.025	0.016
65	α-bisabolol	0.031	0.023	0.019	0.011	0.030	0.018
66	nootkatone	0.056	0.037	0.037	0.012	0.068	0.032
	Hydrocarbons	56.436	58.610	60.508	42.195	78.258	61.798
	Monoterpenes	54.711	57.196	59.200	40.748	76.811	60.505
	Sesquiterpenes	1.725	1.414	1.308	0.954	1.894	1.293
	Oxygenated compounds	43.277	41.190	39.320	21.540	57.261	38.663
	Carbonyl compounds	0.580	0.516	0.510	0.379	1.033	0.827
	Alcohols	9.735	9.151	9.062	1.850	20.491	8.331
	Esters	32.962	31.523	29.748	17.167	41.484	29.477

* The elution order of these components on 60 m SE-52 column is (Z)-β-farnesene, α-humulene, cis-β-santalene; t = trace

Experimental

The research was carried out on three industrial bergamot oils produced in July 1995 by "sfumatrice" and four laboratory-prepared oils produced from May to July 1995. The laboratory-prepared oils were obtained by cold-pressing the fruits applying manual pressure on the rind to release the oil which was collected on a watch glass, after which it was transferred to a test tube, centrifuged and analyzed. All samples were analyzed by GC using a SE-52 column, 25 m x 0.32 mm for quantitative results, as previously reported (6). The component identification of the volatile fraction was carried out by GC/MS (quadrupole) using SE-52 and Carbowax 20 M capillary columns, 60 m x 0.32 mm (11).

The enantiomeric distribution of linalool and linalyl acetate was carried out by GC on a Carlo Erba chromatograph 5160 Mega series, equipped with a Shimadzu data processor C-R3A using the following experimental conditions: capillary column, 25 m x 0.25 mm coated with a diethyl,terbutylsilyl-β-cyclodextrin (MEGA, Legnano, Italy); column temperature, 40°C to 140°C at 2.5°C/min; injector and detector temperature, 280°C; carrier gas, hydrogen, 0.70 kg/cm^2; split injection; detector, FID. Coumarins and psoralens were analyzed by normal phase HPLC as previously reported (7).

Results and Discussion

Volatile Fraction: For each sample, the quantitative composition (as a relative percentage of the area of peaks) for each component, as well as the total amount of hydrocarbons, monoterpenes, sesquiterpenes, carbonyl compounds, alcohols and esters was calculated. These data relative to the industrial oils are reported in Table I, together with the average composition of the Italian bergamot oils previously analyzed (10). Figure 1 shows the chromatogram of an industrial bergamot oil obtained by GC using a SE-52 column (25 m).

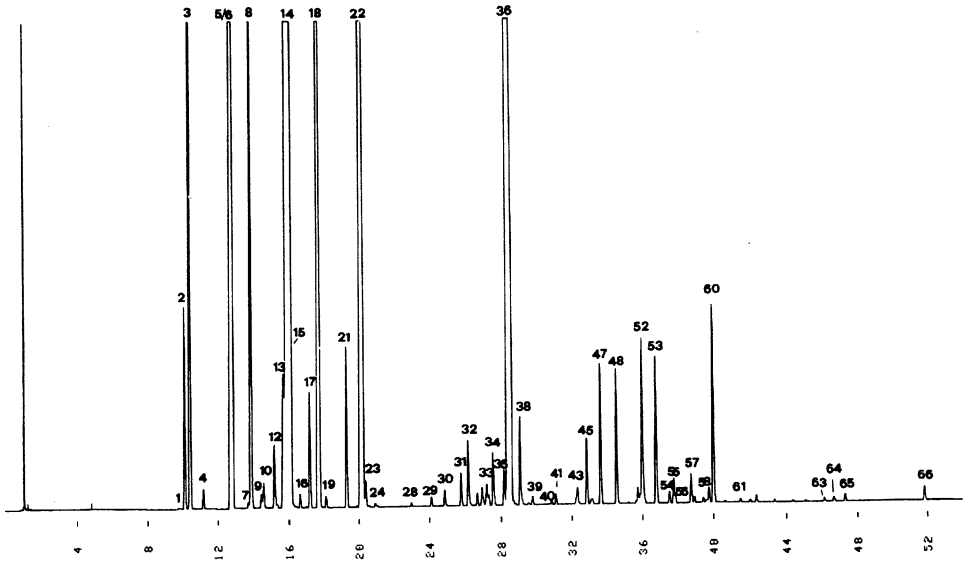

Figure 1. SE-52 chromatogram of an Uruguayan industrial bergamot oil. For the identification of the components, see Table I

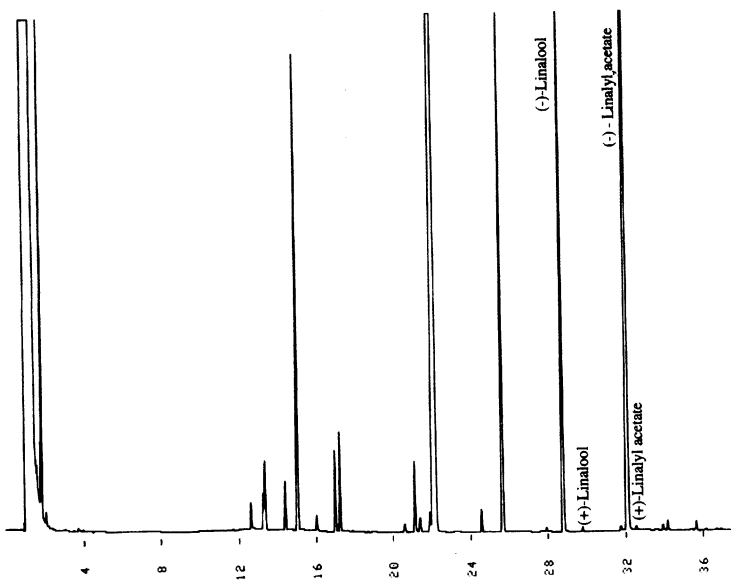

Figure 2. Chiral chromatogram of an Uruguayan industrial bergamot oil

As can be seen from Table I and Figure 1, 68 components were identified representing an average of 99.6% of the volatile fraction. The main components were limonene, linalyl acetate, linalool, sabinene + β-pinene, myrcene and γ-terpinene which constitute altogether about 94% of the whole oil. The oils were characterized by a high content of esters (29.7-32.9%) and alcohols (9.0-9.7%) where about 98% of these classes of substances was represented by linalyl acetate (28.8-31.8%) and linalool (8.9-9.5%) respectively.

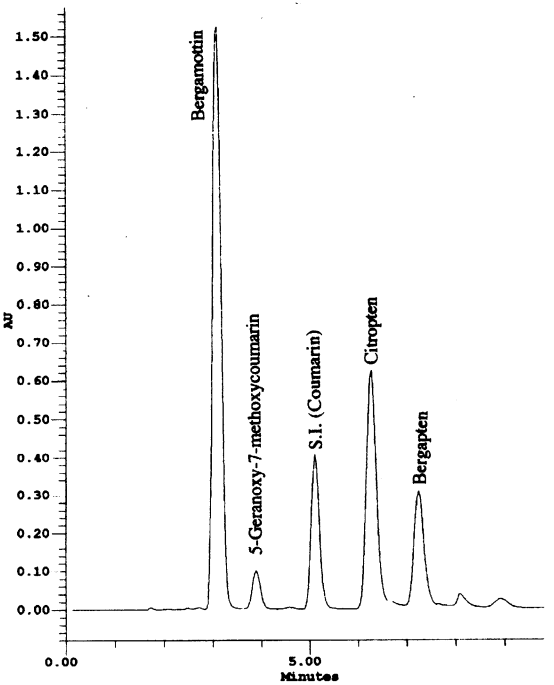

Figure 3. HPLC chromatogram of an Uruguayan industrial bergamot oil

Eleven sesquiterpenes were identified, their content ranging from 1.3% to 1.7% in the oils analyzed. Only small amounts of carbonyl compounds were found (0.51-0.58%), with neral and geranal being the main components.

A comparison between Uruguayan bergamot oils with Italian bergamot oils revealed that they both had a similar composition. In fact the content of each component and substance class for Uruguayan bergamot oil is close to the average values reported for the Italian oils, although the Uruguayan oils showed a slightly higher content of oxygenated compounds and a slightly lower content of monoterpenes than those of Italian origin. As a result, it is difficult to differentiate between the oils of two different origins.

The composition of laboratory-prepared Uruguayan bergamot oil and the grouping of components into classes can be seen in Table II. From this data compositional changes that have taken place during the production season are shown, with the major differences being the changes in limonene and linalool content. For example, limonene increases from 27.4% in June to 41.4% in July, while linalool decreases from 30.9% in June to 12.6% in July.

The linalool content of Uruguayan bergamot oil distinguishes industrial oils from May and June laboratory-prepared oils. The oil produced in July showed a composition similar to that of the industrial oils. This pattern can be explained considering that industrial oils are also produced in July. The higher content of linalool and alcohols in the laboratory-prepared oil produced in July, compared to the industrial oils, is due to the absence of the recycled water which reduces the amount of the water-soluble substances in the industrially produced oils.

The Enantiomeric Ratio of Linalool and Linalyl Acetate: The chromatogram of an Uruguayan bergamot oil obtained by GC using chiral columns is reported in Figure 2. The value of (+)/(–)-linalool and (+)/(–)-linalyl acetate in the Uruguayan oils analyzed compared with those of Italian bergamot oils can be seen in Table III. (+)-Linalool is present in trace amounts, not exceeding 0.6% of the total linalool content. (+)-Linalyl acetate is also present in trace amounts; however, this time it is lower than 0.6% of

Table II. Chemical composition of laboratory-prepared Uruguayan bergamot oils

		May 30, 1995	June 6, 1995	June 13, 1995	July 19, 1995
1	tricyclene	0.002	0.002	0.002	t
2	α-thujene	0.196	0.175	0.179	0.250
3	α-pinene	0.825	0.701	0.720	0.996
4	camphene	0.023	0.019	0.018	0.025
5	sabinene	4.971	4.173	3.934	5.766
6	β-pinene				
7	6-methyl-5-hepten-2-one	t	t	t	t
8	myrcene	0.850	0.673	0.765	0.953
9	octanal	0.024	0.017	0.020	0.025
10	α-phellandrene	0.053	0.050	0.470	0.050
11	δ-3-carene	t	t	t	t
12	α-terpinene	0.105	0.085	0.085	0.118
13	p-cymene	35.606	0.083	31.389	0.278
14	limonene + β-phellandrene		27.454		41.347
15	1,8-cineole	t	t	t	t
16	(Z)-β-ocimene	0.016	0.023	0.018	0.028
17	(E)-β-ocimene	0.160	0.169	0.148	0.189
18	γ-terpinene	4.438	3.837	3.800	5.819
19	cis-sabinene hydrate	0.047	0.028	0.028	0.044
20	octanol	0.007	0.002	t	t
21	terpinolene	0.207	0.172	0.170	0.247
22	linalool	24.983	31.300	25.022	12.607
23	nonanal	0.035	0.036	0.040	0.060
24	heptyl acetate	0.011	0.010	0.010	0.009
25	cis-limonene oxide	0.002	t	0.002	t
26	trans-limonene oxide	0.006	0.005	0.002	t
27	camphor	t	t	t	t
28	citronellal	0.008	0.006	0.007	0.007
29	terpinen-4-ol	0.016	0.013	0.013	0.020
30	α-terpineol	0.092	0.075	0.067	0.068
31	decanal	0.050	0.054	0.048	0.046
32	octyl acetate	0.058	0.059	0.057	0.087
33	nerol	0.126	0.097	0.089	0.048
34	neral	0.214	0.208	0.212	0.209
35	trans-sabinene hydrate acetate	0.065	0.049	0.040	0.068
36	linalyl acetate	24.625	28.400	30.890	28.520
37	geraniol	t	t	t	t
38	geranial + perillaldehyde	0.325	0.330	0.329	0.310
39	bornyl acetate	0.014	0.013	0.015	0.013
40	undecanal	0.017	0.022	0.021	0.005
41	nonyl acetate	0.026	0.025	0.031	0.018
42	methyl geranate	0.006	0.009	0.013	0.005
43	linalyl propionate	0.059	0.063	0.071	0.036
44	δ-elemene	0.011	0.011	0.014	t
45	α-terpinyl acetate	0.057	0.042	0.057	0.109
46	citronellyl acetate	0.024	0.026	0.031	0.026
47	neryl acetate	0.137	0.133	0.179	0.208
48	geranyl acetate	0.119	0.088	0.099	0.169
49	dodecanal	0.004	t	t	0.020
50	decyl acetate	0.004	t	t	0.018

Table II. Continued

		May 30, 1995	June 6, 1995	June 13, 1995	July 19, 1995
51	cis-α-bergamotene	0.030	0.030	0.026	0.015
52	β-caryophyllene	0.247	0.269	0.270	0.311
53	trans-α-bergamotene	0.214	0.197	0.297	0.233
54	α-humulene*	0.031	0.035	0.034	0.027
55	(Z)-β-farnesene*	0.035	0.035	0.021	0.028
56	cis-β-santalene*	0.040	0.052	0.040	0.020
57	germacrene D	0.066	0.055	0.062	0.031
58	bicyclogermacrene	0.027	0.029	0.030	0.031
59	(E,E)-α-farnesene	t	t	t	t
60	β-bisabolene	0.340	0.312	0.276	0.326
61	(E)-nerolidol	0.017	0.015	0.013	0.015
62	tetradecanal	0.004	0.003	0.004	0.004
63	2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol	0.013	0.012	0.012	0.009
64	campherenol	0.013	0.011	0.011	0.014
65	α-bisabolol	0.018	0.012	0.011	0.014
66	nootkatone	0.017	0.020	0.023	0.039
	Hydrocarbons	48.493	38.641	42.345	57.088
	Monoterpenes	47.452	37.616	41.275	56.066
	Sesquiterpenes	1.041	1.025	1.070	1.022
	Oxygenated compounds	51.243	61.183	57.467	42.850
	Carbonyl compounds	0.698	0.696	0.704	0.725
	Alcohols	25.332	31.565	25.266	12.839
	Esters	25.205	28.917	31.493	29.286

* The elution order of these components on 60 m SE-52 column is (Z)-β-farnesene, α-humulene, cis-β-santalene; t = trace

Table III. Enantiomeric ratio of linalool and linalyl acetate in the bergamot oils analyzed

	Linalool		Linalyl acetate	
	(–)	(+)	(–)	(+)
Uruguayan laboratory-prepared oils	99.5-99.8	0.5-0.2	99.6-99.8	04-0.2
Uruguayan industrial oils	99.4-99.6	0.6-0.4	99.5-99.8	0.5-0.2
Italian oils	100.0-99.4	trace-0.6	100.0-99.4	trace-0.6

the total linalyl acetate content. For comparison purposes, the same enantiomeric ratios were found in the Italian oils.

Coumarin and Psorlen Content: Figure 3 shows a HPLC chromatogram of an industrial Uruguayan oil. The coumarins and psoralens found in the industrial and laboratory-prepared oils and the values obtained for the Italian oils are shown in Table IV. Bergamottin, 5-geranoxy-7-methoxycoumarin, citropten and bergapten were identified. In the industrial oils, bergamottin and 5-geranoxy-7-methoxycoumarin content falls within the range reported for Italian oils, although that of bergamottin is lower, while that of 5-geranoxy-7-methoxycoumarin is higher than the average values found in Italian oils. However, citropten and bergapten were similar to the Italian minimum values.

Laboratory-prepared oils showed a lower content of bergamottin and 5-geranoxy-7-methoxy-coumarin and a higher content of citropten and bergapten than the industrial oils. In conclusion, it can be stated that from a compositional standpoint Uruguayan bergamot oils could be considered oils of good quality. They are similar to Italian bergamot oils, and they can only be distinguished by their coumarin and psoralen content of the nonvolatile residue.

Table IV. Major coumarins and psoralens found in bergamot oils of different origins (mg/100 g)

	Uruguayan oils			Italian oils		
	1	2	3	$\bar{X}$	Min	Max
Industrial oils						
Bergamottin	1749	1672	1655	1870	1020	2750
5-Geranoxy-7-MeO-coumarin	153	150	153	130	80	220
Citropten	146	144	146	220	140	350
Bergapten	112	111	112	210	110	320
Laboratory-prepared oils	**May 30, 1995**		**June 6, 1995**	**June 13, 1995**		**July 19, 1995**
Bergamottin	1041		973	845		1256
5-Geranoxy-7-MeO-coumarin	74		72	58		104
Citropten	225		230	218		236
Bergapten	129		163	153		103

Acknowledgments

The authors thank the Instituto Italo Latino Americano which allowed the collaboration between the Universities of Montevideo and Messina and Dr. Dante Irurtia who supplied bergamot oils and fruits.

References

1. R. Huet and C. Dupuis, *Bergamot essential oil in Africa and Corsica.* Fruits, **23**, 301-311 (1968)
2. R. Huet, *Etude comparative de l'huile essentielle de bergamote provenant d'Italie, de Corse et de Cote d'Ivoire.* Rivista Ital. EPPOS, **63**, 310-313 (1981).
3. M. Koketsu, M. T. Magalhaes, V. C. Wilberg and M.G.R. Donalisio, *Oleos Essenciais de frutos citricos cultivados no Brazil.* Bol. Pesqui EMBRAPA Cent. Technol. Agric. Aliment., (7), 21 pp (1983).
4. K.H.C. Baser, Y. Özek and M. Tutas, *Composition of cold pressed bergamot oil from Turkey.* J. Essent. Oil Res., **7**, 341-342 (1995).
5. G. Dugo, G. Lamonica, A. Cotroneo, A. Trozzi, F. Crispo, G. Licandro and D. Gioffré, *Sulla genuinità delle essenze agrumarie. Nota XVII. La composizione della frazione volatile dell'essenza di bergamotto calabrese.* Essenz. Deriv. Agrum., **57**, 456-544 (1987).
6. G. Dugo, A. Cotroneo, A. Verzera, M. G. Donato, R. Del Duce, G. Licandro and F. Crispo, *Genuineness characters of Calabrian Bergamot Essential Oil.* Flav. Fragr. J., **6**, 39-56 (1991).
7. L. Mondello, I. Stagno d'Alcontres, R. Del Duce and F. Crispo, *On the genuineness of citrus essential oils. Part XL. The composition of the coumarins and psoralens of calabrian bergamot essential oils (Citrus bergamia Risso).* Flav. Fragr. J., **8**, 17-24 (1993).
8. A. Cotroneo, I. Stagno d'Alcontres and A. Trozzi, *On the genuineness of citrus essential oils. Part XXXIV. Detection of added reconstituted bergamot oil in genuine bergamot essential oil by high resolution gas chromatography with capillary columns.* Flav. Fragr. J., **7**, 15-17 (1992).
9. L. Mondello, K. D. Bartle, P. Dugo, P. Gans and G. Dugo, *Automated LC-GC: a powerful method for essential oils analysis. Coupled LC-GC-MS (ITD) for bergamot oil analysis.* J. Microcol. Sep., **6**, 237-244 (1994).
10. A. Verzera, G. Lamonica, L. Mondello, A. Trozzi and G. Dugo, *The composition of bergamot oil.* Perfum. Flavor., **21**(6), 19-34 (1996).
11. L. Mondello, P. Dugo, A. Basile, G. Dugo and K. D. Bartle, *Interactive use of linear retention indices, on polar and apolar columns, with a MS-library for reliable identification of complex mixtures.* J. Microcol. Sep., **7**, 581-591 (1995).