



Short report

Chemical and biological diversity of Bergamot (*Citrus bergamia*) in relation to environmental factors

Giancarlo A. Statti^{a,*}, Filomena Conforti^a, Gianni Sacchetti^b,
Mariavittoria Muzzoli^b, Caterina Agrimonti^c,
Francesco Menichini^a

^a*Dipartimento di Scienze Farmaceutiche, Università degli Studi della Calabria, I-87036 Rende (CS), Italy*

^b*Dipartimento delle Risorse Naturali e culturali, Laboratorio Biologia Farmaceutica e Biotrasformazione, Università di Ferrara, corso Porta Mare 2, I-44100 Ferrara, Italy*

^c*Dipartimento di Scienze Ambientali, Sezione genetica e biotecnologia ambientali, Università degli Studi di Parma, Parco Area delle Scienze, 11/A, I-43100 Parma, Italy*

Received 3 September 2003; accepted in revised form 12 December 2003

Abstract

Oil of bergamot is receiving renewed popularity in aromatherapy. The biovariability of *Citrus bergamia* grown wild in Calabria (Italy) was investigated as far as chemical markers (linalool, linalyl acetate and bergapten) content and antioxidant and antifungal activities of the methanolic extracts. The average content in the markers presents slight variations with the altitude and more evident changes with the latitude of the areas of plant collection.

© 2003 Elsevier B.V. All rights reserved.

Keywords: *Citrus bergamia* Risso; Biodiversity; Bergapten; Linalool; Linalyl acetate

Plant. Seven samples of *Citrus bergamia* Risso fruits (CB1–CB7), been collected in Calabria in different zones for altitude and solar exposure in November 2000 and

*Corresponding author. Tel.: +39-0984-493063; fax: +39-0984-493298.

E-mail address: g.statti@unical.it (G.A. Statti).

Table 1
Sites of collection of *C. bergamia* in Calabria, Italy

Sample	Site	Alt.	Coordinate	Soil
CB1	Reggio Calabria	30 m	15°74'05' long. 38°09'68' lat.	Clayey
CB2	Melito p. salvo	50 m	15°78'71' long. 37°91'94' lat.	Clayey
CB3	Gallico	17 m	15°39'93' long. 38°10'52' lat.	Clayey
CB4	Reggio Calabria	23 m	15°39'36' long. 38°04'96' lat.	Clayey
CB5	Melito p. salvo	33 m	15°45'11' long. 37°55'66' lat.	Clayey
CB6	Pellaro	30 m	15°39'70' long. 38°02'55' lat.	Clayey
CB7	Palazzi	19 m	16°03'51' long. 37°55'51' lat.	Clayey

identified by Prof. G. Cesca (Botanical Garden of the University of Calabria, Italy) (Table 1).

Use in traditional medicine. The essence of *C. bergamia* is used for its antiseptic properties. Oil of bergamot is receiving renewed popularity in aromatherapy [1].

Previously isolated classes of constituents. Terpenes, coumarins [2–4].

Tested material. From each fruit sample (500 g) the peel has been submitted to maceration with MeOH (3 extractions of 48 h) and the extracts were treated with pentane. In order to isolate phytochemical markers (linalool, linalyl acetate and bergapten) the *n*-pentane extracts have been submitted to Si-gel CC (*n*-hexane/acetone 98:2). The qualitative data of the chromatographic fractions have been determined through GC-MS (Hewlett-Packard gas-chromatograph, model 5890, equipped with to mass spectrometer, model 5972, and controlled by HP software equipped with capillary column 30 m, 0.25 mm) using programmed temperature from 60 to 280 °C (rate 16°/min); the detector and the injector have been set to a

Table 2
Levels of markers of *C. bergamia* samples

Sample	Plant (g)	Pentane extract (g)	Bergapten (mg)	Linalool (mg)	Linalyl acetate (mg)	Linalool/Linalyl acetate
CB1	500	3.94	20.86 ± 0.004	18.28 ± 0.003	148.44 ± 0.003	0.123
CB2	500	1.7	8.01 ± 0.003	0.053 ± 0.005	0.81 ± 0.005	0.065
CB3	500	2.08	42.71 ± 0.004	18.59 ± 0.004	39.18 ± 0.004	0.474
CB4	500	0.72	14.98 ± 0.002	3.14 ± 0.002	11.54 ± 0.004	0.272
CB5	500	0.83	32.55 ± 0.005	2.02 ± 0.001	13.15 ± 0.003	0.153
CB6	500	0.56	13.77 ± 0.004	4.62 ± 0.003	10.14 ± 0.005	0.455
CB7	500	0.92	22.93 ± 0.01	11.06 ± 0.004	20.22 ± 0.003	0.575

Values are mean ± S.D.
N=3.

Table 3
Biological activity of methanolic extracts of *C. bergamia*

Test	CB1	CB2	CB3	CB4	CB5	CB6	CB7
DPPH assay*							
10 µg/ml	7.57±0.4	8.15±0.5	1.95±0.1	2.29±0.2	1.83±0.1	3.90±0.3	4.13±0.2
20 µg/ml	9.51±0.6	11.45±0.7	5.51±0.3	5.51±0.3	5.51±0.3	4.13±0.3	7.24±0.4
50 µg/ml	15.55±0.8	16.79±1.1	13.56±0.9	12.18±0.8	13.67±0.8	10.80±0.8	13.17±0.9
β-carotene bleaching assay*							
RAA 60 min	0.47±0.031	0.49±0.032	0.45±0.030	0.41±0.029	0.39±0.028	0.31±0.023	0.41±0.029
RAA 120 min	0.29±0.02	0.31±0.02	0.35±0.023	0.32±0.022	0.29±0.02	0.27±0.019	0.31±0.02
Antifungal assay**							
<i>P. ultimum</i>							
100 µg/ml	2.1±0.2	+	3.7±0.7	+	0.9±0.4	8.3±0.6	0.9±0.4
500 µg/ml	36.7±1.5	+	19.8±0.8	14.2±0.7	20.2±1.0	34.2±1.2	5.8±0.9
<i>T. mentagrophytes</i>							
100 µg/ml	+	+	+	+	+	+	+
500 µg/ml	6.5±0.3	11.2±1.0	8.6±0.6	4.3±0.6	9.3±0.8	15.7±0.4	1.4±0.5

N=3.

*BHA was taken as positive control.

**Control was set up at 0.3% of dimethylsulfoxide (DMSO); Miconazole (200 µg/ml) was used as positive control.

All the values are the average of three determinations ±S.D.

temperature of 280 °C and 250 °C, respectively (split vent flow 1 ml min⁻¹). The quantitative determination of the selected markers has been performed by means GC (Shimadzu, model gc 17, series a, equipped with FID detector and with capillary column 30 m, 0.25 mm, controlled by Borwin software) through chromatographic comparison with external standards, which were purchased from Sigma, Germany. Analysis has been conducted in isotherm (110 °C); the detector and the injector have been set to a temperature of 280 °C and 250 °C, respectively. All determinations were performed in triplicate and averaged.

Studied activity. DPPH [5] and β -carotene bleaching [6] tests were used to evaluate the antioxidant activity. Antifungal activity [7,8] was performed on the phytopathogen *Pythium ultimum* Trow and the dermatophyte *Trichophyton mentagrophytes* var. *mentagrophytes* by means of the disc diffusion assay.

Results. Tables 2 and 3.

Discussion and conclusions. The maximum yield of pentane fractions was obtained from the samples CB1 (3.94 g) and CB3 (2.08 g) both deriving from the northern area of the Tyrrhenian slope. The altitude influences slightly the bergapten content (CB3 17 m, yield 2%; CB7 19 m, yield 2.5%) that varies instead in more evident way with the latitude. It is known that ratio linalool/linalyl acetate affects the aroma of the essence of bergamot. A better essence is observed if they ratio has a low value. For the samples analysed in the present work, the ratio between the two markers ranged from 0.065 (CB2) to 0.575 (CB7).

As regards the biological activity the sample CB2 showed the highest antioxidant activity. The extracts of bergamot assayed at the dose of 100 μ g/ml showed some activity against both fungal feters, particularly *T. mentagrophytes*. At 500 μ g/ml, some extracts displayed significant inhibition of *P. ultimum* as following: CB1 (36.7%) and CB6 (34.2%); against *T. mentagrophytes* the highest values were obtained with CB2 (11.2%) and CB6 (15.7%).

With the reference to phytochemical considerations and biological activity data, it is therefore possible to address the proper chemotype of *C. bergamia* for phytomedicine and cosmetic applications.

Acknowledgments

The authors thank Prof. G. Cesca of the Botanical Garden at the University of Calabria, Italy, for supplying the herb samples used in this study.

References

- [1] Stevensen CJ. Clin Dermatol 1998;16:689.
- [2] Subra P, Vega A. J Chromatogr 1997;771:241.

- [3] Dugo P, Mondello L, Stancanelli R, Dugo G. *J Pharm Biomed Analysis* 2000;24:147.
- [4] Wang L-H, Tso M. *J Pharm Biomed Analysis* 2002;30:593.
- [5] Wang M, Li J, Rangarajan M, Shao Y, LaVoie EJ, Huang TC, Ho CT. *J Agric Food Chem* 1998;46:48 693.
- [6] Taga MS, Miller EE, Pratt DE. *J Am Oil Chem Soc* 1984;61:928.
- [7] Maris D, Romagnoli C, Sacchetti G, Vicentini CB, Bruni A. *Med Mycol* 1998;36:379.
- [8] Maris D, Romagnoli C, Sacchetti G, Vicentini CB, Fabiano A, Bruni A. *J Phytophatol* 2000;148:395.