Chemical Composition of Some Traditional Herbal Drug Preparations: Essential Oil and Aromatic Water of Costmary (*Balsamita suaveolens* Pers.)

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The compositions of the essential oil and the aromatic water of costmary (*Balsamita suaveolens* Pers.) cultivated in Tuscany were investigated. They represent the main ingredients of some traditional preparations sold commercially. The essential oil as such and the *n*-hexane extract of the aromatic water were analyzed by GC and GC-MS. Both samples were found to be rich in monoterpenes. Eighty-five compounds were identified, accounting for 95.1 and 95.4% of the essential oil and *n*-hexane extract of aromatic water, respectively. Carvone was the main compound (43.5% in the essential oil and 74.9% in the *n*-hexane extract of aromatic water). In addition, solid phase microextraction was used to sample the volatile organic compounds emitted from the fresh plant and from the aromatic water, and carvone was again the main component, amounting to 46.2 and 41.3%, respectively.

Keywords: *Balsamita suaveolens Pers.; Chrysanthemum balsamita L.; costmary; essential oil; aromatic water; SPME analysis*

INTRODUCTION

Balsamita suaveolens Pers. (syn. *Chrysanthemum balsamita* L., *Tanacetum balsamita* L., Asteraceae) is a well-known herbal drug and has been used since the time of the Egyptians, Greeks, and Romans. Other synonyms are *Balsamita major* Dod, *Pyrethrum balsamita* Willd., and *Balsamita vulgaris* Willd. (*1*). The name "Balsamita" or "balsam herb" is due to a soft, pleasant, and aromatic balsamic odor of the volatile oil contained in the glands on the lower surface of the leaves (*1*). This plant is known by several common names such as "herbe Sainte-Marie", "Balsamita mas", "maudlin", "Achillea ageratum", and "Balsamita foemina". It is more widely called "costmary" (from the Latin "costus", an oriental plant from which the roots were used as a spice and as a preservative, and from "Mary", in reference to the biblical Mary) and "alecost" because it was used as a flavoring in and to clarify beer. Finally, this plant is also known as "bibleleaf" because its broad, long leaves have often been used as aromatic bookmarks in the Bible (*1*). The species is native to Asia Minor, Asia, and Australia but has now become naturalized in many parts of southern Europe, where it is well-known and used in folk medicine mainly for antimicrobial properties due to its essential oil (*2*).

Previous investigations on the essential oil of plants from Romania, Poland, Germany, and Russia showed the presence of carvone or camphor as the main constituents, ranging from 9.2 to 70.0% and from 0 to 91.0%, respectively (*3*−*7*). Recently, an extensive investigation of the constituents of essential oil from the leaves and flowers of a cultivated sample of costmary from Lithuania has also been reported (*8*). Carvone and α-thujone were found to be the major constituents of the oil, ranging from 51.8 to 68.0% and from 9.0 to 16.1%, respectively. On the basis of the dominant terpene derivative of the essential oil, four chemotypes of costmary have been defined: camphor-type (*7*), carvone-type (*7*), a camphor/thujone-type (*9*), and, most recently, the carvone chemotype producing considerable amounts of α-thujone (*8*).

The aim of this investigation was to determine the composition of both the essential oil and aromatic water obtained by traditional extraction equipment from plant material cultivated in Tuscany. These data are useful both to evaluate the adaptive process of the plant, because it does not reach the flowering stage in this climate, and to assess the quality/safety profile of these products traditionally used as both flavors and fragrances.

Different liquid preparations and tablets based on the essential oil and aromatic water of *B. suaveolens* are produced in Florence, using plant material cultivated in Tuscany. For these preparations original recipes of a Dominican monk, Angiolo Marchissi, have been used since 1614. These preparations are traditionally used for their sedative properties, although no pharmacological investigations have supported this application so far. The essential oil is used to prepare some products (0.03% w/w), whereas the aromatic water is employed for the formulation of others (31.23 and 94.75% w/w). More than 150000 tablets and more than 560 L of liquid preparations are marketed every year.

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MATERIALS AND METHODS

Materials. A voucher specimen of the plant has been deposited at the Department "Economico, Estimativo, Agrario e Forestale", Faculty of Agraria, University of Florence, Italy. The *B. suaveolens* plants investigated in this work were cultivated in the Florence area in a dry and sunny but well-irrigated rich land. Propagation was effected by division of 2-year-old deep-rooted buds from the mother plant in early spring, spaced 30 cm apart and fertilized with NPK 20:10:10 and ammonium nitrate in the spring. The plants were cropped the second year after planting and renewed every three years. Aerial parts of *B. suaveolens* collected in July 1999, the time of the maximum development stage in Italy, were submitted to traditional hydrodistillation in the "Officina Profumo Farmaceutica di S. Maria Novella". Thus, the whole aerial fresh plant (7.5 kg) was treated with 50 L of water and boiled for 2 h to obtain 15 L of distilled water saturated with essential oil (the so-called aromatic water) and 3.5 g of essential oil. A quantity of 25 mL of aromatic water was extracted with 5 mL of *n*-hexane three times (2 + 2 + 1 mL). The organic layers were combined, dried with anhydrous Na_2SO_4, and, after filtration, concentrated under nitrogen at room temperature to obtain 1.7 mg (0.0068% of fresh weight).

Methods. *GC analyses* were accomplished with an HP-5890 series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm i.d., 0.25 μm film thickness). The following temperature program was used: 60 °C for 10 min, ramp of 5 °C/min up to 220 °C; injector and detector temperatures, 250 °C; carrier gas, nitrogen (2 mL/min); detector, dual FID; split ratio, 1:30; injection, 0.5 μL. Identification of the components was performed, for both columns, by comparison of their retention times with those of pure authentic samples and by mean of their Kovats indices relative to the series of *n*-hydrocarbons. The relative proportions of the essential oil constituents were percentages obtained by FID peak-area normalization, all relative response factors being taken as 1.

GC-EIMS analyses were also performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm i.d.; coating thickness = 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperatures, 220 and 240 °C, respectively; oven temperature, programmed from 60 to 240 °C at 3 °C/min; carrier gas, helium at 1 mL/min; injection, 0.2 μL (10% hexane solution); split ratio, 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their Kovats indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data (*10*−*15*). Moreover, all of the molecular weights of the identified substances were confirmed by GC-CIMS using MeOH as CI reagent gas, operating under the same conditions as described for GC-EIMS analyses.

SPME analyses were performed on Supelco SPME devices coated with poly(dimethylsiloxane) (PDMS, 100 μm) to sample the headspace of three fresh basal leaves (total weight = 25 g, collected at maturity stage from a 2-year-old plant) and 10 mL of aromatic water inserted into a 100 mL glass conical flask and allowed to equilibrate for 20 min. After the equilibration time, the fiber was exposed to the headspace for 15 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC-MS system, operating in the same conditions as above except that the splitless injection mode was used and the injector temperature was 250 °C.

RESULTS AND DISCUSSION

Constituents and percentages relative to total area of essential oil and the *n*-hexane extract of aromatic water were obtained by GC and GC-MS (Table 1). In addition, SPME analysis of fresh plant and aromatic water are reported in Table 1. GC and GC-MS analyses showed that the essential oil and the *n*-hexane extract of aromatic water and the plant were rich in monoterpenes and that carvone was the main compound, representing 43.5% of the constituents of the essential oil and 74.9% of the constituents of the *n*-hexane extract of aromatic water. Furthermore, carvone represented 46.3% in the SPME analysis of fresh plant and 41.3% in the SPME analysis of aromatic water (Table 1).

The other major compounds present in the essential oil were α-thujone (15.6%), β-bisabolene (4.5%), germacrene D (4.2%), 1,8-cineole (3.4%), β-thujone (2.1%), *cis*-dihydrocarvone (1.8%), T-muurolol (1.7), *epi*-cubebol (1.3%), *p*-mentha-1(7),5-dien-2-ol (1.2%), selin-11-en-4-α-ol (1.1%), and δ-cadinene (1.1%); minor constituents included *trans*-γ-cadinene, pinocarvone, β-copaen-4-α-ol, 1-*epi*-cubenol, *cis*-carveol, limonene, *p*-cymene, isoamyl-2-methylbutyrate, sabinene, *trans*-carveol, and α-pinene. Eighty compounds were present in the essential oil. To our knowledge, 32 of them have not previously been reported in costmary leaves.

In addition to carvone the *n*-hexane extract of aromatic water showed the presence of α-thujone (6.2%), 1,8-cineole (3.5%), *cis*-carveol (2.2%), and other minor constituents, including β-thujone, chrysanthenone, *cis*-verbenol, pinocarvone, *trans*-pinocarveol, borneol, 4-terpineol, myrtenal, *cis*-dihydrocarvone, *trans*-carveol, and *trans*-carvone oxide (Table 1). To our knowledge this is the first investigation of the *n*-hexane extract of aromatic water. It is interesting to note that the *n*-hexane extract of aromatic water content was quite different from that of essential oil. Five of the 27 identified compounds were not found in the essential oil, with chrysanthenone being the main one (1.8%).

Furthermore, this study represents the first report of SPME analysis of both costmary fresh plant and aromatic water. The SPME technique is a fast, solventless technique that permits the establishment of an equilibrium between the sample matrix, the headspace above the sample, and a stationary phase coated on a fused silica fiber. The adsorbed analytes are successively thermally desorbed from the fiber in the injector port of a gas chromatograph. This technique permits rapid sampling of the volatiles emitted by a fresh plant. Fresh plant also showed the presence of β-bisabolene (10.1%), α-thujone (6.9%), germacrene D (5.9%), (*E*)-3-hexenol acetate (5.7%), and (*E*)-γ-bisabolene (2.3%), whereas α-thujone (15.9%), 1,8-cineole (14.9%), α-terpinene (6.4%), and *p*-mentha-1(7),5-dien-2-ol (2.3%) were present in the aromatic water. Among the 52 components identified in the fresh plant, some were not present either in the essential oil or in the aromatic water and were possibly undetected due to their low concentration. Among them, the principal constituents were represented by (*E*)-3-hexenol acetate (5.7%) and bicyclogermacrene (1.2%).

These results show that the GC profiles of the essential oil and the *n*-hexane extract of aromatic water are different, even through carvone and α-thujone represent the dominant constituents of both, whereas camphor was not evident. Thus, according to recent literature data (*8*) the sample we have investigated seems to be defined as belonging to the fourth chemotype, namely, the carvone one producing considerable amounts of α-thujone. Also, the composition of the essential oil was quite similar to that reported (*8*).

Another aspect to be considered concerns the safety of the marketed products due to the fact they are also

Table 1. Percentage Composition of Essential Oil (EO), Aromatic Water (AW), and Leaves of *B. suaveolens* Pers. by GC-MS and SPME/GC-MS

compound[a]	ID method[b]	RI[c]	EO[d]	*n*-hexane extract AW[d]	SPME leaves[d]	SPME AW[d]	compound[a]	ID method[b]	RI[c]	EO[d]	*n*-hexane extract AW[d]	SPME leaves[d]	SPME AW[d]
(*E*)-3-hexenol*	MS	852			tr[e]		verbenone	MS	1205			0.12	0.27
isopentyl acetate*	MS	875			tr		*trans*-carveol	MS	1219	0.35	1.25	0.63	0.78
α-thujene	MS	932	0.24				*cis*-carveol	MS	1230	0.98	2.19	0.71	1.39
S-ethyl pentanethioate*	MS	938	tr			0.11	carvone	Co/MS	1245	43.54	74.92	46.23	41.27
α-pinene	MS	940	tr		0.14		carvenone*	MS	1255			0.12	
camphene	Co/MS	955	tr		0.21		*cis*-chrysanthenyl acetate	MS	1263	0.51	0.10	0.22	
thuja-2,4(10)-diene*	MS	958	tr				*cis*-carvone oxide*	MS	1268	tr	0.10		0.10
benzaldehyde	MS	963				0.10	*trans*-carvone oxide	MS	1279	0.28	0.68	tr	0.37
sabinene	Co/MS	978	0.38		1.62		isobornyl acetate*	MS	1286	tr	0.10	tr	tr
β-pinene	Co/MS	982	0.16		0.12		bornyl acetate	Co/MS	1287				0.18
6-methyl-5-hepten-2-one*	MS	987				0.10	1-tridecene*	MS	1295	tr			
myrcene	MS	992	0.25			0.21	carvacrol	Co/MS	1300		0.10		
mesitylene*	MS	995	tr				isodihydrocarveol acetate*	MS	1326			0.20	
ethyl hexanoate*	MS	998	tr				*trans*-carvyl acetate	MS	1338	0.32		0.28	tr
(*E*)-3-hexenol acetate*	MS	1005			5.72		α-cubebene*	MS	1353	tr			
α-phellandrene*	MS	1007				0.20	*cis*-carvyl acetate	MS	1364	0.24		0.17	tr
Δ^3-carene	MS	1012				tr	cyclosativene*	MS	1370	tr			
3-methylbutyl butanoate	MS	1013	tr				α-copaene	MS	1377	0.32		0.39	
3-methylbutyl-2-methylpropanoate	MS	1017	tr				β-bourbonene*	MS	1386	tr			
1,4-cineole*	MS	1018		tr		tr	β-cubebene	MS	1391	tr		0.12	
α-terpinene	MS	1020	tr	tr		6.43	α-gurjunene*	MS	1409	tr			
p-cymene	Co/MS	1028	0.65			0.80	β-caryophyllene	Co/MS	1420	0.22		0.48	
limonene	Co/MS	1032	0.82		0.14	0.12	β-gurjunene*	MS	1432	tr		0.11	
β-phellandrene*	MS	1033	tr				*cis*-muurola-3,5-diene*	MS	1447	tr			
1,8-cineole	Co/MS	1035	3.42	3.51	2.33	14.95	(*E*)-β-farnesene	MS	1455	0.16		0.49	
butyl-2-methylbutanoate	MS	1044	tr				α-humulene*	MS	1456	tr		tr	
(*E*)-ocimene*	MS	1052	tr				α-patchoulene*	MS	1457	tr			
γ-terpinene	Co/MS	1064	0.14		tr		alloaromadendrene*	MS	1461			tr	
cis-sabinene hydrate	MS	1070	tr		0.22		β-cadinene*	MS	1462	tr			
terpinolene	MS	1089	tr				γ-muurolene*	MS	1477	tr			
p-cymenene	MS	1091	tr		tr		germacrene D*	MS	1481	4.24		5.91	
linalool	Co/MS	1101		0.10		0.25	(*Z*,*E*)-α-farnesene*	MS	1491			0.30	
isoamyl-2-methylbutyrate*	MS	1103	0.31		0.25		*epi*-cubebol	MS	1494	1.30			
nonanal*	MS	1104	0.12				bicyclogermacrene*	MS	1495			1.18	
α-thujone	Co/MS	1105	15.61	6.22	6.92	15.93	α-muurolene	MS	1500	0.20			
isopentyl isovalerate*	MS	1106	0.65				β-himachalene*	MS	1500			0.18	
1,3,8-*p*-mentha-1.3,8-triene	MS	1113				0.10	β-bisabolene	MS	1510	4.53	tr	10.14	
β-thujone	Co/MS	1116	2.10	0.55	0.62	1.75	*trans*-γ-cadinene	MS	1513	1.02		1.10	
chrysanthenone*	MS	1125		1.76			δ-cadinene	MS	1524	1.09	tr		
cis-*p*-mentha-2,8-dien-1-ol	MS	1128			2.73	1.25	β-sesquiphellandrene*	MS	1525			0.22	
trans-sabinol*	MS	1140	0.16			0.88	*trans*-calamenene	MS	1533	0.38			
cis-verbenol	MS	1141	0.19	0.74	0.58	0.41	(*E*)-γ-bisabolene*	MS	1535	0.60		2.26	
trans-*p*-mentha-2,8-dien-1-ol	MS	1142			0.60		cadina-1,4-diene	MS	1535	tr			
trans-pinocarveol	MS	1147	0.31	0.46	0.16	1.03	*trans*-nerolidol	MS	1565	tr			
trans-verbenol	MS	1148		tr		0.10	spathulenol	MS	1577	0.19			
sabinaketone*	MS	1158			0.14	0.15	caryophyllene oxide	MS	1582	tr			
pinocarvone	MS	1164	1.00	0.64	0.27	1.85	globulol*	MS	1584	tr			
borneol	Co/MS	1167	0.20	0.35	0.12	0.48	β-copaen-4-α-ol*	MS	1586	0.91			
isopinocamphone*	MS	1175	tr	0.10	tr	0.19	1-*epi*-cubenol	MS	1628	0.98			
4-terpineol	Co/MS	1179	0.16	0.29	tr	0.47	T-muurolol	MS	1642	1.66			
α-thujenal*	MS	1182				0.10	selin-11-en-4-α-ol	MS	1653	1.12	tr		
p-mentha-1(7),5-dien-2-ol*	MS	1183	1.23		0.97	2.28							
myrtenal*	MS	1194	tr	0.23		0.67	total			95.07	95.36	96.39	97.90
cis-dihydrocarvone	MS	1195	1.83	0.97		1.35							
dihydrocarveol*	MS	1195			0.97	1.12							
trans-dihydrocarvone*	MS	1202	tr			0.16							

[a] Compounds are listed in order of their elution from an HP-5 apolar column. Those compounds marked with an asterisk (*) have not been previously reported in *B. suaveolens* Pers. leaves. [b] Co/MS, peak identifications are based on standard comparison with internal standard; MS, peak identifications are based on MS comparison with file spectra. [c] Retention indices on an HP-5 apolar column. [d] Relative percentage of the identified volatile based on the FID chromatographic area. [e] tr, traces (amounts >0.1%).

used orally. The main constituent, carvone, is reported to have a very low toxicity, with an oral LD_{50} in rats of 1640 mg/kg (*16*). On the other hand, thujones, both α and β forms, which can cause some adverse effects after oral ingestion, are present at 18% in the essential oil and 7% in the aromatic water (*16*). Taking into account the total amount of essential oil in the tablets (<1.8 mg pro die, corresponding to 0.3 mg of thujone) and the formulation of the liquid preparations (∼1.5 mg of essential oil and ∼1.5 g of aromatic water pro die, corresponding to 110 mg of total thujone), the contents of both α and β forms of thujone are lower than toxic dosages reported in the literature (*16*).

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