Seasonal Variation in the Essential Oil Composition of *Origanum majorana* L. Cultivated in Egypt

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The hydrodistilled essential oil content of *Origanum majorana* L. (Lamiaceae) ranged from 2.5–3% with the maximal value (3%) in spring. Analysis of the oil by GC/MS resulted in the identification of 15, 15, 11, and 14 components in the oils prepared in spring, summer, autumn, and winter, respectively. The composition of the essential oils differed quantitatively and qualitatively according to the time of collection. Thymol (38.4%) and *cis*-sabinene hydrate (25.3%) were the major components in spring plants. Terpinen-4-ol (37.4%, 20.5%, 16.3%) was a major component in the summer, autumn and winter oils, respectively. *cis*-Sabinene hydrate (54.4%) was major in winter plants while terpinolene (43.1%) was the main component in autumn plants. Other components detected in lower amounts in all oil samples were sabinene and *p*-cymene (up to 7.4% and 13.9% in autumn), and α-terpinene (up to 13.3% in summer).

Key words: Origanum majorana, Essential Oil, Seasonal Variation

Introduction

Origanum majorana L. (= *Majorana hortensis* Moench.), sweet marjoram, is a perennial, evergreen subshrub which grows in South Europe, North Africa and Turkey (Bown, 2002). *O. majorana* has been used to treat colds and rhinitis (Bown, 2002). The herb and essential oil is used against cramps, depression, gastrointestinal problems, headaches, and as a diuretic. The essential oil is used externally for chest congestion, muscle aches and arthritis. Warm olive oil infused with sweet marjoram is a reported remedy for ear infections. It can be prepared as an infusion, mouth wash, poultice; the oil is an ingredient in ointments and compound preparations. The oil of *O. majorana* is used commercially to scent soaps, lotions and perfumes (Marderosian and Beutler, 2002; Gruenwald *et al*., 2004).

The composition of the essential oils from various *Origanum* species has been investigated. Components of the essential oil of *Origanum* species show great variation according to the plant habit. Two chemotypes of marjoram are reported (Guenther, 1952), a terpinen-4-ol/sabinene hydrate-rich type and a thymol/carvacrol-rich type (Fischer *et al*., 1987; Sarer *et al*., 1982).

The present study was planned with the aim to evaluate the influence of the harvest time on the essential oil composition of the aerial parts of *O. majorana* cultivated in Egypt. In this respect, samples of dried aerial parts, collected along the four seasons, were analyzed using gas chromatography/mass spectrometry (GC/MS). This was performed as a trial to establish the best conditions for oil production.

Material and Methods

Plant material

Samples of aerial parts of *O. majorana* were obtained, at the different seasons, during 2006/2007 from plants cultivated in the Experimental Station of Medicinal Plants, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Giza, Egypt. Identification of samples was carried out as previously mentioned (Soliman *et al*., 2007). Shade-dried samples were coarsely powdered and packed in amber, tightly closed containers until use.

Preparation, characterization and analysis of the essential oils

Samples (500 g, each), collected in January, April, July and October, were separately subjected to hydrodistillation. The percentage yield was calculated on dry weight basis. The essential

oil in each case was dried over anhydrous sodium sulfate and kept refrigerated for further analysis. The specific gravity and refractive index of each of the tested samples were determined according to The Egyptian Pharmacopoeia (1984).

GC/MS analyses of the different essential oil samples were performed using a Thermo Trace GC 2000 (Thermo Quest, TX, USA)/MS Finnigan mat SSQ 7000 system. The instrument was equipped with a DB-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, USA); carrier gas: helium at a flow rate of 1 ml/min; injection temperature: 220 °C; oven temperature programme: initial temperature 40 °C, isothermal for 3 min, then heating to 160 °C at 4 °C/min, followed by 10 °C/min to 280 °C; ionization mode: EI; ion source: 70 eV; mass range: 40–500 amu.

Identification of the essential oil constituents was achieved by library search on a Wiley 275 L GC-MS data base and by comparing the retention indices and mass fragmentation patterns with those of the available references as well as of published data (Davis, 1990; Adams, 2004). The quantitative estimation was carried out by peak area measurement. A series of authentic *n*-alkanes was subjected to GLC under the same experimental conditions, and the retention indices of the oil constituents were calculated. The individual components were determined by computerized peak area measurement. Compounds of the essential oil, their retention indices and relative percentage composition are compiled (Table I).

Results and Discussion

A marked variation was observed in the percentage yield of the hydrodistilled essential oil prepared from the aerial parts of *O. majorana* cultivated in Egypt at different harvest times. Samples, harvested at the four seasons (winter, spring, summer, and autumn) gave 2.8%, 3%, 2.5%, 2.5%, respectively, of a colourless to pale yellow essential oil with characteristic odour. The yield of *O. majorana* essential oil in a previous work ranged from 0.5–0.9% (Heinz, 1981). Recent reports showed that this yield varied from

Table I. Results of GC/MS analysis of the essential oil of the aerial parts of *O. majorana* at the four seasons.

Peak no.	Compound	Rt[a]	KI[b]	[M$^+$]	Base peak	Percentage			
						Spring (Flowering)	Summer (Fruiting)	Autumn (Fruiting)	Winter (Pre-flowering)
1	Sabinene	18.46	975	136	93	4.5	6.1	7.4	4.2
2	β-Pinene	19.1	979	136	93	1.0	0.9	0.4	0.3
3	α-Phellandrene	19.89	1003	136	93	0.1	-	-	-
4	α-Terpinene	20.36	1017	136	121	4.4	13.3	0.4	2.8
5	*p*-Cymene	20.94	1025	134	119	2.3	4.2	13.9	3.3
6	γ-Terpinene	22.18	1060	136	93	8.3	18.3	-	5.3
7	*trans*-Sabinene hydrate	22.87	1070	154	43	–	0.2	5.1	5.6
8	Terpinolene	23.17	1089	136	93	2.9	2.6	43.1	0.3
9	*cis*-Sabinene hydrate	24.12	1098	154	43	25.3	7.4	–	54.4
10	*p*-Menth-2-en-1-ol	25.14	1122	154	43	0.3	0.9	0.5	0.5
11	*trans*-2-*p*-Menthen-1-ol	25.89	1141	154	43	0.1	0.5	–	–
12	Terpinen-4-ol	27.38	1177	154	71	7.7	37.4	20.5	16.3
13	α-Terpineol	28.21	1189	154	59	1.3	2.7	6.4	4.1
14	Thymol methyl ether	29.2	1245	164	149	0.1	–	–	–
15	Linalyl acetate	29.09	1257	190	43	–	–	–	0.9
16	Thymol	33.12	1290	150	135	38.4	–	–	–
17	δ-Elemene	32.07	1338	204	121	–	0.5	–	–
18	β-Caryophyllene	35.13	1419	204	41	1.5	2.6	0.7	0.9
19	Bicyclogermacrene	36.98	1500	204	121	–	1.7	–	0.6
20	Spathulenol	38.84	1578	220	43	–	–	0.3	–

[a] Rt, retention time in min.
[b] KI, Kovat's index according to Adams (2004).

Table II. Classes of compounds identified in the essential oil of *O. majorana* at the four seasons.

Class	Percentage			
	Spring (Flowering)	Summer (Fruiting)	Autumn (Fruiting)	Winter (Pre-flowering)
Hydrocarbons	[25.0]	[50.0]	[66.0]	[17.6]
Monoterpenoids	23.5	45.6	65.3	16.1
Sesquiterpenoids	1.5	4.7	0.7	1.4
Oxygenated compounds	[73.2]	[49.1]	[32.7]	[81.7]
Monoterpenoids	73.2	49.1	32.5	81.7
Sesquiterpenoids	–		0.3	–
Total identified	98.2	99.1	98.7	99.3

1–3% (Gruenwald *et al*., 2004). In the present study, the yield showed the highest percentage during the flowering period in spring (3%); the percentage yield was considerably less before and after the flowering period (2.8% in winter and 2.5% in summer and autumn).

On the other hand, the influence of seasonal variation on the refractive index and specific gravity of the different oils was not obvious. As a matter of fact, the refractive indices recorded at 25 ºC were very close ranging from 1.4784–1.4788 with an average of 1.4786. The specific gravity of all the oils, determined at 25 ºC, was 0.85.

Analysis of the oil by GC/MS resulted in the identification of 15, 15, 11, and 14 components in the oils prepared in spring, summer, autumn, and winter, respectively. The composition of the essential oils differed quantitatively and qualitatively according to the time of collection. Thymol (38.4%) and *cis*-sabinene hydrate (25.3%) were the major components in spring plants. Terpinen-4-ol (37.4%, 20.5%, 16.3%) was a major component in the summer, autumn and winter oils, respectively. *cis*-Sabinene hydrate (54.4%) was a major in winter plants while terpinolene (43.1%) was the main component in autumn plants. Other components detected in lower amounts in all oil samples were sabinene and *p*-cymene (up to 7.4% and 13.9% in autumn), and α-terpinene (up to 13.3% in summer).

The present study revealed that Egyptian marjoram oil displayed characteristics of the first chemotype during summer, autumn and winter, with total monoterpene alcohols amounting to 49.1%, 32.5% and 81.7% respectively (Table II). However, the oil prepared in spring was rich in thymol (38.4%) comparable at the same time to total monoterpene alcohols (37.7%). Hence, the oil prepared during spring could be used as a substitute for any of the thymol-containing essential oils. However, the oil production during winter offers a product with the characteristic flavour and fragrance of marjoram oil since it was reported that terpinen-4-ol (>20%) together with *cis*-sabinene hydrate (3–18%) are responsible for the characteristic flavour and fragrance of marjoram oil (Vági *et al.*, 2005). The above data can be of importance in the chemotaxonomic classification of the Egyptian *O. majorana*. At the same time, the Egyptian plant gave an excellent yield that can be attributed to environmental factors.

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