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COMPARATIVE CHEMICAL ANALYSIS OF *ORIGANUM MAJORANA* ESSENTIAL OILS FROM SERBIA AND EGYPT

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Abstract. *The chemical composition of commercial marjoram (*Origanum majorana*) essential oils sourced from Serbia and Egypt was investigated using GC and GC-MS. The major volatile compounds in both oils were terpinen-4-ol and γ-terpinene, constituting over a third of the oils based on GC peak areas. However, there were significant differences between the two oils in terms of their* cis- *and* trans-*sabinene hydrate content, which are commercially important monoterpenes. The Egyptian oil contained 12.1%* trans-*sabinene hydrate and 3.5%* cis-*sabinene hydrate, while the Serbian oil contained a total of 7.3%* cis- *and* trans-*sabinene hydrates in a 1:1.7 ratio.*

Key words: *Origanum majorana, essential oil, terpinen-4-ol, trans-sabinene hydrate, cis-sabinene hydrate*

1. INTRODUCTION

Marjoram (*Origanum majorana* L., Lamiaceae) is a perennial aromatic herb native to the Mediterranean region and is cultivated in several countries in southern Europe, North America, and Asia (Wittmann et al., 2020). Although it is occasionally confused with another *Origanum* species (*Origanum vulgare*, commonly known as oregano or wild marjoram, in contrast to sweet marjoram, *O. majorana*), it significantly differs in its chemical and aromatic profile. The monoterpene-dominated essential oil of marjoram has a mild and sweet aroma, often described as warm and calming, which stands in stark contrast to the phenol-rich oil of oregano, known for its stronger and more pungent smell (Cinbilgel and Kurt, 2019).

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Both *Origanum* species have significant economic importance. Although oregano oil has a higher global demand[1] and commands nearly double the price of marjoram oil[2], there is also a steady demand and a growing market for marjoram oil. While the demand is mostly driven by its use in aromatherapy, cosmetics, and natural personal care products (such as soaps, lotions, and massage oils), its (ethno)medicinal use should not be underestimated. Marjoram oil has been reported to have antioxidant, antimicrobial, anti-inflammatory, anti-acetylcholinesterase, anticancer, antidepressant, and analgesic activities (Bina and Rahimi, 2017, Kakouri et al., 2022). In recent years, it has also been evaluated as a new natural and eco-friendly pesticide, with its insecticidal, larvicidal, repellent, and fumigant activities thoroughly investigated (Kakouri et al., 2022). The large margin of safety (demonstrated for this oil in a mouse study, Bina and Rahimi, 2017) has likely contributed significantly to its popularity.

The most common *O. majorana* chemotype is characterized by a high abundance of terpinen-4-ol and *cis*-sabinene hydrate. These two compounds, along with *cis*-sabinene hydrate acetate, are recognized as the primary contributors to its sweet flavor (Kakouri et al., 2022, and references therein). However, the exact percentage of these monoterpenes can vary significantly (for a comprehensive review of the major constituents in *O. majorana* essential oil of different geographical origin, see Kakouri et al., 2022). Some patterns were, however, noted by Circella et al., who reported that increased temperature and longer periods of sunlight favored the formation of *cis*- and *trans*-sabinene hydrates over terpinen-4-ol (Circella et al., 1995).

Motivated by the growing popularity of marjoram essential oil in Serbia, we decided to perform a detailed chemical analysis of two commercial oil samples, originating from Serbia and Egypt, available on the domestic market. The analysis was performed by GC and GC-MS and was also facilitated by chromatographic separation on silica gel.

2. MATERIALS AND METHOD

2.1. Chemicals

Essential oil samples were acquired commercially. All solvents (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Authentic chemical samples were obtained from Merck (Darmstadt, Germany) and Carl Roth (Karlsruhe, Germany) in the highest available purity. For the determination of retention indices, a commercial *n*-alkane mixture (Sigma-Aldrich, St. Louis, MO, USA) ranging from heptane to eicosane (C_7–C_{40}) was used.

2.2. GC and GC-MS

The GC-MS analyses were performed on a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column DB-5MS (5% diphenylsiloxane, 95% dimethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm; Agilent Technologies, Palo Alto, CA, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface temperatures were set to 250 °C and 320 °C, respectively. The oven temperature was programmed to increase from 70 to 290 °C at a rate of 5 °C/min, followed by

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[1] US$ 8.22 billion global market compared to US$ 533 million for marjoram in 2023 (source: marketresearch.biz).

[2] Pricing data was taken from amazon.com.

an isothermal hold for 10 minutes. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. A sample (10 µL of the oil in 1 mL Et_2O) was injected in pulsed-split mode (with a flow rate of 1.5 mL/min for the first 0.5 min, then 1.0 mL/min for the remainder of the analysis; split ratio 40:1). MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range of 35–500 amu, with a scan time of 0.34 s. The GC (FID) analyses were performed under the same experimental conditions using the same column as described for GC-MS. The percentage composition was calculated from the GC peak areas without the use of correction factors. Linear retention indices were calculated for all identified components using standards of *n*-alkanes (C_9–C_{17}). AMDIS software was used for chromatogram deconvolution and mass spectral libraries (Wiley 7, NIST 14, MassFinder 2.3, and Adams library) were searched with NIST MS Search software.

2.3. Dry-flash chromatography

Egyptian *O. majorana* essential oil (10 ml, Sample B, Fig. 1) was subjected to dry-flash chromatography on silica gel (gradient elution, from pure *n*-hexane to pure diethyl ether) and 15 fractions (I – XV, Table 1) were obtained (eluent volume of each fraction was 100 ml): fraction I (*n*-hexane, 4.27 g), fraction II (*n*-hexane, 205 mg), fraction III (*n*-hexane, 14 mg), fraction IV (2%, *v*/*v*, Et_2O in *n*-hexane, 2 mg), fraction V (4% Et_2O, 2 mg), fraction VI (6% Et_2O, 630 mg), fraction VII (10% Et_2O, 1.02 g), fraction VIII (15% Et_2O, 450 mg), fraction IX (20% Et_2O, 500 mg), fraction X (30% Et_2O, 370 mg), fraction XI (40% Et_2O, 500 mg), fraction XII (50% Et_2O, 130 mg), fraction XIII (50% Et_2O, 50 mg), fraction XIV (66% Et_2O, 3 mg), fraction XV (100% Et_2O, 22 mg).

Gravity chromatography was used to purify *cis*-sabinene hydrate from fraction IX. The conditions were as follows: a 500 mg sample was applied to 25 g of silica gel, and gradient elution was performed with 0% to 20% (*v*/*v*) diethyl ether in *n*-hexane. Pure *cis*-sabinene hydrate (40 mg) was obtained in fraction 45. Fractions XI–XIII contained pure *trans*-sabinene hydrate, as determined by TLC and GC-MS and were not further purified.

3. RESULTS AND DISCUSSION

The list of identified constituents of two *Origanum majorana* essential oils (originating from Serbia, sample A, and Egypt, sample B) is presented in Table 1. Their characterization by GC and GC-MS allowed the identification of 61 compounds in total: 45 compounds representing 98.3% of the oil composition in sample A, and 52 compounds accounting for 99.0% of the oil composition in sample B. The total-ion chromatograms (TICs) of the analyzed oils (samples A and B) are shown in Fig. 1. The monoterpenoid fraction was the most abundant, comprising ca. 95% of the oils. The monoterpenoids were unevenly distributed between hydrocarbons (39.9% and 53.4% for samples A and B, respectively) and oxygenated derivatives (55.4% and 41.9%, respectively). The essential oil composition of Serbian marjoram showed terpinen-4-ol (31.7%), γ-terpinene (13.1%), *p*-cymene (8.2%), α-terpinene (6.5%), and *trans*-sabinene hydrate (4.6%) as the main constituents. Terpinen-4-ol was also the major component of essential oil of Egyptian marjoram (17.0%), which contained a significant amount of γ-terpinene (14.5%). However, the contents of other dominant oil constituents of sample B are slightly different from those of sample A: *trans*-sabinene hydrate (12.1 vs. 4.6%), α-terpinene (11.4 vs. 6.5%), sabinene (10.1 vs. 3.0%), and *p*-cymene (2.4 vs. 8.2%).

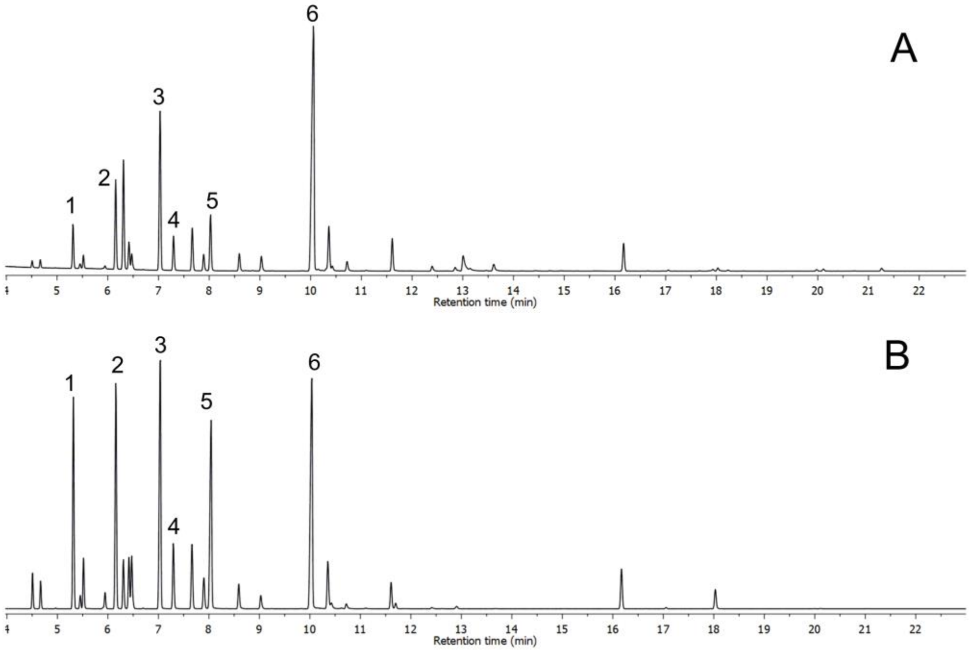

Fig. 1 Total ion current chromatograms of the essential oils from Serbia (sample A) and Egypt (sample B). 1 – sabinene; 2 – α-terpinene; 3 – γ-terpinene; 4 – *cis*-sabinene hydrate; 5 – *trans*-sabinene hydrate; 6 – terpinen-4-ol

To facilitate identification, the Egyptian oil was fractionated using dry-flash chromatography on silica gel. All hydrocarbon components of the oil eluted with pure *n*-hexane (fractions I–III, Table 1), while the oxygenated fraction eluted starting from fraction VI (6% diethyl ether in *n*-hexane, *v*/*v*). This pre-separation step significantly simplified the identification process, allowing for the detection of 11 constituents (marked with # in Table 1) that were not detectable in the chromatogram prior to chromatography.

Dry-flash chromatography of the Egyptian *O. majorana* oil proved to be an excellent and rapid method for isolating sabinene hydrates. Fractions XI–XIII consisted of high-purity (>95%) *trans*-sabinene hydrate, while additional column chromatography was required to purify *cis*-sabinene hydrate from fraction IX.

While the total amount of sabinene hydrates and terpinen-4-ol was slightly higher in the Serbian *O. majorana* sample (39% vs. 32% in the Egyptian sample), the most significant difference between the two essential oils was their sabinene hydrate content. The Egyptian oil (sample B, Table 1) contained more than double the amount of these two isomers (15.6%) compared to the Serbian sample (7.3%, sample A). The ratio of the two isomers also differed between the samples: in both oils, the *trans* isomer was dominant, with *trans/cis* ratios of 1.7 and 3.5 in samples A and B, respectively (Table 1). The results that were obtained for the Serbian oil agreed with results of Milenković et al. (2021) who also investigated *O. majorana* essential oil from southern Serbia and reported high content of terpinen-4-ol (34.4 – 36.8%) and low amounts of sabinene hydrates (ca. 3% for the *cis*-isomer while *trans*-sabinene hydrate was not even detected).

Table 1 Chemical composition of the *O. majorana* essential oil from Serbia (A) and Egypt (B)

Compound	RI[a]	A %[b]	B %	Fractions	Compound class[e]	Identification method[f]
α-Thujene	927	0.4	1.4	I-II	MT	MS, RI
α-Pinene	937	0.5	1.2	I-II	MT	MS, RI, CoI
Camphene	955	tr[c]	tr	/[d]	MT	MS, RI, CoI
Sabinene	976	3.0	10.1	I-II	MT	MS, RI
3-Octanone[#]	984	-	tr	VI	O	MS, RI, CoI
β-Pinene	985	0.3	0.5	I-II	MT	MS, RI, CoI
Myrcene	989	0.9	2.3	I-II	MT	MS, RI
Dehydro-1,8-cineole	994	-	tr	VI	MT	MS, RI
(*E*)-3-Hexenyl acetate[#]	1007	-	tr	VI	O	MS, RI
α-Phellandrene	1011	0.3	0.8	I-II	MT	MS, RI
α-Terpinene	1021	6.5	11.4	I-II	MT	MS, RI, CoI
p-Cymene	1027	8.2	2.4	I-II	MT	MS, RI, CoI
Limonene	1032	2.0	2.4	I-II	MT	MS, RI, CoI
β-Phellandrene	1034	1.3	2.9	I-II	MT	MS, RI
1,8-Cineole	1035	tr	tr	VI-VII	MT[*]	MS, RI, CoI
(*E*)-β-Ocimene	1044	tr	tr	/	MT	MS, RI
γ-Terpinene	1059	13.1	14.5	I-II	MT	MS, RI, CoI
cis-Sabinene hydrate	1071	2.7	3.5	VII-XI	MT[*]	MS, RI
Terpinolene	1087	3.4	3.5	I-II	MT	MS, RI, CoI
p-Cymenene[#]	1096	-	tr	II	MT	MS, RI
Linalool	1097	1.3	1.5	VI-IX	MT[*]	MS, RI, CoI
trans-Sabinene hydrate	1103	4.6	12.1	IX-XIII	MT[*]	MS, RI
cis-*p*-Menth-2-en-1-ol	1125	1.4	1.3	VII-XI	MT[*]	MS, RI
α-Campholenal[#]	1129	-	tr	VI	MT[*]	MS, RI
1-Terpineol	1136	tr	tr	/	MT[*]	MS, RI
trans-Pinocarveol[#]	1142	-	tr	IX	MT[*]	MS, RI, CoI
trans-*p*-Menth-2-en-1-ol	1143	1.3	0.8	VII-XI	MT[*]	MS, RI
Isomenthone[#]	1167	-	tr	VI	MT[*]	MS, RI
Borneol	1175	tr	tr	IX-X	MT[*]	MS, RI, CoI
Terpinen-4-ol	1185	31.7	17.0	VI-IX	MT[*]	MS, RI, CoI
Cryptone	1192	tr	tr	I	MT[*]	MS, RI
α-Terpineol	1197	4.2	2.9	IX-XI	MT[*]	MS, RI, CoI
cis-Piperitol	1200	0.5	0.4	VI-IX	MT[*]	MS, RI
cis-Dihydro carvone[#]	1201	-	tr	VI	MT[*]	MS, RI, CoI
trans-Dihydro carvone	1207	-	tr	VI-VIII	MT[*]	MS, RI, CoI
trans-Piperitol	1212	0.9	0.4	VII-X	MT[*]	MS, RI
trans-Chrysanthenyl acetate	1225	tr	tr	/	MT[*]	MS, RI
Linalool acetate	1245	2.8	1.4	VI	MT[*]	MS, RI, CoI
trans-Sabinene hydrate acetate	1249	-	0.4	VI	MT[*]	MS, RI
Citronellyl formate	1272	-	tr	/	MT[*]	MS, RI
cis-Verbenyl acetate	1276	0.5	tr	XIV-XV	MT[*]	MS, RI

Isobornyl acetate	1284	-	tr	VI	MT*	MS, RI
trans-Verbenyl acetate	1295	0.4	-	-	MT*	MS, RI
Terpinen-4-ol acetate	1296	0.9	0.2	VI	MT*	MS, RI
Thymol	1299	2.2	-	-	MT*	MS, RI, CoI
Tridecane	1300	tr	tr	/	O	MS, RI, CoI
Neryl acetate	1357	tr	-	-	MT*	MS, RI, CoI
Geranyl acetate	1376	tr	-	-	MT*	MS, RI, CoI
β-Bourbonene	1388	tr	-	-	ST	MS, RI
(*E*)-Caryophyllene	1427	2.5	2.5	I-II	ST	MS, RI, CoI
6,9-Guaiadiene	1445	tr	tr		ST	MS, RI
α-Humulene	1460	tr	tr	II	ST	MS, RI, CoI
cis-Muurola-4(14),5-diene	1485	tr	-	-	ST	MS, RI
Viridiflorene	1496	0.2	-	-	ST	MS, RI
Bicyclogermacrene	1500	0.3	1.2	II-III	ST	MS, RI
β-Bisabolene	1510	tr	-	-	ST	MS, RI
δ-Cadinene	1523	tr	-	-	ST	MS, RI
Spathulenol#	1585	-	tr	II-III	ST*	MS, RI
Caryophyllene oxide#	1588	-	tr	I-II	ST*	MS, RI, CoI
Humulene epoxide II#	1618	-	tr	III	ST*	MS, RI
Isospathulenol#	1642	-	tr	II-III	ST*	MS, RI
Total identified [%]		98.3	99.0			
MT-Monoterpenes		39.9	53.4			
MT*-Oxygenated monoterpenes		55.4	41.9			
ST- Sesquiterpenes		3.0	3.7			
ST*- Oxygenated sesquiterpenes		tr	tr			
O- Other		tr	tr			

[a] Linear retention index (RI) determined experimentally on the DB-5MS column relative to a series of C_9 – C_{17} *n*-alkanes. [b] Values are the means of three individual analyses.
[c] tr, trace amounts (< 0.05%). [d] Compound was not detected in any of the chromatographic fractions.
[e] The abbreviations of the compound classes are given at the end of the table.
[f] Compound identification: RI, retention indices matching with literature data; MS, mass spectra matching; CoI, coinjection with a pure reference compound. # Compound was detected in a chromatographic fraction chromatogram, but not in the chromatogram of the oil.

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REFERENCES

Bina, F., Rahimi, R., 2017. J. Evid. Based. Complementary Altern. Med., 22, 175–185. doi: 10.1177/2156587216650793

Cinbilgel, I., Kurt, Y., 2019. Journal of Herbal Medicine, 16, 100257. doi:10.1016/j.hermed.2019.100257

Circella, G., Franz, C., Novak, J., Resch, H., 1995. Flavour Fragr. J., 10, 371–374. doi: 10.1002/ffj.2730100607

Kakouri, E., Daferera, D., Kanakis, C., Revelou, P.-K., Kaparakou, E.H., Dervisoglou, S., Perdikis, D., Tarantilis, P.A., 2022. Life, 12, 1982. doi: 10.3390/life12121982

Milenković, L., Ilić, Z.S., Šunić, L., Tmušić, N., Stanojević, L., Stanojević, J., Cvetković, D., 2021. Saudi J. Biol. Sci., 28, 6532–6543. doi: 10.1016/j.sjbs.2021.07.018

Wittmann, S., Jüttner, I., Mempel, H., 2020. Agronomy, 10, 1769. doi: 10.3390/agronomy10111769

KOMPARATIVNA HEMIJSKA ANALIZA ETARSKIH ULJA BILJNE VRSTE *ORIGANUM MAJORANA* IZ SRBIJE I EGIPTA

*Hemijski sastav komercijalnih etarskih ulja majorana (*Origanum majorana*) iz Srbije i Egipta je određen pomoću GC i GC-MS-a. Glavni sastojci oba etarska ulja, terpinen-4-ol i γ-terpinen, činili su preko trećine ulja (određeno na osnovu površine ispod pikova u GC-hromatogramu). Međutim, uočene su i značajne razlike u sadržaju* cis- *i* trans-*sabinen-hidrata, dva komercijano važna monoterpena. Ulje iz Egipta sadržalo je 12,1%* trans-*sabinen-hidrata i 3,5%* cis-*sabinen-hidrata, dok je ulje iz Srbija sadržalo ukupno 7,3%* cis- *i* trans-*sabinen-hidrata u odnosu 1:1,7.*

Ključne reči: *Origanum majorana, etarsko ulje, terpinen-4-ol, trans-sabinen-hidrat, cis-sabinen- hidrat*