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ANALYSIS OF THE ESSENTIAL OIL COMPOUNDS OF *ORIGANUM SYRIACUM* L.

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Syrian oregano (*Origanum syriacum* L., Lamiaceae) is a very popular culinary herb that has been used through the ages in traditional medicine mainly in Lebanon and the Arab world. The subject of this study was the microscopic analysis of the herbal drug Origani syriaci folium, as well as the chemical analysis of its essential oil components. Plant materials originated from Arabsalim – South Lebanon and were collected in 2010. The presence of very dense clusters of multicellular covering trichomes (510 μm × 18.47 μm) and peltate secretory glands (∅ 87.95 μm) with eight cells were observed. The essential oil of Origani syriaci folium was isolated and quantified using hydro-distillation according to SPhC 1997. The content of essential oil was 95 ml/1000 g, i.e. 9.5% (V/m). The volatile constituents of Origani syriaci folium were qualitatively and quantitatively evaluated using SPME GC/MS. The two dominant constituents identified in Syrian oregano leaves were carvacrol (78.4%) and thymol (17.9%).

Keywords: *Origanum syriacum L. – Syrian oregano leaves – microscopy – SPME GC/MS – carvacrol – thymol*

INTRODUCTION

Origanum syriacum L. (syn. *Amaracus syriacus* L., *Majorana crassa*, *Origanum maru* L.) also known as Syrian oregano and white oregano, in Arabic za'atar (زعتر) (Seidermann, 2005), is a very popular culinary herb that has been used through the ages in traditional medicine mainly in Lebanon and the Arab world. The Persian philosopher and doctor Avicenna (ibn Sīnā; 980 – 1037 AD) describes in his world famous "The Canon of Medicine" the effects of *Origanum syriacum*: "it is a good analgesic for joint pain, chewing the leaves relieves both gum and toothache. When rubbed upon chest it relieves bronchitis, it has beneficial effect on the liver and stomach and

a strong anthelmintic effect" (Abū ʿAlī al-Ḥusayn ibn ʿAbd Allāh ibn Sīnā, 1593). David Ben Omar from Antioch (Dāwūd ibn 'Umar al-Anṭākī; 1534 – 1592 AD), a pharmacist, doctor, and astronomist, wrote in his book "*Tadkirat ūlī al-albāb wa-al-jāmi' li-al-'ajab al-'ujāb"* that *Origanum syriacum* was used as "an antidote for many poisons, a carminative, for detoxification of the organism, as a blood thinner, for loss of appetite, as an anthelmintic and food preservative" (Dāwūd ibn 'Umar al-Anṭākī, 1986).
Origanum syriacum L. is a perennial herb, 60 cm – 90 cm high, with creeping woody roots, branched woody, hairy stems. Leaves are opposite, shortly or subsessile (petiolate to 8 mm), ovate, 5 mm – 35 mm × 4 mm – 23 mm and hairy, margins are entire or remotely serrate, the apex is obtuse. The upper leaf surface is darker; the lower leaf surface is brighter with secretory glands. Flowers are shortly petiolate, hairy. Bracts obovate or elliptic, 2 mm – 5 mm × 1.5 mm – 3.5 mm, acute or obtuse, entire or denticulate. A two-lipped pale purple corolla 4.5 mm – 7.5 mm and a five-toothed tubular campanulas calyx (Alma et al., 2003, Kintzios, 2004). *O. syriacum* grows mainly in the East Mediterranean Region: South Anatolia, Cyprus, Syria, Lebanon, Jordan, Egypt, Sinai Peninsula (Seidermann, 2005).
In recent years, *O. syriacum* has drawn attention for its antioxidant activity and acetylcholinesterase inhibition (Alzheimer's disease) (Loizzo et al., 2009, Zein, 2011); antifungal activity (*Alternaria solani, Aspergillus niger, Botrytis cinerea, Cladosporium* sp., *Fusarium oxysporum, Penicillium* sp., *Verticillium dahlia, Saccharomyces cerevisiae*) (Kintzios, 2004, Daouk, 1995, Abou-Jawdah, 2002, 2004); antibacterial activity (*Bacillus brevis, Bacillus megaterium, Bacillus subtilis, Mycobacterium smegmatis, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Klebsiella oxytoca, Enterococcus faecalis, Escherichia coli, Micrococcus luteus, Yersinia enterocolitica*) (Kintzios, 2004); analgesic activity; antiflogistic activity (atherosclerosis, Alzheimer's disease), antirheumatic, expectorant, sedative, antiparasitic and antihelminthic activities (Zein, 2011).

MATERIAL AND METHODS

Plant material and sample preparation

In this study, the leaves of Syrian oregano (*Origanum syriacum* L., Lamiaceae) were collected in Arabsalim – South Lebanon in 2010, they were air-dried and then manually diminished. For microscopic analysis, a sample of these leaves was adjusted by chloral hydrate. Essential oil isolation from Syrian oregano leaves was done as follows: 30.0 g of dried cut Syrian oregano leaves were distilled with water for 4 hours using Clevenger apparatus. The distilled essential oil was isolated, dissolved in hexane, dried over anhydrous sodium sulphate, and stored in vials at 4 °C – 6 °C for further use (CPhS 1, 1997). For GS/MS a sample of 0.5 g of air-dried and cut Oregano leaves was used.

Chemicals

Chloral hydrate p.a. (MEDIKA, Slovak Republic), anhydrous sodium sulphate and hexane p.a. (REACHEM, Slovak Republic), distilled water.

Equipment

For microscopic analysis: Optical microscope: LEICA (D) DME, trinocular, planachromatic objective lens, zoom objective 20×, tube ½; digital camera: LEICA EC 3 Mpix; software: LEICA application suite 2.4.0 R1, LAS EZ ver. 1.3.0. For essential oil isolation a standard Clevenger apparatus was used. The composition of essential oil was analysed on an AGILENT 6890/5973N GC/MS (Santa Clara, CA, USA), CTC Combi Dispenser PAL (CTC Analytics AG, Zwingen, CH). The operating conditions were as follows: I. GC parameters: a) analytical column: SLB-5ms (Sigma-Aldrich, Saint Louis, MO, USA); dimensions: 30 m × 250 µm, film thickness: 0.25 µm, stationary phase: 5 % diphenyl – 95 % dimethylpolysiloxane; b) analytical temperature: 60 °C (1 min isothermal), temperature program: 8 °C/min to 260 °C (after 4 min isothermal); c) SPME method – solid phase microextraction; d) injector temperature: 240 °C; e) sample: 0.5 g of drug (Origani syriaci folium); f) the carrier gas was helium with a constant flow rate of 1.0 ml/min (average linear flow rate of 37 cm/s). II. MS parameters: a') positive ion electron ionisation (EI^+) (electron energy 70 eV) quadrupole analyzer; b') scanning module: mass range of 40 amu to 500 amu, 3.15 scans/s; c') software: MSD ChemStation D.02.00.275 (Agilent) (Héthelyi, 2011, 2012, Galambosi, 2010).
The essential oil components were identified using the library of spectra of the AGILENT instrument (MSD ChemStation D.02.00.275) and Kovats indices and partly using authentic samples.

RESULTS AND DISCUSSION

Essential oil and hydroxycinnamic acid derivatives are typical active compounds of the Lamiaceae family plants. In 1945 the Swedish botanist Erdtman subdivided this taxonomic family into two major subfamilies: the Lamioideae and the Nepetoideae. The Lamioideae are characterized by tricolpate, binucleate pollen, albuminous seeds, spatulate embryos, the presence of iridoid glycosides, lower content of essential oils and rosmarinic acid but higher content of phenylpropanoid glycosides, whereas the Nepetoideae have hexacolpate, trinucleate pollen, exalbuminous seeds, investing embryos, the presence of volatile terpenoids, mainly monoterpenes and high content of essential oil and rosmarinic acid. Both subfamilies contain caffeic acid and its derivatives (Erdtman, 1945, Cantino, 1992).
Origanum syriacum L. is an aromatic herb belonging to the subfamily Nepetoideae. The leaves of *O. syriacum* collected in Arabsalim – South Lebanon were analysed using a microscope after being adjusted by chloral hydrate. The presence of very dense clusters of multicellular covering trichomes was observed (510 µm × 18.47 µm).

These are likely to mechanically protect the typical peltate secretory glands (∅ 87.95 µm) of Lamiaceae plants with eight secretory cells. Peltate glands lie underneath the covering trichomes, so they are protected from external environmental changes, e.g. in temperature and humidity; hence they remain intact, their contents don't evaporate that easy, and the aromatic properties of Origani syriaci folium increase (Figures 1 – 4) (Farhat, 2012).

Figure 1 Peltate secretory gland with 8 secretory cells (top view)

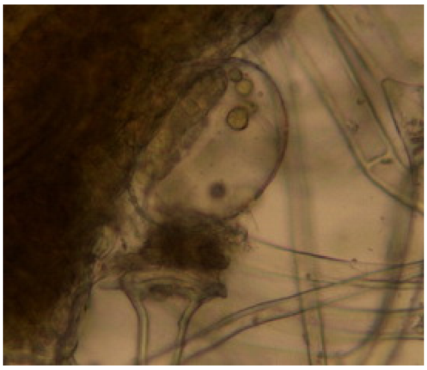

Figure 2 Peltate secretory gland (side view)

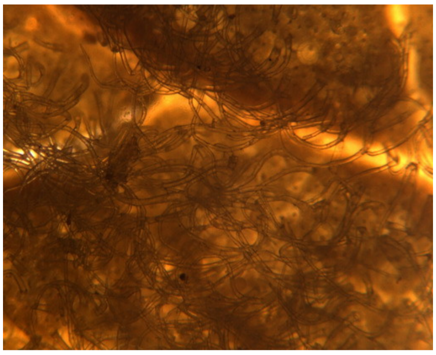

Figure 3 Multicellular covering trichomes

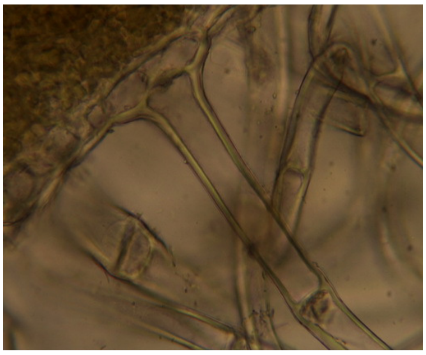

Figure 4 Multicellular covering trichome – basal cell

A greenish yellow essential oil with pungent smell was isolated from dry O. syriaci folium. The oil content was determined to be 95 ml/1000 g drug, i.e. 9.5% (V/m). This drug is not officinal in the European Pharmacopoeia (Ph. Eur. 7) but Origani herba is. The Pharmacopoeia defines Origani herba as dried leaves and flowers separated from the stems of *Origanum onites* L. or *Origanum vulgare* L. subsp. *hirtum* (Link) I etsw., or a mixture of both species. Origani herba of pharmacopoeial quality should contain 25 ml of essential oil per 1000 g anhydrous drug. Thus the content of essential oil in Origani syriaci folium in our study was almost four times higher than the pharmacopoeial quality standard (Origani herba) (Farhat 2012, Ph. Eur 7, 2011).

The volatile compounds in *Origanum syriacum* leaves collected in Arabsalim – South Lebanon were analysed and identified using SPME GC/MS. Carvacrol (78.4%) and thymol (17.9%) were the main abundant components (Table 1, Figure 5).

Table 1 Essential oil components in Origani syriaci folium

RI	Essential oil components	RT [min]	Content [%]
1190	Unknown	11.16	0.3
1249	Thymoquinone	12.44	2.5
1290	Thymol	13.18	17.9
1298	Carvacrol	13.39	78.4
1418	β-Caryophyllene	15.46	0.8

RI – Kovats index
RT – Retention time

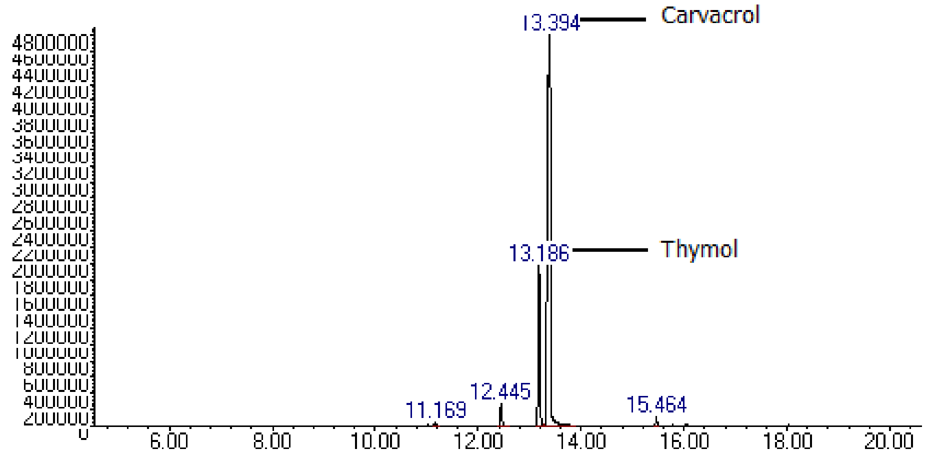

Figure 5 GC/MS chromatogram of Origani syriaci folium showing the two major components in the essential oil: carvacrol (78.4%) and thymol (17.9%)

The chemical composition of *Origanum syriacum* leaves essential oil determined in this study was compared to that in four other studies (Table 2) (Farhat, 2012).

Table 2 Comparison of chemical composition (percentage) of Origani syriaci folii aetheroleum determined in this study with the results of other studies

Study A* (Loizzo et al., 2009)	**Study B*** (Kintzios, 2004)	**Study C**** (Kintzios, 2004)	**Study D*** (Al-Kalaldeh, 2010)	**Our Study**** (Farhat, 2012)
Baskinta Mountain; Lebanon	Dortyol district; Turkey	Kahramanmaras; Turkey	Amman; Jordan	Arabsalim; Lebanon
Thymol (24.7 %)	γ-Terpinene (27.8%)	**Carvacrol** (64.1%)	**Carvacrol** (41.10%)	**Carvacrol** (78.4%)
Carvacrol (17.6 %)	**Carvacrol** (27.0%)	*p*-Cymene (12.3%)	*p*-Cymene (30.2%)	**Thymol** (17.9%)
γ-Terpinene (12.6 %)	*p*-Cymene (15.7%)	–	γ-Terpinene (4.3%)	Thymoquinone (2.5%)
***p*-Cymene (8.7 %)**	β-Caryophyllene(12.6%)	–	*cis*-Sabinene hydrate (3.2%)	β-Caryophyllene (0.8%)

* determined from a sample of Origani syriaci folii aetheroleum
** determined from a sample of Origani syriaci folium

The essential oil of *Origanum syriacum* L. var. *sinaicum* (cultivated in El Arish, Egypt) obtained by hydrodistillation was analysed by GC/MS. The following monoterpenes were identified: thymol (24% – 29%), *cis*-sabinene hydrate (18% – 20%), *γ*-terpinene (13% – 15%), *p*-cymene (5% – 8%) and terpinen-4-ol (4% – 8%) (Baser, 2003).
The major components in *O. syriacum* var. *bevanii* collected in Turkey were carvacrol (42.5%) and thymol (24.8%) (Baser, 1993).
The studies show variation in essential oil components in *Origanum syriacum* growing in different regions of different countries. These variations in quality, quantity and composition can be due to climate, soil composition, geographical location, seasonal variation, plant organ, age, vegetative cycle stage and harvesting time (Zein, 2011).
The European Pharmacopoeia (Ph. Eur. 7, 2011) requires that the sum of percentage of both carvacrol and thymol should be minimum of 60% in the Origani herba essential oil. In this study, the major contents of Origani syriaci folium were monoterpenes, mainly carvacrol (78.4%) and thymol (17.9%). The sum of percentage was 96.3%.
Thymol and carvacrol are isomers found mostly in the family Lamiaceae, such as the genera *Thymus* and *Origanum.* These monoterpenes are responsible for the antibacterial and antiphlogistic activities of the respective herbal drugs.

CONCLUSION

Thymol and carvacrol are isomers found mainly in the family Lamiaceae, such as the genera *Thymus* and *Origanum.* These monoterpenes are responsible for the antibacterial, antiphlogistic and antioxidant activities of the respective herbal drugs, which explain their wide use in traditional medicine. The same reason applies for recent studies that are being conducted on these two monoterpenes, the purpose being their use as a "natural cure" or at least as a part of various adjuvant therapies.

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REFERENCES

Abou-Jawdah Y, Sobh H, Salameh A. Antimycotic activities of selected plant flora, growing wild in Lebanon, against phytopathogenic fungi. J Agric Food Chem. 2002;50:3208–3213.

Abou-Jawdah Y, Wardan R, Sobh H, Salameh A. Antifungal activities of extracts from selected Lebanese wild plants against plant pathogenic fungi. Phytopathol Mediter. 2004;43:377–386.

Abū ʿAlī al-Ḥusayn ibn ʿAbd Allāh ibn Sīnā. Al-Qanun fi al-Tibb – The Canon of Medicine. Rome; 1593. p. 221–227 (in Arabic).

Al-Kalaldeh JZ, Abu-Dahab R, Afifi FU. Volatile oil composition and antiproliferative activity of *Laurus nobilis, Origanum syriacum, Origanum vulgare*, and *Salvia triloba* against human breast adenocarcinoma cells. Nutr Res. 2010;30:271–278.

Alma MH, Mavi A, Yildirim A, Digrak M, Hirata T. Screening chemical composition and in vitro antioxidant and antimicrobial activities of the essential oils from *Origanum syriacum* L. growing in Turkey. Biol Pharm Bull. 2003;26:1725–1729.

Baser KHC, Kurkcuoglu M, Demirci B, Ozek T. The essential oil of *Origanum syriacum* L. var. *sinaicum* (Boiss.) Ietswaart. Flavour Frag J. 2003;18:98–99.

Baser KHC, Ozek T, Tumen G, Sezik E. Composition of the essential oils of Turkish *Origanum* species with commercial importance. JEOR. 1993;5:619–623.

Cantino PD, Harley RM, Wagstaff SJ. Genera of Labiatae: Status and Classification, In Harley RM, Reynolds T (eds.). Advances in Labiatae Science. Kew: Royal Botanic Gardens; 1992. p. 511–555.

Codex Pharmaceuticus Slovacus. 1st ed. Bratislava: Herba; 1997, 354 p (in Slovak).

Daouk R, Dagher S, Sattout E. Antifungal activity of the essential oil of *Origanum syriacum* L. J Food Protect. 1995;58:1147–1149.

Dāwūd ibn ʻUmar al-Anṭākī. Tadkirat ūlī al-albāb wa-al-jāmiʻ li-al-ʻajab al-ʻujāb. Al-Maktaba Al-Thaqafiya. Beirut; 1986 (in Arabic).

Erdtman G. Pollen morphology and plant taxonomy. IV. Labiatae, Verbenaceae and Avicenniaceae. Svensk Botanisk Tidskrift. 1945;39:277–285.

European Pharmacopoeia. 7th ed. Strasbourg: Council of Europe; 2011.

Farhat M. Phytochemical analysis of Lebanese za'atar [Thesis for the PharmDr. degree]. Bratislava: Comenius University, Faculty of Pharmacy, Department of Pharmacognosy and Botany; 2012.

Galambosi B, Galambosiné Szebeni Zs, Héthelyi BÉ, Szarka Sz. The common oregano red and white flowers volatile matter content and components. J Oil Soap Cosmet. 2010;59:89–95 (in Hungarian).

Héthelyi BÉ, Szarka Sz, Galambosi B, Lemberkovics É, Szőke É. Finland cultivated medicinal, spices and aromatic plants – phytochemical analysis with modern instrumental analysis (GC, GC/MS, SPME-GC/MS) method. Paper presented at: 18th Workshop on Plant Breeding. Hungarian Academy of Sciences. March 6, 2012; Budapest, H., p. 48 (in Hungarian).

Héthelyi BÉ, Szarka Sz, Galambosi B. The Finnish-Hungarian scientific cooperation in specific areas: Finland cultivated medicinal herbs and aromatic plants, their plant chemical analysis by GC, GC/MS, and SPME-GC/MS methods. J Oil Soap Cosmet. 2011;60(suppl):73–88 (in Hungarian).

Kintzios SE. *Oregano*: The Genera *Origanum* and *Lippia*. 1st ed. Taylor & Francis; 2004.

Loizzo MR, Menichini F, Conforti F, Tundis R, Bonesi M, Saab AM. Chemical analysis, antioxidant, antiinflammatory and anticholinesterase activities of *Origanum ehrenbergii* Boiss and *Origanum syriacum* L. essential oils. Food Chem. 2009;117:174–180.

Seidemann J. World Spice Plants: Economic Usage, Botany, Taxonomy. 1st ed. Berlin, Heidelberg: Springer-Verlag; 2005.

Zein S, Awada S, Rachidi S, Hajj A, Krivoruschko E, Kanaan H. Chemical analysis of essential oil from Lebanese wild and cultivated *Origanum syriacum* L. (Lamiaceae) before and after flowering. J Med Plants Res. 2011;5:379–387.

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ANALÝZA SILICE *ORIGANUM SYRIACUM* L.

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Pamajorán sýrsky (*Origanum syriacum* L., Lamiaceae) je veľmi populárna kulinárska rastlina, ktorá sa od pradávna používala v tradičnej medicíne, najmä v Libanone a v arabskom svete. Predmetom našej práce bola mikroskopická analýza Origani syriaci folium, izolácia silice z listov *O. syriacum* a identifikácia jej prchavých zložiek. Rastlinný materiál pochádzal z južného Libanonu (Arabsalim), z roku 2010. Pri mikroskopickej analýze sa zistila prítomnosť veľmi hustých zoskupení viacbunkových krycích trichómov (510 µm × 18,47 µm) a žliazky typu Lamiaceae s ôsmimi secernujúcimi bunkami (∅ 87.95 µm). Z drogy Origani syriaci folium sa izolovala silica pomocou destilácie s vodou, obsah silice bol 95 ml/1000 g, resp. 9,5 % (V/m). Prchavé zložky sa identifikovali pomocou SPME GC/MS. V listoch pamajoránu sýrskeho sa v najväčšom množstve nachádzali karvakrol (78,4 %) a tymol (17,9 %).

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