Journal of Medicinal Plants Research Vol. 5(1), pp. 58-62, 4 January, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Analysis of essential oils of three wild medicinal plants in Albania

A. Ibraliu[1,2]*, X. Mi[2], Mihailo Ristić[3], Zora Dajic Stefanovic[4] and J. Shehu[1]

[1]Department of Crop Production, Faculty of Agriculture and Environment, Agricultural University of Tirana, Kodër Kamëz Tirana, Albania.
[2]Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, 70593 Stuttgart, Germany.
[3]Institute of Medicinal Plant Research "Dr Josif Pančić", 11000 Belgrade, Serbia.
[4]Faculty of Agriculture, University of Belgrade, 11080 Belgrade-Zemun, Serbia.

Accepted 8 December, 2010

Seventeen populations of *Orignanum vulgare* (four populations), *Thymus capitatus* (four populations) and *Satureja montana* (*Origanum*) were collected from different agro-climatically diverse sites in Albania. Their essential oils were subjected to detailed GC/FID and GC/MS analyses to determine similarities and differences among them for their chemical composition, especially the major compounds, such as Carvacrol and Thymol, which carry economic importance in medicinal plant industry and food industry. The concentrations of Carvacrol varied from 21.07 to 77.79%; Thymol from 0.72 to 39.9%; y-Terpinene from 4 to 13.8% and p-Cymene from 0.74 to 17.4%. From the cluster analysis (CA) accomplished by using the statistical software R, we came to two conclusions. (1) The collections of *O. vulgare* subsp. hirtum (rich in Carvacrol) and *Th. capitatus* (balanced concentration of compound) made from identical locations belonged to two different sub-groups, whereas the populations of *S. montana* were divided into these two groups based on their oil compositions. (2) There were no appreciable environmental effects on oil concentration.

Key words: *Orignanum vulgare*, *Thymus capitatus*, *Satureja montana*, essential oils, cluster analysis.

INTRODUCTION

Origanum vulgare L., *Thymus capitatus* (L.) Hoffmanns and Link and *Satureja montana* L., all belonging to Lamiaceae family, are Mediterranean aromatic shrubs, and are present in Northern, Southern, East and Western parts of Albania (Ibraliu et al., 2010; Vangjeli et al., 1996). These are endangered species and are included in Albanian National Red Data Book. All of these three plants produce essential oils which are rich in phenolic compounds (Carvacrol or Thymol are the dominant phenols in their essential oils) and are used as oregano spices (Kokkini et al., 2003). In addition to the essential oils, their extracts are used as remedies for many folk diseases (Lawrence, 1979). Both the essential oil (obtained by leaves distillation) and the oleoresin are used in the pharmaceutical and food industries as a flavoring agent, in the making of liqueurs and in perfumery (Piccaglia, 1998). The *O. vulgare*, *T. capitatus* and *S. montana* are characterized by a high variation for their oil compounds. In the literature, there are several studies in the phenol-rich (Carvacrol, Thymol) together with two hydrocarbons (γ-Terpinene and p-Cymene) and the substrates of the phenol. These studies showed a high content of Carvacrol, Thymol, γ-Terpinene and p-Cymene, representing the essential oil composition, *T.capitatus* (Karousou et al., 2005), *O. vulgare* (Sivropoulou et al., 1996) and *S. montana* (Ćavar et al., 2008). "Thymol" and "Carvacrol" are negatively correlated and there exists a pathway for these four chemical compounds (Russo et al., 1998). The statistical analysis

*Corresponding author. E-mail: albanibraliu@ubt.edu.al. Tel: 355 4 2353 873. Fax: 355 42353 874.

was carried out by R, which is widely used statistical software and was invented by Robert Gentleman and Ross Ihaka. It is currently maintained by the R core-development team (R Development Core Team, 2006).

MATERIALS AND METHODS

Plant material

All leaves of plants were collected from different locations in Albania from July to August 2006. The number of individuals from which leaves were collected was between 20 and 30 from each site, in proportion to the morphological variability observed. The determination of the taxonomical status for each population was performed *in situ.* The plant materials were dried at room temperature (1 month). The voucher specimens are deposited with the Crop Production Department, Faculty of Agriculture and Environment, Agricultural University of Tirana, Albania.

Isolation of the essential oil

The essential oil was isolated from 100 g of dried leaf samples by hydro-distillation for 3 h using a Clevenger-type apparatus. The resulting essential oil was dried over anhydrous sodium sulphate and stored at 4°C. The oil solution in ethanol (1%) was used for chromatographic analysis. The oil yield was determined by gravimetery. The percentages of component percentages were calculated as the mean value on column HP-20M and HP-101 for duplicate analyses.

Analytical gas chromatography (GC/FID)

The GC/FID analysis of the oil was carried out on a Hewlett-Packard HP-5890 Series II GC apparatus equipped with split-splitless injector and automatic liquid sampler (ALS), attached to HP-5 column (25 m 0.32 mm, 0.52 µm film thickness) and fitted to flame ionisation detector (FID). Carrier gas (hydrogen) flow rate was 1 ml/min, split ratio was 1:30, injector temperature was 250°C, and detector temperature was 300°C, while column temperature was linearly programmed from 40-260°C (at rate of 4°/min). Solutions of essential oil samples in ethanol (~1%) were consecutively injected by ALS (1 µl, split mode). Area percent reports, obtained as a result of standard processing of chromatograms, were used as base for the quantification purposes.

Gas chromatography/mass spectrometry (GC/MS)

The same analytical conditions as those mentioned for GC/FID were employed for GC/MS analysis, along with column HP-5MS (30 m 0.25 mm, 0.25 µm film thickness), using Hewlett-Packard HP G 1800 C Series II GCD system. Instead of hydrogen, helium was used as carrier gas. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV) in m/z range of 40-450. Sample solutions in ethanol (~1 %) were injected by ALS (200 nl, split mode).

Identification of components

The components of the oil were identified by comparison of their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1), compared to those from available literature (Adams, 2007). They were used as additional tool to approve MS findings.

Estimation for correlation

The analyses were carried out with R statistical software by using R packages cluster, labdsv and Google map. The advantages to using R is not only because of its open source that people can use freely, but also because of its good features, such as simple grammar rules and statistical functions for beginners, C and FORTRAN port for sophistic users, literate programming technology for software project management and tens of thousands of packages with a well organized website for users and authors to interact with each other. The phenotypic data of plants are collected and stored in MS excel and imported from a basic core function of R. By using R-package Rgooglemap, we searched the locations on maps.google.com and generated the geographical map with attitude and climate information (Table 1 and Figure 1). This gave us a first overview of the possible potential groups that may be formed by collections. Then we used R package cluster and labdsv to perform Principal Component Analysis (PCA).

RESULTS AND DISCUSSION

In our study, 17 populations of three different medicinal plants species (*O. vulgare*, *Th. capitatus*, *S. montana*), all belonging to the family of Lamiaceae, collected from 17 localities in Albania were characterized for four compounds involved in the compounds, namely Thymol, Carvacrol, y-Terpinene, and p-Cymene. The sums of these four compounds based on their volume are: 78.5 to 89.7% for the essential oil in *O. vulgare;* 69.4 to 87.8% in *Th. capitatus* and 64.5 to 75.9% for *S. montana*. Thus, the sum of these compounds was larger than 50.0% of the essential oil in the species (Table 2). By using R-package, cluster and labdsv, we carried out a PCA to categorize the collections. These clusters were identified based on reference to GC data for these four compounds of the essential oils.

The essential oils are categorized by different concentrations of Thymol (0.72 to 39.9%), Carvacrol (21.07 to 77.79%), γ-Terpinene (4 to 13.8%) and p-Cymene (0.74 to 17.4%). Carvacrol, Thymol, p-Cymene and y-Terpinene have been reported as major components of *S. montana* by Ibraliu et al. (2010) who obtained 2.2 to 56.0% Carvacrol; 0.4 to 27.3% Thymol; 1.1 to 17.4% p-Cymene; 0.31-8.86% for y-Terpinene and their total being in the range of 41 to 69%. It has also been reported as the major compound of the oil by Kurcuoglu et al. (2001) in *S. boissieri* (41%), Sefidkon and Jamzad (2005) in *S. mutica* (31%), Sefidkon et al. (2006) and Mihajilov-Krstev et al. (2009) in *S. hortensis* (48-67 %), and by Karousou et al. (2005) in *S. thymbra* (66%). The high content of Carvacrol, Thymol, y -terpinene and p-Cymene (Poulose and Croteau, 1978), characterizes all “oregano” type essential oils (Kokinni, 1997).

In the classification according to oil components, we find that the p Cymene and γ-Terpinene are in the same

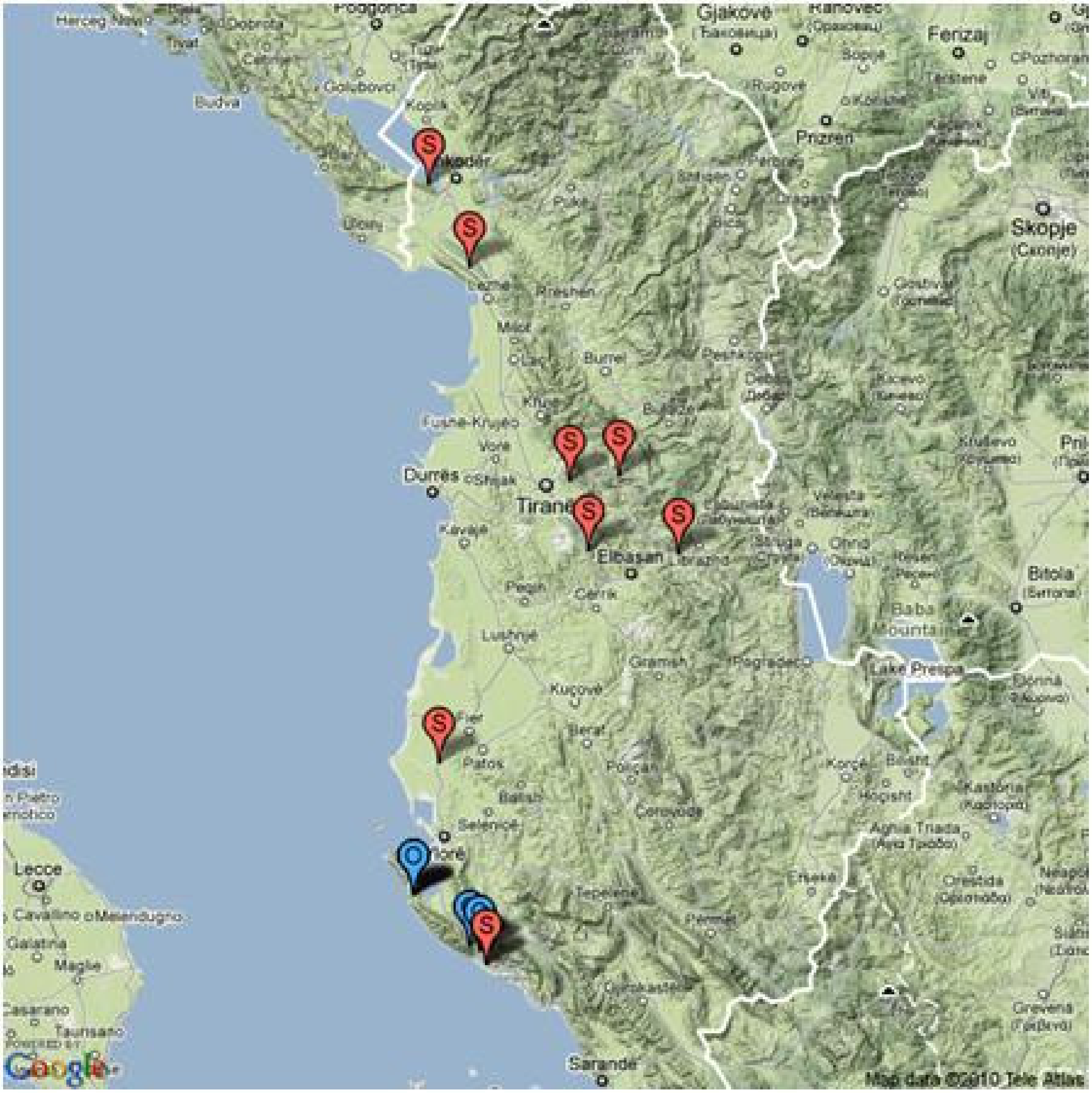

Figure 1. All localization from where collection were made, map generated by maps.google.com: four blue flags represent locations from where both *O. vulgare* sub.hirtum and *Th. capitatus* were collected and red flags the locations from where *S. montana* were collected.

same group and have positive correlation. In other studies, p-Cymene and γ-Terpinene were also reported to have positive correlation (Arrebola et al., 1993) but Thymol and Carvacrol were reported to be negatively correlated (Alissandrakis et al., 2007; D'Antuono et al., 2000). By using this information, we classified the plants into four groups: one group for *O. vulgare* (O1-O4), one group for *Th. capitatus* (T5-T8) and two groups for *S. montana* (S5-S8 and S9-S13). We plotted the geographical information and the botanical information on these four groups together with the Google map (Figure 2). This showed that one group of *S. montana* (S5-S8) is in the South and their oil compounds are also similar to the *Th. capitatus* (T1-T4), which is Carvacrol rich. The second group of *S. montana* (S9-S13) is in the North and their oil compounds are quite different (Figure 1), and are closer to the *O. vulgare* (O1-O4) group. This analysis showed that the altitude and climate of the collection locations did not influence the essential oil concentration too much. For example, the collections made from 1400 m high mountains, that is, O. *vulgare* from L4, *Th. capitatus* from L4 and the *S. montana* from location L5

Table 1. Characterization of 13 locations from where different collections were made: four locations (L1-L4) for *O. vulgare* sub.hirtum and *Th. capitatus* and nine locations (L5-L13) for *S. montana*.

No.	Location name	Altitude (masl)	Latitude (N)	Longitude (E)	Climatic details
		O. vulgare* sub.hirtum and *Th. capitatus			
L1	Karaburun	289	40°19’	19°23’	SMMCZ b
L2	Pashaliman	282	40°19’	19°23’	SMHCZ c
L3	Llogora	992	40°11’	19°36’	SMMCZ
L4	Maja Thanasit	1349	40°12’	19°34’	SMMCZ
		S. montana			
L5	Llogora	992	40°11’	19°36’	SMMCZ
L6	Llogora	205	40°09’	19°37’	SMMCZ
L7	Dajti Mountain	436	41°20	19°53’	CMMCZ f
L8	Ura-Mifolit	58	40°39’	19°28’	CFMCZ g
L9	Lezhe	16	41°51’	19°34’	NMHCZ d
L10	Shiroke, Shkoder	43	42°03’	19°26’	NMHCZ
L11	Dajti Mountain	1400	41°21’	20°03’	CMMCZ
L12	Qafe Kerrabe	750	41°10’	19°57’	CMMCZ
L13	Librazhd	217	41°9’	20°14’	SEMHCZ e

M a - Mediterranean, SMMCZ b - South Mediterranean Mountain Climate Sub-Zone, SMHCZ c - South Mediterranean Hilly Climate Sub-Zone, NMHCZ d - North Mediterranean Hilly Climate Sub-Zone, SEMHCZ e - South East Mediterranean Hilly Climate Sub-Zone, CMMCZ f - Centre Mediterranean Mountain Climate Sub-Zone, CFMCZ g -Central Field Mediterranean Climate Sub-Zone.

Table 2. Qualitative and quantitative composition of different collections of *O. vulgare* subsp.hirtum (O1-O4 collected from L1-L4), *Th. capitatus* (T1-T4 from L1-L4) and *S. montana* (S5-S13 collected from L5-L13) in leaves collected from 13 locations of Albania.

Compounds	***O. vulgare sub.hirtum***				***Th. capitatus***				***S. montana***								
	O1	**O2**	**O3**	**O4**	**T1**	**T2**	**T3**	**T4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**
Thymol	35.7	39.2	39.9	29.4	0.8	1.6	0.8	0.7	1.3	2.2	1.5	0.7	0.4	2.9	12.4	27.3	1,45
Carvacrol	27.8	32.4	32.8	32.4	77.8	57.8	71.5	78	54	39.8	56.8	61.9	55.9	39.5	37.5	21.1	40.6
γTerpinene	11.0	8.3	8.2	8.6	3.7	2.6	3.6	4	13.1	13.8	9.2	9.3	4.8	4.9	8.9	5.3	5,16
pCymene	10.0	8.9	8.8	8.1	5.5	7.5	8.4	5	2.9	0.7	1.4	1.3	14.8	15.3	13.3	16.2	17.4
Total oil (%)	84.5	88.9	89.7	78.5	87.8	69.4	84.3	87	72	56.5	68.8	73.2	76	62.7	72.2	69.8	64,6

were not much different from the other collections. However the *S. montana* from location L11 and L12 showed a relatively higher Thymol concentration. Further investigation of these two locations (Location 11 and 12) is required. The study also provided evidences that the gene action or

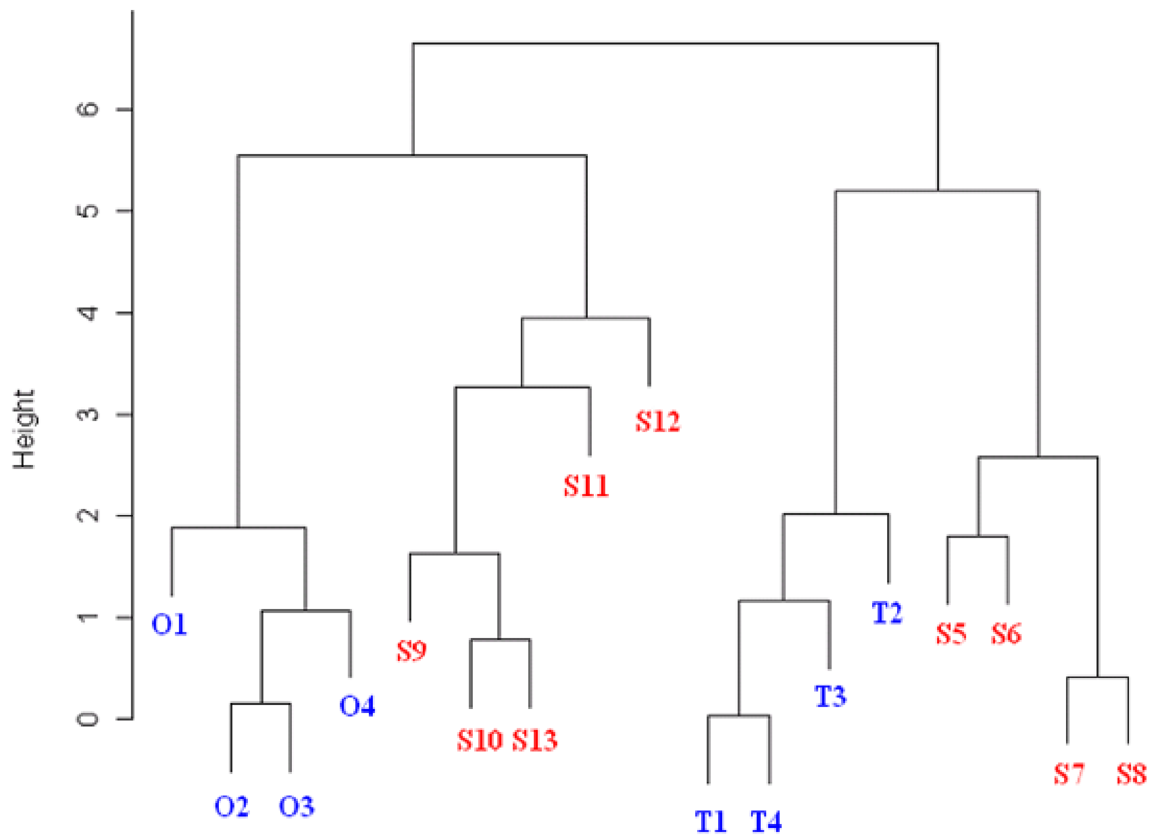

Figure 2. Dendrogram of 17 collections (O1-O4 of *O. vulgare* sub.hirtum, T1-T4 of *Th. capitatus*, and S5-S13 of *S. montana*) developed with cluster analysis based on oil compounds.

enzymes controlling the production of Carvacrol are not complicated. A molecular marker analysis is expected to provide evidence and reveal the genetic structures of *O. vulgare*, *Th. capitatus* and *S. montana*

REFERENCES

Adams RP (2007). Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Ed., Allured Publishing Corporation, Carol Stream, Illinois, USA.

Alissandrakis E, Petros A, Tarantilis PCH, Moschos P (2007). Comparison of the Volatile Composition in Thyme Honeys from Several Origins in Greece.J. Agric. Food Chem., 55: 8152–8157.

Arrebola M, Leonor M, Concepción N, José J, Francisco AO (1993). Variations in yield and composition of the essential oil of *Satureja obovat.* Phytochemistry, 35: 22, 83-93.

Cavar S, Maksimović M, Solić M, Jerković-Mujkić A, Bešta R (2008). Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. Food Chem., 111: 648-653.

D'Antuono L, Filippo G, Galletti C, Paola B (2000). Variability of Essential Oil Content and Composition of *Origanum vulgare* L. Populations from a North Mediterranean Area (Liguria Region, Northern Italy). Ann. Bot., 86, 471-478

Karousou R, Koureas D, Kokkini S (2005). Essential oil composition is related to the natural habitats: *Coridothymus capitatus* and *Satureja thymbra* in NATURA 2000 sites of Crete. Phytochem., 66: 2668-2673.

Kokkini S, Karousou R, Hanlidou E (2003). Herbs of the Labiatae. In: Caballero, B., Trugo L, Finglas P (Eds.), Encyclopedia of Food Sciences and Nutrition, second ed. Academic Press, London. 3082-3090.

Kokkini S (1997). Taxonomy, diversity and distribution of Origanum species. In: Padulosi S, ed. Oregano. Proceedings of the IPGRI International Workshop on Oregano, Valenzano (Bari), Italy. Rome: IPGRI, 2-12.

Lawrence BM (1979). Essential Oils 1979-1980. Allured Publishing. Carol Stream, Illinois, USA.

Poulose J, Rodney C (1978). Biosynthesis of aromatic monoterpenes : Conversion of γ-terpinene to p-cymene and thymol in *Thymus vulgaris* L. Arch. Biochem. Biophys., 187: 307-314.

Ibraliu A, Baldev S. Dhillon, Faslia N, Stich B (2010). Variability of essential oil composition in Albanian accessions of *Satureja montana* L. J. Med. Plant. Res., 4(14): 1359-1364.

Piccaglia R (1998). Aromatic plants: A world of flavouring compounds. Agro. Food. Ind. Hi. Technol., 9: 12–15.

R Development Core Team (2006). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Russo M, Galletti, GC, Bocchini P, Carnacini A (1998). Essential Oil Chemical Composition of Wild Populations of Italian Oregano Spice (*Origanum vulgare* ssp. hirtum (Link) Ietswaart): A Preliminary Evaluation of Their Use in Chemotaxonomy by Cluster Analysis. 1. Inflorescences. J. Agric. Food Chem., 46 (9): 3741–3746

Sivropoulou A, Papanikolaou E, Nikolaou C, Kokkini S, Lanaras T, Arsenaki M (1996). Antimicrobial and Cytotoxic Activities of *Origanum* Essential Oils. J. Agric. Food Chem., 44: 1202–1205.

Vangjeli J, Ruci B, Mullaj A (1996). Red Book of Albanian Flora. Institute of Biological Research, Academy of Science of Albania, Tirana, Albania.