NPC Natural Product Communications

2015
Vol. 10
No. 7
1293 - 1296

Characterization of Essential Oil Components from Aromatic Plants that Grow Wild in the "Piana del Sele" (Salerno, Southern Italy) using Gas Chromatography-Mass Spectrometry

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Received: January 22nd, 2015; Accepted: March 6th, 2015

Essential oils from *Rosmarinus officinalis*, *Salvia officinalis*, *Thymus vulgaris*, *Melissa officinalis* and *Mentha spicata* growing wild in the "Piana del Sele" (Salerno, Southern Italy) have been extracted by hydro-distillation, quantified and characterized by gas chromatography coupled with flame ionization detection (FID) and mass-spectrometry (MS). Sixty-nine compounds were identified and classified according to their chemical classes. The results showed that the composition of the essential oils was extremely variable and specific for each botanical species. Hydrocarbons were the most abundant class in all essential oils except for sage where aldehydes and ketones were the most representative compounds. Only for thyme was a higher content of alcohols found.

Keywords: Essential oils, Hydro-distillation, *Rosmarinus officinalis*, *Salvia officinalis*, *Thymus vulgaris*, *Melissa officinalis*, *Mentha spicata*, Gas chromatography-mass spectrometry.

Essential oils are natural product extracted from a great variety of plants [1], traditionally obtained by hydro-distillation, steam distillation, Soxhlet extraction and simultaneous distillation-extraction. The chemical composition of essential oils is very complex and differs for each species or subspecies and is characteristic for each. Furthermore, among the same subspecies, there are different chemotypes due to environmental, seasonal, or other factors, which are responsible for compositional changes in the essential oils [2,3,4].

Nowadays, the use of essential oils in pharmaceutical, sanitary, cosmetic, agricultural and food industriesis increasingly diffuse, both for the growing interest of consumers in ingredients from natural sources and also because of concern about potentially harmful synthetic additives [5]. Often essential oils are added as natural food antioxidants, thanks to their well reported ability to retard lipid oxidation, and their antioxidant capacity is currently accepted as a criterion to monitor the impact of food processing in the nutraceutical value of certain food product [6].

By the middle of the 20th century, the role of essential oils had been reduced almost entirely in the fields mentioned above, while their use in pharmaceutical preparations had declined [7]. However recent research has reported that the beneficial properties of aromatic plants are partially attributed to essential oils. The science of utilizing naturally extracted aromatic essences from plants to balance, harmonize and promote health, mind and spirit can be defined as "aromatherapy". This seeks to unify physiological, psychological and spiritual processes to enhance an individual's innate healing process [8].

In this study, samples of essentials oils were obtained from plant species growing wild in the "Piana del Sele", Campania (Southern Italy). The "Piana del Sele", also called "Piana di Paestum" or "Piana di Eboli", is a plain of about 500 Km^2, which extends along the Sele river in the province of Salerno. It is bound on the north by Piacentini mountains, to the East by the Sele Valley, to the south by the Campania-Lucania segment of the Apennine arc, and to the west by the Tyrrhenian Sea. The "Piana del Sele" is one of the most industrialized regions of Southern Italy and is a national area for the cultivation of vegetables for ready-to use fresh meals and for the production of aromatic plants for large-scale retail distribution and exportation. Today, in the "Piana del Sele" about 150 ha are cultivated with aromatic plants, predominantly sage, rosemary, basil, mint, thyme, marjoram, and savory, that are used by the food industry. These herbs and aromatic plants from Campania are currently used as fresh and dry herbs, for preparing beverages (teas, infusion, liqueurs, elixirs), for cosmetics (soaps, cream), to create gardens and botanical trails in order to enhance their balsamic and beneficial functions, and to produce herbal products (biocides, natural colorants, balsamic pillows). Therefore, the possibility of obtaining quality essential oils from these herbs and aromatic plantsis not excluded.

Essential oils obtained from rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), thyme (*Thymus vulgaris*), lemon balm (*Melissa officinalis*) and mint spicata (*Mentha spicata*) were studied for the valorization of the territory of the "Piana del Sele" (Salerno, Southern Italy), where these aromatic plants grow wild [9]. These species were chosen for their widespread occurrence in the "Piana del Sele" and for their economic importance to the world-wide spice market. Each component of the essential oil was quantified by a gas chromatographic technique coupled with flame ionization detection (FID), and chemically characterized by mass spectrometry (MS). The composition of each essential oil was compared with that of other essential oils already reported in the literature.

The essential oil of rosemary is a natural antioxidant and may be an alternative source of compounds capable of protecting lipids in foods [10]. Moreover, antibacterical activity has been observed

Table 1: Quantitative composition (mg/g) of the essential oils from *Rosmarinus officinalis (RMA and RMB)*, *Salvia officinalis (S)*, *Thymus vulgaris (T)*, *Melissa officinalis (M)* and *Mentha spicata (Ms)*. Value are given as mean ± SD (n=3).

Compound	Retention time (min)			Composition (mg/g)					
	GC-FID	GC-MS	Identification	RMA	RMB	S	T	M	Ms
Hydrocarbons									
n-Octane	3.37	5.42	NIST02/Wiley275	-	-	1.4 ± 0.0	-	-	-
Cyclopropane, 1,1, dimethyl-2-(2-methyl-2)propenyl	4.15	6.28	NIST02/Wiley275	-	-	3.4 ± 0.0	-	-	-
Tricyclene	5.39	7.44	NIST02/Wiley275	3.1 ± 0.0	3.8 ± 0.0	-	-	-	-
Origanene	5.47	7.51	Pure standard	4.6 ± 0.0	2.1 ± 0.0	5.4 ± 0.0	19.7 ± 0.0	-	3.2 ± 0.0
α-Pinene	5.60	7.62	Pure standard	190.6 ± 1.3	287.7 ± 0.0	21.7 ± 0.1	6.2 ± 0.0	-	11.2 ± 0.0
Camphene	5.90	7.88	Pure standard	79.7 ± 0.2	77.4 ± 0.2	31.7 ± 0.1	2.8 ± 0.0	-	1.2 ± 0.1
Sabinene	6.37	8.39	NIST02/Wiley275	1.4 ± 0.0	-	5.7 ± 0.0	3.0 ± 0.0	-	39.0 ± 0.1
β-Pinene	6.45	8.47	Pure standard	40.1 ± 0.0	41.8 ± 0.3	41.6 ± 0.2	-	-	213.3 ± 1.5
3-Octanone	6.59	8.62	NIST02/Wiley275	-	14.2 ±1.3	-	6.0 ± 0.0	-	-
β-Myrcene	6.70	8.77	NIST02/Wiley275	-	-	42.2 ± 0.0	8.9 ± 0.0	-	17.6 ± 0.0
o-Cymene	6.75	8.79	Pure standard	13.6 ± 0.0	33.5 ± 0.0	-	-	-	-
α-Phellandrene	6.99	9.02	NIST02/Wiley275	36.1 ± 0.1	2.5 ± 0.0	1.0 ± 0.0	2.7 ± 0.1	-	-
α-Terpinene	7.23	9.24	Pure standard	10.7 ± 0.0	5.2 ± 0.0	4.7 ± 0.0	41.7 ± 0.4	-	-
p-Cymene	7.40	9.45	Pure standard	7.0 ± 0.0	2.7 ± 0.1	3.7 ± 0.0	196.1 ± 1.2	-	-
Limonene	7.49	9.51	Pure standard	-	35.9 ± 0.0	-	-	-	250.6 ± 0.8
2-Thujene	7.50	9.56	NIST02/Wiley275	83.8 ± 0.0	-	-	6.3 ± 0.1	-	-
β-*cis*-Ocimene	7.64	9.68	NIST02/Wiley275	-	-	-	1.4 ± 0.0	3.5 ± 0.0	10.1 ± 0.0
β-*trans*-Ocimene	7.87	9.88	NIST02/Wiley275	-	-	-	-	6.3 ± 0.0	2.2 ± 0.0
γ-Terpinene	8.10	10.12	Pure standard	18.6 ± 0.0	9.2 ± 0.0	8.3 ± 0.1	200.1 ± 0.4	-	10.0 ± 0.1
cis-Sabinene hydrate	8.30	10.34	NIST02/Wiley275	2.9 ± 0.0	-	2.1 ± 0.0	13.7 ± 0.0	-	7.9 ± 0.0
α-Terpinolene	8.80	10.88	Pure standard	11.1 ± 0.0	11.7 ± 0.1	5.9 ± 0.0	-	-	-
2-Isopropyl-1-methoxy-4-methylbenzene	14.58	16.61	NIST02/Wiley275	-	-	-	38.9 ± 0.0	-	-
Copaene	24.74	26.78	NIST02/Wiley275	-	-	-	-	23.0 ± 0.1	-
β-Bourbonene	25.35	27.42	NIST02/Wiley275	-	-	-	-	4.9 ± 0.2	-
1,8 Isopropenyl-1,5-dimethyl-1,5-cyclodecadiene	25.91	27.98	NIST02/Wiley275	-	-	-	-	1.3 ± 0.0	-
β-Caryophyllene	27.45	29.44	Pure standard	28.7 ± 0.0	46.2 ± 0.0	57.4 ± 0.3	13.4 ± 0.1	287.7 ± 1.4	99.4 ± 0.4
Aromadendrene	28.45	30.51	NIST02/Wiley275	-	-	3.1 ± 0.0	-	-	-
α-Caryophyllene	29.15	31.22	NIST02/Wiley275	3.5 ± 0.0	4.5 ± 0.1	22.6 ± 0.2	-	25.0 ± 0.0	-
β-Farnesene	29.62	31.64	NIST02/Wiley275	-	-	-	-	8.6 ± 0.0	-
α-Cubebene	30.42	32.48	Pure standard	-	-	1.9 ± 0.0	4.7 ± 0.0	309.1 ± 1.6	-
α-Bergamotene	31.21	33.57	NIST02/Wiley275	-	-	-	-	28.0 ± 0.0	-
β-Bisobolene	31.64	33.66	NIST02/Wiley275	-	-	-	24.1 ± 0.2	-	-
α-Farnesene	31.69	33.69	NIST02/Wiley275	-	-	-	-	18.6 ± 0.0	-
β-Cadinene	32.12	34.27	NIST02/Wiley275	-	-	2.0 ± 0.0	-	41.7 ± 0.1	-
γ-Muurolene	33.89	35.91	NIST02/Wiley275	-	-	-	-	13.2 ± 0.0	-
Caryophyllene oxide	34.14	36.17	NIST02/Wiley275	-	-	-	-	6.2 ± 0.2	-
Viridiflorene	34.48	36.51	NIST02/Wiley275	-	-	-	-	-	19.8 ± 0.0
Alcohols									
τ-Yerbenol	6.00	8.06	NIST02/Wiley275	2.0 ± 0.0	5.3 ± 0.0	-	-	-	-
Amylvinylcarbinol	6.43	8.44	NIST02/Wiley275	-	-	-	10.9 ± 0.1	-	-
3-Octanol	6.76	8.86	Pure standard	-	-	-	2.5 ± 0.0	-	5.1 ± 0.0
β-Linalol	9.04	11.12	NIST02/Wiley275	5.0 ± 0.0	17.1 ± 0.0	3.2 ± 0.1	4.3 ± 0.0	-	-
trans-Pinocarveol	10.23	12.27	Pure standard	-	-	-	-	-	36.5 ± 0.0
Sabinol	10.28	12.32	NIST02/Wiley275	-	-	1.8 ± 0.0	-	-	-
Borneol	11.12	13.14	Pure standard	32.3 ± 0.1	46.7 ± 0.0	7.1 ± 0.2	5.4 ± 0.0	-	-
4-Terpineol	11.54	13.58	NIST02/Wiley275	9.8 ± 0.1	9.0 ± 0.0	-	3.2 ± 0.0	-	-
Carvomenthol	11.55	13.67	NIST02/Wiley275	-	-	4.8 ± 0.0	-	-	-
γ-Terpineol	12.05	14.07	NIST02/Wiley275	14.3 ± 0.0	9.5 ± 0.1	3.3 ± 0.0	-	-	-
Myrtenol	12.30	14.22	NIST02/Wiley275	-	1.2 ± 0.0	-	-	-	-
Thymol	17.71	19.78	Pure standard	-	-	-	200.8 ± 0.4	-	-
Carvacrol	18.37	20.44	Pure standard	-	-	-	85.0 ± 0.0	-	-
γ-Cadinol	36.65	38.72	NIST02/Wiley275	-	-	-	-	14.4 ± 0.0	-
γ-Muurarol	37.24	39.43	NIST02/Wiley275	-	-	-	-	15.5 ± 0.1	-
Aldehydes and ketones									
Eucalyptol	7.55	9.61	Pure standard	199.2 ± 0.2	86.1 ± 0.2	205.7 ± 1.2	2.1 ± 0.0	-	-
α-Thujone	9.32	11.46	Pure standard	-	-	228.2 ± 0.8	-	-	-
β-Thujone	9.58	11.62	Pure standard	-	-	62.6 ± 0.2	-	-	-
Chrysanthenone	9.82	11.81	NIST02/Wiley275	1.8 ± 0.0	11.8 ± 0.0	-	-	-	-
Isothujal	10.09	12.07	NIST02/Wiley275	-	-	1.1 ± 0.0	-	-	-
Camphor	10.43	12.48	Pure standard	139.2 ± 1.9	79.2 ± 0.4	200.4 ± 1.4	-	-	-
Pinocarvon	11.03	13.12	NIST02/Wiley275	-	-	-	-	-	44.0 ± 0.0
2-(1-Cyclopent-1-enyl-1-methylethyl)cyclopentanone	11.22	13.28	NIST02/Wiley275	-	2.9 ± 0.0	-	-	-	-
3-Pinanone	11.45	13.56	NIST02/Wiley275	-	11.5 ± 0.0	-	-	-	137.5 ± 1.6
Murtenal	12.32	14.34	NIST02/Wiley275	-	-	-	-	-	30.9 ± 0.0
cis-Verbenone	12.87	14.89	NIST02/Wiley275	12.6 ± 0.0	46.4 ± 0.0	-	-	-	-
Neral	14.41	16.42	Pure standard	-	-	-	-	24.4 ± 0.0	-
Geranial	16.19	18.26	Pure standard	-	-	-	-	46.9 ± 0.2	-
Others									
β-Citronellal	10.64	12.67	NIST02/Wiley275	-	-	-	-	42.1 ± 0.0	-
Methylthymolether	14.09	16.10	NIST02/Wiley275	-	-	-	40.6 ± 0.1	-	-
Methylcitronellate	15.57	17.61	NIST02/Wiley275	-	-	-	-	7.2 ± 0.0	-
α-Bornylacetate	17.18	19.22	NIST02/Wiley275	44.1 ± 0.0	71.6 ± 0.0	-	-	2.2 ± 0.0	-

against some spoilage organisms, such as *Pseudomonas fluorescens* and *Brochothrix thermoplasta* [11]. Sage essential oil also has antioxidant properties and can be used as an alternative to rosemary oil for the protection and preservation of nutraceutical products [12]. The aromatic and medicinal properties of *Thymus* species have made them one of the most popular groups of plants throughout the world. Tonic, carminative, digestive and expectorant properties [13]have been reported, but thyme species also have strong

antibacterial, antifungal and antioxidant activities in food [14]. Lemon balm (*Melissa officinalis*) is distributed in all Mediterranean countries [15] where it is used for several diseases that include catarrh, fever, flatulence and headaches. Also, antioxidant and antimicrobial activity against Gram-positive bacteria and some Gram-negative bacteria such as *Psudomonas aeruginosa*, *Escherichia coli*, *Salmonella enteritidis*, *S. typhi* and *Shigella* strains was demonstrated [16].There are little literature data on the composition of mint essential oils and previous studies concerning the antimicrobial properties of the essential oil of mint are difficult to compare. As reported by Sivropoulou *et al*. [17], this is due to the great differences between *Mentha* species and, to a lesser extent, to differences in the experimental techniques applied.

Table 1 shows the composition of each essential oil; all the compounds were classified according to their chemical classes and retention time (min).

In the essential oil extracted from rosemary-A, twenty-six compounds were identified, belonging to different types of chemical classes. Hydrocarbons were the most representative (53.7%), followed by aldehydes and ketones (35.4%), alcohols (6.4%) and other molecules (4.4%). Among the hydrocarbons, α-pinene was the most abundant (190.6 mg/g). Other hydrocarbons were present in percentages lower than 2%. Borneol was the most abundant alcohol with a content of 32.3 mg/g. Eucalyptol and camphor were the most representative aldehydes with concentrations of199.2 mg/g and 139.2 mg/g, respectively. Among other compounds, α-bornylacetate (44.1 mg/g) was found in RM-A oil (chromatograms not shown, the data are reported in Table 1).

In the essential oil of rosemary-B, chromatographic analysis allowed the identification of twenty-eight compounds belonging to hydrocarbons, (59.2%), aldehydes and ketones (24.3%), alcohols (9.1%) and others (7.3%). The composition of rosemary-B significantly differed from that of rosemary A. The percentage of α-pinene was higher than 33.7% (content of α-pinene detected in rosemary-B: 287.7 mg/g), as well as that of β-pinene that was greater than 4.1% (content of β-pinene detected in rosemary-B: 41.8 mg/g). The concentrations of eucalyptol and camphor detected in rosemary-B (86.1 mg/g and 79.2 mg/g respectively) were significantly lower than those of rosemary-A (chromatograms not shown, the data are reported in Table 1). The high content of eucalyptol, α-pinene and camphor was typical of essential oils extracted from rosemary plant that grown on the Mediterranean coast [18].

In sage essential oil twenty-nine compounds were identified belonging to the classes of aldehydes and ketones (70.9%), hydrocarbons (27.0%) and alcohols (2.1%). Three compounds were dominant: α-thujone (228.2 mg/g), eucalyptol (205.7 mg/g) and camphor (200.4 mg/g). Camphene (31.7mg/g), β-pinene (41.6 mg/g), β-myrcene (42.2 mg/g) and β-caryophyllene (57.4mg/g), and the ketone β-thujone (62.6 mg/g) were other compounds present in a percentage higher than 3% (chromatograms not shown, the data are reported in Table 1). The composition of the sage essential oil was in accordance with the earlier published data [19].

The major classes of molecules of thyme essential oil were alcohols (33.0%) and hydrocarbons (62.4%). The chemical composition of the oil was characterized by high amounts of thymol (200.8 mg/g), γ-terpinene (200.1 mg/g) and *p*-cymene (196.1 mg/g) (chromatograms not shown, the data are reported in Table 1). Carvacrol and thymolare known for their wide spectrum of antimicrobial activity. Due to their hydrophobic nature, the molecules interact with the lipid bilayer of cytoplastic membrane causing loss of integrity and leakage of cellular material such as ions, ATP and nucleic acid [20, 21]

Lemon balm (*Melissa officinalis*) is an aromatic plant, widely distributed in the Mediterranean and as well as *Rosmarinus officinalis*, belongs to the family of *Laminaceae*. Its essential oil contained twenty-one substances, most of which were hydrocarbons (83.6%), aldehydes and ketones (7.6%). β-Caryophyllene and α-cubebene were predominant, their contents being 287.7 mg/g and 309.1 mg/g, respectively. Neral (24.4 mg/g) and geranial (46.9 mg/g) were also found (chromatograms not shown, the data are reported in Table 1).

*Mentha spicata*is commonly known as spearmint or garden mint [22]. In the essential oil of *M. spicata*, eighteen compounds were identified, most of them hydrocarbons (72.9%), aldehydes and ketones (22.6%). Limonene was the most abundant component (250.6 mg/g), followed byβ-pinene(213.3 mg/g), 3-pinanone (137.5 mg/g) and β-caryophyllene (99.4 mg/g) (chromatograms not shown, the data are reported in Table 1). Several authors reported the presence of limonene in different species of mint plants [23, 24].

In this work essential oils obtained from aromatic plants and herbs that grow wild in dry meadows, calcareous grassland, and crags in the fields located in the "Piana del Sele" (Salerno, Southern Italy) were studied. Our results show that quality essential oils could be obtained from these herbs and aromatic plants.

Except for sage (*Salvia officinalis*), in all the essential oils analyzed hydrocarbons were the most abundant class of compounds. The percentages of hydrocarbons in RM-A, RM-B, Sage, Thyme, Lemon balm and Mint spicata were 53.8%, 59.2%, 27.0%, 62.4%, 83.6% and 72.9%, respectively. The chemical compositions of rosemary-A and rosemary-B were different. In particular, rosemary-B contained more α-pinene and β-pinene than rosemary A. In sage, aldehydes and ketones were the most abundant, the percentage being 70.9%. Compared with the other samples, thyme essential oil was characterized by a higher content of alcohols (33.0%), mainlycarvacrol and thymol. There are few studies on the chemical composition of essential oils of *Mentha spicata*. In particular, this oil showed a high concentration of limonene (250.6 mg/g), whose beneficial properties have already been reported in the literature. Consequently, the results obtained could lay the groundwork for the development of the "Piana del Sele" area from the point of view of the aromatic plants that grow there spontaneously.

Experimental

Sampling and distillation: All plants analyzed (rosemary, sage, thyme, lemon balm and mint spicata) were collected in the "Piana del Sele", in fields located in Acerno and in Capaccio Scalo (only rosemary-B). All the plants were collected from the wild and none was subjected to any kind of agronomic treatment. Immediately after harvest, which took place manually, samples of each collection were minced and subjected to hydrodistillation according to the standard procedure reported in the Pharmacopoeia [25]. The distillation was performed during 2 h using a chamber of 20 L capacity (Albrigi Luigi s.r.l., Varese, Italy). Oil samples were centrifuged and the supernatant was dried with sodium sulfate.

Identification and quantification of essential oil compounds: A solution of essential oil in *n*-hexane (1:100) (0.2 μL)was injected into the gas chromatograph (mod. 6890N Network GC System, Agilent Technologies Palo Alto, CA) coupled with a mass spectrometer (mod. 5973 Network Mass Selective Detector, Agilent

Technologies Palo Alto, CA) and equipped with a HP-5MS column (30 m length;0.25 mm internal diameter; 0.25 μm film thickness; Agilent Technologies, Palo Alto, CA). Helium gas at a flow rate of 1.2 mL/min (linear velocity: 33 cm/s) was used as carrier. Injection was made into a split-splitless injector (split ratio 50:1) at 200°C. The oven temperature program was the following: 45°C for 5 min.; increase of 7°C/min. up to 100°C, held for 15 min, from 100°C to 150°C with an increment of 5°C per min, held for 2 min. The MSD transfer line was set at a temperature of 250°C; MSD temperature of the quadrupole was 150°C and ionization temperature was 230°C. Mass spectra were acquired at 70 eV energy and the scan acquisition was performed in the range between 35 and 300 m/z. To perform quantitative analysis, samples prepared as previously described were injected into a gas chromatograph mod. 6850 Series II Network GC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID), a programmed temperature vaporizer (PTV) injector and a HP-5MS column (30 m, i.d. 0.25 mm; 0.25 μm film thickness; Agilent Technologies, Palo Alto, CA, USA). PTV injector temperature program was 60°C for 10 s increased 9°C/min to 200°C for 2 min. Carrier gas was helium at 1.0 mL/min. One μL volume injection was made with a split ratio of 50:1; the oven temperature program was the same as that used for GC-MS analysis and the FID temperature was held at 300°C. For quantitative analysis the method suggested by Bayramoglu *et al*., was used [26]. The components of the essential oils were identified by comparison of their retention time (min) with those of pure standard listed in Table 1 (Sigma Aldrich, St. Louis, MO, USA) and by matching their mass spectra with those available in NIST/NBS and WILEY 275 libraries.

All determinations and experiments were performed in triplicate and the results were reported as the average value of 3 determinations. Standard deviation was calculated by using Microsoft Excel program (Microsoft Corporation, Redmond, Wash, USA).

References

[1] Teissedre PL, Waterhouse AL. (**2000**) Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties. *Journal of Agriculture and Food Chemistry*, ***48***, 3801-3805.

[2] Daferera DJ, Tarantilis PA, Polissiou MG. (**2002**) Characterization of essential oils from *Lamiaceae* species by Fourier transform Raman spectroscopy. *Journal of Agriculture and Food Chemistry*, ***50***, 5503-5507.

[3] Skoula M, El Hilali I, Makris AM. (**1999**) Evaluation of the genetic diversity of *Salvia fruticosa* Mill. Clones using RAPD markers and comparison with the essential oil profiles. *Biochemical Systematics and Ecology*, ***27***, 559-568.

[4] Tzakou O, Verykokidou E, RoussisV, Chinou I. (**1998**) Chemical composition and antibacterial properties of *Thymus longicaulis* subsp. *chaoubardii* oils: Three chemotypes in the same population. *Journal of Essential Oil Research*, ***10***, 97-99.

[5] Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R.(**2005**) Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry*, ***91***, 621-632.

[6] Cabrera AC, Prieto JM. (**2010**) Application of artificial neural networks to the prediction of the antioxidant activity of essential oils in two experimental *in vitro* models. *Food Chemistry*, ***118***, 141-146.

[7] Edris AE (**2007**) Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. *Phytotherapy Research*, ***21***, 308-323.

[8] Bakkali F, Averbeck S, Averbeck D, Idaomar M. (**2008**) Biological effects of essential oils - A review. *Food and Chemical Toxicology*, ***46***, 446-475.

[9] Sivropoulou A, Papanikolaou E, Nikolaou C, Kokkini S, Lanaras T, Arsenakis M. (**1996**) Antimicrobial and cytotoxic activities of *Origanum* essential oils. *Journal of Agriculture and Food Chemistry*, ***44***, 1202-1205.

[10] Rezzoug SA, Boutekedjiret C, Allaf K. (**2005**) Optimization of operating conditions of rosemary essential oil extraction by fast controlled pressure drop process using response surface methodology. *Journal of Food Engineering*, ***71***, 9-17.

[11] Valero M, Salmerón MC.(**2003**) Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth. *International Journal of Food Microbiology*, ***85***, 73-81.

[12] Shahidi F. (**2000**) Antioxidants in food and food antioxidants. *Molecular Nutrition & Food Research*, ***44***, 158-163.

[13] Zargari A. (**1992**) *Medicinal plants. 5*th ed, Vol. IV. Tehran University Press, Iran, 28-42.

[14] Stahl-Biskup E, Saez F. (**2002**) *Thyme - The genus Thymus*. Taylor & Francis, London, 75-125.

[15] Sari AO. (**2002**) Yield characteristics and essential oil composition of lemon balm (*Melissa officinalis* L.) grown in the Aegean Region of Turkey. *Turkish Journal of Agriculture and Forestry*, ***26***, 217-224.

[16] Mimica-Dukic N, Bozin B, Sokovic M, Natasa S.(**2004**) Antimicrobial and antioxidant activities of *Melissa officinalis* L. (*Lamiaceae*) essential oil. *Journal of Agriculture and Food Chemistry*, ***52***, 2485-2489.

[17] Jordán MJ, Lax V, Rota MC, Lorán S, Sotomayor JA. (**2013**) Effect of bioclimatic area on the essential oil composition and antibacterial activity of *Rosmarinus officinalis* L. *Food Control*, ***30***, 463-468.

[18] Santos-Gomes, P.C., Fernandes-Ferreira, M. (**2001**) Organ- and season-dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites. *Journal of Agriculture and Food Chemistry*, ***49***, 2908-2916.

[19] Helander IM, Alakomi HL, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris LGM, Von Wright A. (**1998**) Characterization of the action of selected essential oil components on gram-negative bacteria. *Journal of Agriculture and Food Chemistry*, ***46***, 3590-3595.

[20] Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE. (**2001**) A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *European Journal of Applied Microbiology and Biotechnology*, ***91***, 453-462.

[21] Miguel MG (**2010**) Antioxidant and anti-inflammatory activities of essential oils: A short review. *Molecules*, ***15***, 9252-9287.

[22] Zambonelli A, Zechini D'Aulerio A, Bianchi A, Albasini A. (**1996**) Effects of essential oils on phytopathogenic fungi in vitro. *Journal of Phytopathology*, ***144***, 491-494.

[23] Senatore F. (**1996**) Influence of harvesting time on yield and composition of the essential oil of a thyme (*Thymus pulegioides* L.) growing wild in Campania (Southern Italy). *Journal of Agriculture and Food Chemistry*, ***44***, 1327-1332.

[24] Kanatt SR, Chander R, Sharma A.(**2007**) Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. *Food Chemistry*, ***100***, 451-458.

[25] European Pharmacopoeia. 5th ed., Strasbourg. Council of Europe (**2005**).

[26] Bayramoglu B, Sahin S, Sumnu G. (**2009**) Extraction of essential oil from laurel leaves by using microwaves. *Separation Science and Technology*, ***44***, 722-733.