Chemical Composition of the Essential Oil of *Satureja myrtifolia* (Boiss. & Hohen.) from Lebanon

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Abstract: *Satureja myrtifolia* (Boiss. & Hohen.) Greuter & Burdeta medicinal plant belonging to the Lamiaceae family was collected from south of Lebanon and hydro-distilled by Clevenger method. Essential oil composition from aerial parts was analyzed by GC-MS technique. The odor of essential oil is characteristic, and clear yellow liquid oil was obtained after hydro-distillation. The yield of the essential oil was 1.25±0.02 % of dry matter (w/w). Thirty nine volatile components were identified in the *Satureja myrtifolia* oil, which shows a high amount of hydrocarbons class (57.82±0.1 %). Other classes were also identified such assesquiterpene hydrocarbons (12.96±0.1 %), oxygenated sesquiterpenes (10.65±0.2 %), phenolic compounds (10.32±0.1 %), acids (5.53±0.1 %), and monoterpenes hydrocarbons (2.21±0.1 %).In addition, a comparison with the unique study performed on *Satureja myrtifolia* was also carried out.

Key words: *Satureja myrtifolia*, Medicinal plant, Essential oil, Lebanon.

Introduction

Lebanon is known for its rich biodiversity especially with plants having medicinal properties. In fact, 2607 wild species from which 92 are endemics can be found in only 10452 km^2 [1]. Among this biodiversity, *S .myrtifolia* (Syn: *Micromeria myrtifolia* Boiss. & Hohen) is a perennial plant that grows in rocky slopes (often limestone) exposed to sunlight, and mainly found in the Eastern Mediterranean regions (Turkey, Crete, Karpathos, Cyprus, Greece, Palestine, Jordan, Egypt, Lebanon and Syria). The aerial parts of *S. myrtifolia* are used as medicinal teas and infusions, in folk medicine for its carminative, appetizer, stimulant and pain relieving such as stomach ache [2-6]. The ethno medicinal use of *S. myrtifolia* for digestive perturbation, nervous system regulation, headache and hepatic diseases with *Ruta chalapensis* [5]. In Lebanon this plant is known as "Zoufa, Zoufi or Achnan Daoud" and is used in the traditional medicine for pneumonia, respiratory infections, cough, stomach ache, mouth ulcer, gastritis, cardiotonic, febrifuge, diuretic, stomachic, expectorant internally, while the cooled infusion is used as a gargle for laryngitis and as antiseptic externally [1].

Chemical composition of aromatic and medicinal plants depends on cultivation area, climatic conditions, vegetation phase, genetic modifications and other factors [7,8]; therefore characterization

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of flora present in different growing sites, countries and geographical zones is an important task [9]. Factors that determine the composition and the yield of essential oil are numerous and in some instances it is difficult to segregate these factors from each other, since many are inter dependent and influence one another [10]. These variables may include seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized and post harvest drying and storage [10,11]. There are many reports in the literature showing the variation in the yield and chemical composition of the essential oil with respect to geographical regions. Ozek *et al.* [12] have studied the essential oil composition of *S. myrtifolia* from Turkey chemotype. They found that β-caryophyllene (42.56 %), patchoulane (8.69 %), germacrene D (7.00 %), and δ-cadinene (6.99 %) were the main components of the oil. To the best of our knowledge, since 1992 (Ozek study) to date, no other study was carried on essential oil composition of *S. myrtifolia* from other Mediterranean countries.

The main goal of this study is to examine the chemical composition of essential oil *of S. myrtifolia* from Lebanon origin and to compare it to the unique Turkish study performed on this species.

Experimental section

Plant material

S. myrtifolia plant biomass was collected in south region of Lebanon from kfarhatta village (350 m altitude, longitude 35° 26'47.04", and latitude 33° 30'28.08") during July 2011. *S. myrtifolia* has been authenticated and cataloged in the herbarium of the Faculty of Agronomy (Holy Spirit University of Kaslik, Lebanon) under voucher specimen number MNV175a [1].

Essential oils extraction

Air dried plant (40 g) was mixed with 500 mL of distilled water and the essential oil was isolated by hydrodistillation in a Clevenger type apparatus during 3 h. The obtained essential oil was separated from the water and dried over anhydrous sodium sulphate. Isolation of essential oils was performed in duplicate and the samples of essential oil were stored in a freezer prior to further analysis. The yield of the oil was 1.25±0.02 % of dry matter (w/w).

Essential oil analysis

GC analysis

Analytical gas chromatography was carried out on a Thermo Electron Corporation gas chromatograph fitted with a DB-5 MS capillary column (30 m × 0.25 mm), 0.1 µm film thickness. Helium was the carrier gas (0.7 ml/min). Column temperature was initially 35°C, then gradually increased to 85°C at 5°C /min rate, held for 20 min and finally raised to 300°C at 10°C /min held for 5 min. Diluted samples (1/100, v/v) of 1 µl were injected at 250°C, manually and in the split-less mode. Flame ionization detection (FID) was performed at 310°C.

GC-MS analysis

GC-MS was performed using an Agilent gas chromatograph 6890 coupled with Mass Detector 5975. The 7683 B auto sampler was injecting each time 1 µL of oil sample. GC-MS analysis was carried out using a fused silica capillary column Factor DB-5 MS, measuring 30 m x 0.25 mm internal diameter, film thickener of 0.1 µm; the oven temperature program adopted was 35°C with an increase of 5°C /min until 85°C (20 min) and then to 300°C (10 min) with an increase of 10°C / min. Mass spectra were recorded at 70 eV, Ion source temperature 310°C, Transfer line 320°C, Acquisition: Full Scan 50-400 amu.

GC and GC-MS analysis was also run by using a fused silica HP Innowax polyethylen glycol capillary column (50 m × 0.20 mm), 0.20 µm film thickness. In both cases helium was used as carrier gas.

Most constituents were identified by gas chromatography by comparison of their retention indices (RI) with those of the literature [13] or with those of authentic compounds obtained from Sigma-Aldridch (Lebanon). The retention indices were determined in relation to a homologous series of n-alkanes (C8–C24) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST and Wiley 275 Libraries and our home-made library or with mass spectra from literature [13]. Standards of some EOs

(Essential Oil) of known composition (such as EO of *Rosmarinus officinalis* L. - Phytosun' Aroms, Plelo, France) have been injected in similar conditions to check the retention times and the mass spectra. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Statistical analysis

For GC-MS raw data were imported into the statistical software package MS Stat (ANALYT MTC, Muehlheim, Germany, MS Statistical Software Version 3.02u); the software was looking for differences in the mass fragments (m/z), the retention time and the abundance.

Results and discussion

The yield of the essential oil from air-dried aerial parts of S. myrtifolia was 1.25±0.02 % (dry wt. basis). Thirty nine components were identified in oil and their composition is presented in Table 1. Five major volatile compounds, namely Tricosane (13.65±0.02 %), tetracosane (12.31±0.05 %), caryophyllene oxide (10.65±0.02 %), thymol (10.32±0.03 %), and β-caryophyllene (8.99±0.04 %), were found in oil. Volatile constituents identified in the S. myrtifolia belong to the following classes of organic compounds: Hydrocarbons, sesquiterpenes hydrocarbons, oxygenated sequiterpenes, phenolic compounds, acids and monoterpenes hydrocarbons.

Hydrocarbons were the most abundant class of organic compounds in S. myrtifolia oil which consist 57.82±0.01 % of total oil, followed by sesquiterpene hydrocarbons (12.96±0.01 %) and oxygenated sequiterpenes (10.65±0.01 %). About 10.32±0.03 % of total oil composition consists of thymol, a typical phenolic compound. Also minor classes of organic compounds were identified such acids (5.53±0.01 %), and monoterpenes hydro-

Table 1. Essential oil composition (%) of *S. myrtifolia* aerial parts from Lebanon origin

No.	Compounds	R.I.[ap]	R.I.[p]	Content GC area %	Odour perception[c]
1	α-Pinene	938	1032	t	fresh, camphor, sweet pine, earthy, woody
2	β-Pinene	980	1118	t	n.a
3	Myrcene	993	1174	t	peppery, terpene, spicy, balsam, plastic
4	α-Phellandrene	1004	1033	t	n.a
5	ρ-Cymene	1025	1028	0.11±0.02a	fresh, citrus, terpene, woody, spice
6	Limonene	1030	1023	1.66±0.01b	citrus, orange, fresh, sweet
7	γ-Terpinene	1057	1255	0.44±0.01a	oily, woody, terpene lemon/ lime,tropical, herbal
8	Linalool	1098	1553	0.11±0.01b	flower, lavender
9	Borneol	1167	1719	t	camphor
10	Terpinen-4-ol	1176	1611	0.11±0.01a	turpentine, nutmeg, must
11	Thymol	1239	2198	9.65±0.03a	Spice, woody, camphor, thymol

table 1. (continued).

No.	Compounds	R.I.[ap]	R.I.[p]	Content GC area %	Odour perception[c]
12	Carvacrol	1299	2239	0.66±0.01a	spice, woody, camphor, thymol
13	α-Cubebene	1352	1466	0.11±0.01b	herbal, waxy
14	α-Copaene	1377	1497	0.66±0.02a	woody, spice
15	γ-Bourbonene	1385	1535	0.22±0.01a	herbal
16	α-Cedrene	1411	1568	0.55±0.01b	smoky
17	β-caryophyllene	1415	1612	8.99±0.04a	sweet, woody, spice, clove dry
18	*trans*-α-Bergamotene	1436	1573	0.22±0.01a	woody, warm, tea
19	Germacrene-D	1477	1726	0.11±0.01a	woody, spice
20	α-Curcumene	1483	1784	1.22±0.01b	herbal
21	δ-Cadinene	1526	1773	0.88±0.01a	woody
22	Caryophyllene oxide	1577	2008	10.65±0.02a	herbal, sweet, spice
23	Nonanoic acid	1278	2190	0.11±0.01a	green, fat
24	Tetradecanoic acid	1768	2672	0.88±0.01a	n.a
25	Octanoic acid	2172	3402	0.33±0.01b	sweet, cheese
26	Hexadecanoic acid	1957	2931	4.21±0.01a	n.a
27	α-Ionene	1208	1567	t	
28	Octadecane	1800	1800	0.66±0.01a	alkane
29	Nonadecane	1900	1900	4.32±0.02b	alkane
30	Eicosane	2000	2000	1.55±0.01a	n.a
31	Heneicosane	2100	2100	5.99±0.02a	n.a
32	Docosane	2200	2200	7,65±0.01a	n.a
33	Tricosane	2300	2300	13.65±0.02a	alkane
34	Tetracosane	2400	2400	12,31±0.05a	n.a
35	Pentacosane	2500	2500	3.88±0.02b	n.a
36	Hexacosane	2600	2600	3.88±0.02a	n.a
37	Heptacosane	2700	2700	1.99±0.02a	n.a
38	Octacosane	2800	2800	0.99±0.02a	n.a
39	Nonacosane	2900	2900	0.99±0.02a	n.a
	Monoterpene hydrocarbons (%)			2.21±0.1a	
	Oxygenated monoterpenes (%)			0.22±0.2a	
	Phenolic compounds (%)			9.65±0.0a	
	Sesquiterpene hydrocarbons (%)			12.96±0.1a	
	Oxygenated sesquiterpenes (%)			10.65±0.1a	
	Acids (%)			5.53±0.1a	
	Hydrocarbons (%)			57.82±0.1a	
	Total (%)			99.99±0.2a	

RI[ap] and RI[p] are retention indices calculated using respectively an apolar column (HP-5) and polar column (HP Innowax): Values with different letters (a-b) are significantly different at $p<0.05$.

[c] Odor perception adapted from www.thegoodscentscompany.com

Data are means ± SD of three replicates: t: traces

carbons (2.21±0.01 %). It is known that the parent sesquiterpenes are biosynthesized from geranyl diphosphate by its condensation with isopentenyl diphosphate. It can be suggested that monoterpenes are consumed by the plants faster at late vegetation phases compared to sesquiterpenes.

Comparison of the percentages of the main components in *S. myrtifolia* oil analyzed in our study and previously reported in literature by Ozek *et al.* [12] are summarized in Table 2. Air-dried parts of *S. myrtifolia* Turkish plant where found to produce an oil yield of 0.03 % while the same parts of *S. myrtifolia* Lebanese plant produce 1.25±0.02 % of oil. In present study tricosane was the dominat constituent (13.65±0.02 %) in Lebanese oil, while β-caryophyllene (42.56±0.04 %) was the major constituent in Turkish oil. β-caryophyllene was also present in Lebanese oil but about five fold less (8.99±0.04 %) with regard with the one obtained from Turkish oil (42.56 %). Besides, five other major volatile compounds such as germacrene-D (7.00 %), δ-cadinene (6.99 %), patchoulane (8.69 %), α-humulene (3.01 %), and nerodiol (2.60 %) were present in Turkish oil but not detected in Lebanese oil. On the other hand, five major volatile compounds such as tricosane (13.65±0.02 %), tetracosane (12.31±0.05 %), thymol (9.65±0.03 %), caryophyllene oxide (10.65±0.04 %), docosane (7.65±0.01 %) and heneicosane (5.99±0.02 %) were present in Lebanese oil but not detected in Turkish oil.

It should be noted that the Lebanese plant contained interesting amount of thymol, which was not the case for the Turkish plant. Thymol is characterized by a pleasant aromaticsmell and is known for its antimicrobial, antiseptic and antioxidant properties. This variation in component level is significant, but not impossible because chemical polymorphism is significantly characteristic of many Labiatae family species [14].

The major components in both Turkish and Lebanese *S. myrtifolia* oils were different. The

Table 2. Comparison of main essential oil constituents in *S. myrtifolia* aerial parts from two different geographic origins (Lebanon and Turkey)

Compound	Lebanon[a]	Turkey[b]
β-Caryophyllene	10.65	42.56
Patchoulane	-	8.69
Germacrene-D	-	7.00
δ-Cadinene	0.88	6.99
α-Humulene	-	3.01
Nerolidol	-	2.60
α-Selinene	-	2.32
α-Copaene	-	2.30
α-Murolene	-	1.03
Tricosane	13.65	-
Tetracosane	12.31	-
Caryophyllene oxide	10.65	-
Thymol	9.65	-
Docosane	7.65	-
Heneicosane	5.99	-
Nonadecane	4.32	-
Pentacosane	3.88	-
Hexacosane	3.88	-
Hexadecanoic acid	4.21	-
Limonene	1.66	0.29

Notes: [a]Our study; [b]Ozek et *al.*

odor of these compounds was not similar. Indeed, the absence of thymol compound in Turkish oil which has astronger, spicy and woody smell, suggests that the odor perception of *S. myrtifolia* oil reported from Turkey is different than that of Lebanese oil. However, five major volatile compounds (germacrene-D; δ-cadinene; patchoulane; α-humulene and nerodiol) were not present in the Lebanese oil. These compounds have woody, spicy, smoky, flowery, herbal, green, waxy, floral and citrusodor (Table 1). A remarkable quantitative and qualitative difference exists between both oils (Table 2). These differences may be dependent on climatic conditions prevailing during plant development and their genetic potential [7,8,9]. Indeed, the composition of essential oils as well as other chemical molecules of plants may vary considerably depending on annual climatic changes and genotype [15,16]. These parameters influence the total content of essential oils and their compositions. Unfortunately, weather conditions were not reported in studies performed in Turkey.

Conclusion

The present study contains some new information and gives a significant amount of information about the essential oil chemical composition of *S. myrtifolia* species from Lebanon geographical origin. Our study highlighted the differences in essential oils composition by comparison with the unique study performed up till now. Nevertheless, this comparison may gain in precision if the same analytical methods are used. Further investigations are necessary to ascertain our results and therefore could help for *S. myrtifolia* genetic programs improvement and may be to diversify the industrial uses.

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