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VARIATION OF ESSENTIAL OIL COMPOSITION OF *Myrtus communis* (MYRTACEAE) FROM MONTENGERO

SUMMARY

This study describes the qualitative and quantitative analysis of the essential oil species *Myrtus communis* L. from many different locations. The plant samples were collected from six locations of the Montenegrin coast (Ulcinj, Bar, Budva, Tivat, Kotor, and Herceg Novi). The quantity of the myrtle essential oil for samples from the locations Ulcinj (0.72%) and Herceg Novi (0.8%) are in accordance with the data from the literature (Boelens and Jimenez 1992; Bradesi et al., 1997), while the derived quantities for different locations are notably below previously mentioned values (0.4-0.53%). The analysis of the essential oil from six locations was completed with the help of GC-MS. By analysing results of the chemical composition of the myrtle essential oil from six different locations of the Montenegrin coast, we are able to come to conclusion that there are no main differences in qualitative composition. The main differences were found in the content of Myrtenyl acetate and α-Pinene. Regarding the content of these two compounds, the sample from the southernmost location of the Montenegrin coast (Ulcinj) is expressed most, and it can be classified into distinct chemotype with a high content of Myrtenyl acetate (21.64%) and a small content of α-Pinene (14.67%). The sample from the location Budva is distinctive for somewhat smaller content of Myrtenyl acetate (2.66%). The components from the class of oxidized monoterpenes are dominant in all samples: 1,8 – cineole (18.81-25.68%), linalool (10.05-16.13%), Myrtenyl acetate (2.66-21.64%), and from the class of monoterpene hydrocarbons, it is the α-Pinene that stands out as quantitatively the most common component (14.67-36.77%).

Keywords: *Myrtus communis* L., essential oil, myrtenyl acetate, α-pinene, oxidized monoterpenes, chemotype

INTRODUCTION

Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction but the most commonly used method for commercial production is steam distillation.

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An estimated 3000 essential oils are known, of which 300 are commercially important in fragrance market (Van de Braak and Leijten,1999.).

Essential oils are complex mixers comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Each of these constituents contributes to the beneficial or adverse effects. Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties (Burt, 2004, Kordali et al., 2005, Bakkali et al., 2008). Some oils have been used in cancer treatment (Sylvestre et al., 2006). Some other oils have been used in food preservation (Faid et al., 1995), aromatherapy (Buttner et al., 1996) and fragrance industries (Van de Braak and Leijten, 1999). Essential oils are a rich source of biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils (Milhau et al., 1997). Therefore, in these oils it is reasonable to expect, a variety of plant compounds with specific, as well as general antimicrobial activity and antibiotic potential (Darokar et al., 1998).

Myrtle (*Myrtus communis* L.) is an evergreen shrub belonging to the Myrtaceae family. Myrtus, the Greek name for Myrtle and communis means common plant growing in groups. It grows spontaneously throughout the Mediterranean area and has been used for medicinal, food and spice purposes since ancient times. The leaves and fruit are traditionally used as antiseptic, disinfectant, and hypoglycemic agents. In folk medicine the fruit of the plant is used in the treatment of various infectious diseases, including diarrhea and dysentery, whereas the leaves are used as antiseptic and anti-inflammatory agents, as a mouthwash, for treatments of candidiasis, for healing wounds, as well as in the therapy of urinary diseases (Baytop, 1999.). A striking feature of the plant is the pleasant smell of its essential oil, present in numerous glands, especially in the leaves. The main compounds responsible for the flavour and scent of myrtle oil are monoterpenes: 1,8-cineole, myrtenyl acetate, α-pinene, myrtenol, limonene, etc. The oil composition is highly influenced by the geographic origin of the plant (Lawrence, 1997). According to the numerous published papers on the topic, myrtle essential oil possesses strong antimicrobial activity that makes it a valuable raw material for the cosmetic, pharmaceutical and foodstuff industries. Myrtle has been shown to have antioxidant, anti-inflammatory, antimicrobial, insecticidal and apoptostic activities (Garry et al., 1998, Mahboubi and Ghazian, 2010, Sumbul et al., 2010, Mimic Dukić et al., 2010, Amira et al., 2012).

MATERIAL AND METHODS

Plant material and chemicals

Plant material: For essential oil analysis leaves were collected from the trees of *Myrtus communis* L. 1753 subsp. *tarantina* growing on five locations at Montenegro coastline: Ulcinj (No 2-1818), Bar (No 2-1819), Budva (No 2-1820), Tivat (No 2-1822), Kotor (No 2-1821), and Herceg Novi (No 2-1823), in August 2006. Voucher specimens were prepared and identified by Goran Anačkov, PhD, and deposited at the Herbarium of the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad.

Essential oil isolation and analysis

Essential oils isolation: Air-dried plant materials were submitted to hydro-distillation according to Eur. Pharm. 4 (European Pharmacopeia, 4th ed. 2002), using n-hexane as a collecting solvent. The solvent was removed under vacuum, and the quantities of the essential oils were determined gravimetrically.

GC-MS analysis of essential oil: Qualitative analysis of essential oils was performed by gas chromatography-mass spectrometry (GC-MS). Agilent Technologies 6890N-5975B system was used, with data acquisition parameters as follows: carrier gas - He, flow rate 1.0 mL/min, constant flow mode; injection volume 0.2 μL (split 50:1), inlet temperature 250°C; Agilent Technologies HP-5MS 30 m × 0.25 mm × 0.25 μm column, temperature program: 50°C for 1 min, 5°C/min to 100°C, 9°C /min to 200°C, hold 7.89 min; transfer line temperature 280°C; electron ionization, electron energy 70 eV, scan mode, mass range 35-400 Da, quadruple temperature 150°C, source temperature 230°C. Acquired data were analysed by Agilent Technologies MSD ChemStation software in conjunction with AMDIS (Automated Mass Spectral Deconvolution and Identification System) and NIST MS Search software. Two different mass spectra libraries were used for mass spectra identification: Wiley Registry of Mass Spectral Data 7th Edition (338000 spectra, 289000 unique compounds) (Wiley Registry of Mass Spectral Data, 2005), NIST/EPA/NIH Mass Spectral Library 05 with 190825 spectra, 163198 unique compounds (NIST/EPA/NIH Mass Spectral Library with Search Program, 2005). Identity is confirmed by comparison of Kovat`s retention indices.

RESULTS AND DISCUSSION

The amount of essential oil obtained by hydro-distillation from the dried leaves is presented in Table 1. The oil quantity ranged from 0.4 % (Budva) up to 1.59% (Herceg Novi).

Table 1. Essential oil content (%) in dried leaves of *Myrtus communis* L. collected from five locations from Montenegro coastline.

Sample	Ulcinj	Bar	Budva	Tivat	Kotor	H. Novi
Oil Content %	0.72	0.51	0.4	0.5	0.53	0.8

The value of the quantity of the myrtle essential oil in the examined samples corresponds to the data from the literature (Boelens, M., Jimenez, R., 1992, Bradesi et al., 1997), for the samples from the locations Ulcinj and Herceg Novi (0.8%), while the quantity of oil for the other locations are below this value. (0.40-0.53%). It is interesting to mention that the northernmost location (Herceg Novi) and the southernmost location (Ulcinj) have approximately the same value of the quantity of essential oil. It is also notable that the quantity of essential oil gradually decreases from the south and north of the Montenegrin coast to the

central part (Budva) where it reaches the lowest value (0.40%).

The obtained results confirm that the amount of the essential oil of the certain plant species can be affected by numerous factors such as the time of collection of plant material, the way of processing plant material, the way of oil insulation, as well as the great number of ecological factors such as type of soil, insolation, humidity etc. (Hefendehl and Murray, 1978, Letchamo et al., 1995).

As can be seen from the picture, the quantity of the oxidized monoterpenes in the myrtle essential oils is approximately the same at many locations and it ranges from 48.92-61.6%, except at the location Ulcinj where significantly higher value was recorded (78.39).

The quantity of monoterpenes hydrocarbons in the examined samples of the myrtle essential oils from most locations of the Montenegrin coast is approximately the same and it ranges from 35.5-45.2%, except at the location Ulcinj where significantly smaller value was recorded (17.75%).

The compounds from the class of monoterpenes are not the only present. In the examined samples of the myrtle essential oil, the sesquiterpenoids are also present, but in significantly smaller amount. Among present sesquiterpens compounds, the sesquiterpenic carbohydrates are dominant (1.9-2.5%) while the oxidized sesquiterpens are present in minimal amount (0.00-0.85%).

The aliphatic compounds in the myrtle essential oils are present in very small amounts (0.37-1.92%). The amount of the aliphatic compounds in the myrtle essential oils is approximately equal at most of the examined locations, except at the location Ulcinj where found value was significantly higher.

By analysing the presented results of chemical content of myrtle essential oils from six locations of the Montenegrin coast, it can be concluded that there are no significant differences in qualitative composition. The main differences between the locations are concerning the ratio between Myrtenyl acetate and α-Pinene. In regard to the essential oil from other locations, the myrtle essential oil from location Ulcinj is characteristic because of the high content of Myrtenyl acetate (21.64%), and significantly smaller content of α-Pinene (14.67%). Besides that, it is important to mention that the sample of the myrtle essential oil from this location is characterized by the significantly greater contents of the oxidized monoterpenes and the aliphatic compounds compared to the other samples, while the amount of the monoterpene carbohydrates is significantly smaller. Having all this in mind, the myrtle essential oil from this location is put in a special chemotype.

The sample from the location Budva is characteristic because it had the significantly smaller amount of Myrtenyl acetate (2.66%). In summary, in all samples of the myrtle essential oil the components from the class of oxidized monoterpenes are dominant: 1,8 – cineole (18.81-25.68%), linalool (10.05-16.13%), Myrtenyl acetate (2.66-21.64%), and from the class of monoterpene hydrocarbons it is the α-Pinene that stands out as quantitatively the most common component (14.67-36.77%).

Table 2. Percentage content of volatile compounds in essential oil of the leaves from examined *Myrtus communis* L. from Montenegro.

	Compound	KI[a]	Ulcinj	Bar	Budva	Tivat	Kotor	H. Novi
1	Isobutyl isobutyrate	914	1.89	0.66	0.72	0.67	0.37	0.70
2	α-Thujene	927	0.37	0.45	0.35	0.33	0.30	0.38
3	α-Pinene	934	14.67	23.06	36.77	34.86	33.63	35.86
4	2-β-Pinene	978	0.16	0.22	0.22	0.23	0.25	0.32
5	β-Myrcene	992	0.31	0.30	0.18	0.19	0.24	0.22
6	β-Phellandrene	1006	0.40	0.51	0.24	0.31	0.30	0.32
7	δ-3-Carene	1012	0.48	0.56	0.40	0.41	0.31	0.41
8	α-Terpinene	1018	0.25	0.25	0.16	0.25	0.17	0.24
9	p-Cymene	1026	0.86	1.52	0.69	0.67	0.69	1.15
10	Limonene	1030	4.10	8.30	5.64	3.37	2.78	4.47
11	1,8-Cineole	1032	25.68	21.86	25.46	22.42	18.81	23.91
12	(E)-β-Ocimene	1049	0.35	0.84	0.28	0.28	0.49	0.32
13	γ-Terpinene	1060	0.92	0.88	0.62	0.68	0.61	0.71
14	α-Terpinolene	1090	1.05	0.98	0.70	0.77	0.70	0.76
15	Linalool	1101	10.05	14.04	11.48	11.04	16.13	10.85
16	4-Terpineol	1182	0.28	0.41	0.23	0.27	0.23	0.32
17	Cryptone	1191	tr	0.34	tr	tr	tr	0.16
18	α-Terpineol	1189	3.07	2.98	2.81	3.15	2.64	2.78
19	Myrtenol	1200	0.79	0.80	0.52	0.77	0.49	0.62
20	Geraniol	1256	2.55	3.03	1.82	1.98	2.89	1.61
21	Myrtenyl acetate	1325	21.64	9.17	2.66	8.13	8.58	5.35
22	α-Terpineyl acetate	1355	1.36	0.85	0.48	0.62	0.76	0.53
23	Neryl acetate	1367	0.27	0.24	0.16	0.17	0.24	0.16
24	Geranyl acetate	1385	3.37	2.71	2.29	2.51	2.64	2.32
25	Methyl eugenol	1406	0.84	1.24	1.32	1.14	1.23	1.04
26	(E)-β-Caryophyllene	1420	0.61	0.64	0.67	0.61	0.91	0.52
27	α-Humulene	1460	1.54	1.23	1.56	1.60	1.56	1.29
28	Bicyclogermacrene	1500	tr	tr	tr	tr	tr	0.20
29	Spathulenol	1593	tr	0.54	tr	tr	0.09	0.84
	Total identified		**98.52**	**98.73**	**98.73**	**98.14**	**98.45**	**98.68**

[a] Retention indices relative to C9-C24 n-alkanes on the HP 5MS column. GC, identification based on retention times of authentic compounds on HP 5MS column; MS, tentatively identified on the basis of computer matching of the mass spectra of peaks with the NIST/NBS and Wiley libraries; tr - ratio in essential oil below 0.1%; n.i non identified.

Table 3. The basic classes of compounds (%) in essential oil of *Myrtus communis* L. from the examined locations.

Compound	Ulcinj	Bar	Budva	Tivat	Kotor	H.Novi
Oxygenized monoterpenes	73.39	61.5	48.92	54.95	57.33	52.12
Monoterpene hydrocarbons	17.75	35.5	45.2	41.39	39.28	43.95
Sesquiterpene hydrocarbons	0	0.55	0	0	0.09	0.85
Oxygenated sesquiterpenes	2.18	1.9	2.26	2.25	2.5	2.03

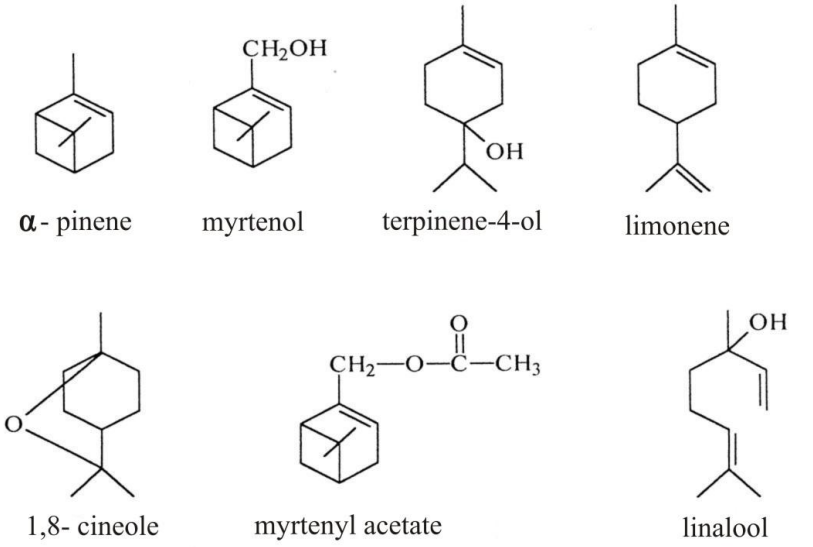

Figure 1. Molecular structures of major compounds in *Myrtus communis* L. essential oil

Statistical Analysis

The t-test was used for statistical analysis. The significance was tested at the $p < 0.05$ level. Values in Tables are averages with standard errors. In experiments, we calculated the percentage of inhibition of mutagenesis (%I) as described by Wall et al., 1988.

CONCLUSION

GC-MS analysis of the essential oil *Myrtus communis* L. from six different locations of the Montenegrin coast shows that there is a significant difference in chemical content of the oil between the location Ulcinj and other locations (Bar, Budva, Tivat, Kotor and Herceg Novi). The myrtle essential oil from the location Ulcinj contains significantly greater amount of Myrtenyl acetate (21.64%) in

comparison to the essential oils from other locations (2.66-9.17%), while the quantity of α-Pinene (14.67%) is significantly smaller (23.06-36.77%), which puts it in a special chemotype. There were also significant differences in the amount of the essential oil between the examined locations and they range from 0.4-0.8%. This is the first report about the chemical composition of the myrtle essential oil from Montenegro.

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