GC-MS ANALYSIS OF THE ESSENTIAL OIL OF
SATUEREJA SUBSPICATA BARTL. EX VIS. OF MOLDOVAN ORIGIN

Ion Dragalina*, Aculina Aricua, Nina Ciocarlanb, Alexandru Ciocarlanac, Victoria Coditaac

*Institute of Chemistry, Academy of Science of Moldova, 3, Academiei str., Chisinau MD 2028, Republic of Moldova
bBotanical Garden (Institute) of Academy of Sciences of Moldova,18, Padurii str., Chisinau MD 2002, Republic of Moldova
tiraspol State University, 5, Gh. Iablocicin str., Chisinau MD 2069, Republic of Moldova
*e-mail: iondragalin@yahoo.com, phone: (+37322) 739769

Abstract. For the first time the results of GC-MS analysis of Satureja subspicata L. oil of Moldovan origin are reported. The chemical profile includes forty-four constituents and consists mostly (97.86%) of phenolic monoterpenes, monoterpenic hydrocarbons, bicyclic sesquiterpenes and their oxygenated derivatives. A substantial quantitative and qualitative chemical differentiation of S. subspicata oil of Moldovan origin and reported oil of Croatian origin were found. The essential oil of S. subspicata plants cultivated in Republic of Moldova belongs to the carvacrol chemotype.

Keywords: Satureja subspicata L., essential oil, chemical profile, GC-MS analysis.

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Introduction
Numerous studies have been conducted on Satureja L. species, growing in different world regions, particularly on its volatile oil composition. According to these data, essential oils from Satureja species have a very complex composition, which includes phenolic monoterpenes, monoterpenic hydrocarbons, bicyclic sesquiterpenes, other terpene derivatives and flavonoids [1-4].

A wide range of biological activities have been reported for different species of Satureja L.: anti-inflammatory [5], anti-HIV [6], anti-diabetic, antioxidant, antimicrobial and anti-hyperlipidemic [7], antifungal [8], antispasmodic and anti-diarrheal [9], vasodilatory [10] and cytotoxic [11].

The present studies are related to Satureja subspicata Bartl. ex Vis. cultivated in the Botanical Garden (Institute) of Academy of Science of Moldova (ASM), Collection of Medicinal Plants. Climate of the Republic of Moldova is favourable for S. subspicata, where plants have normal growth rhythms and successfully attain the generative period.

Up to date a few reports on essential oil composition and biological activity of S. subspicata species have been published. The volatile oil of S. subspicata possesses a big part of activities mentioned before, and is therefore a potential source of antimicrobial ingredients for the food and pharmaceutical industry.

The aim of this work is to reveal the chemical composition of S. subspicata essential oil cultivated in climatic and soil conditions specific for Republic of Moldova by means of GC-MS analysis.

Experimental
Materials
The plant material Satureja subspicata of Moldovan origin (aerial parts with inflorescence) for the chemical analyses was collected during flowering stage in July 2016 from experimental fields of the Botanical Garden (Institute) of ASM. The plants were cultivated in ecological conditions and without fertilizer use. Voucher specimen is deposited in the Herbarium of the Botanical Garden (Institute) of ASM.

Methods
Essential oil of S. subspicata was obtained by hydrodistillation (600 mL) of the aerial parts of the plant (200 g) for 2 hours in a Clevenger apparatus. After phase separation, volatile oil was dried over anhydrous sodium sulphate, and then used for chromatographic measurements.

The GC-MS analysis of the S. subspicata essential oil was carried out on an Agilent Technologies 7890A system with 5975C Mass-Selective Detector (GC-MSD) equipped with split-splitless injector (split, 250°C, split ratio 1:50, 1 μL) and HP-5 ms capillary calibrated column (30 m x 0.25 mm x 0.25 mm); The carrier gas: helium 1.1 mL/min; oven: 70°C-2 min, 5°C/min-200°C-20 min-300°C-5 min; MSD in scan 30-300 amu, 15 min, 30-450 amu, solvent delay 3 min.

Results and discussion
By hydrodistillation of plant material a whitish coloured volatile oil (nD20 = 1.5020) was obtained. The GC-MS analysis of S. subspicata oil resulted in identification of 44 components (see Figure 1 and Table 1). These components represent 97.86 % of the oil and were identified by comparison of the acquired mass spectra with those from apparatus database. The mass spectrum of the major component carvacrol (RT=13.54) is depicted in Figure 2.
According to GC-MS analysis, the most abundant component of *S. subspicata* essential oil is phenolic terpene carvacrol **1** (47.56%), followed by monoterpane hydrocarbons *p*-cimen **2** (6.94%) and *γ*-terpinene **3** (5.54%), alcohols nerol **4** (2.15%), aldehydes (E)-citral **5** (7.23%) and (Z)-citral **6** (5.73%) (Figure 3, Table 1); sesquiterpenes (+)-β-bisabolene **7** (5.80%), β-caryophyllene **8** (2.61%), germacrene B **9** (1.78%) and germacrene D **10** (1.42%) (Figure 4, Table 1). The reported compounds accounted for 97.86% of total content of the essential oil.
It must be mentioned, that the *S. subspicata* essential oil of Moldovan origin showed significant difference in qualitative and quantitative chemical composition in comparison with the reported from native area. *S. subspicata* essential oil of Croatian origin contains carvacrol (16.76%) as major constituent, followed by α-pinene (13.58%), *p*-cymene (10.75%) and γ-terpinene (9.54%) [12]. According to Dunkić V. *et al*., the major component of the essential oil of two *S. subspicata* subspecies (*S. subspicata* ssp. *subspicata* and *S. subspicata* ssp. *liburnica*) is monoterpenic hydrocarbon α-pinene (yield 52.9% and 42.6%, respectively) and it means that both taxa belong to α-pinene chemotype [13]. The carvacrol content (16.8% and 13.5%, respectively) reported by these authors is three times smaller than its content in *S. subspicata* oil of Moldovan origin.

Another team of researchers reported α-pinene (24.2%) as main compound of the essential oil from wild-grown *S. subspicata* in the Mediterranean part of Croatia, while carvacrol constitutes only 2.7% [14]. The *S. subspicata* species originating from Bosnia and Herzegovina contain thymol (28.6%) and carvacrol (27.9%) as main compounds of their essential oils [15].

### Table 1

| No | RT* (min) | Component | %     | No | RT* (min) | Component | %     |
|----|-----------|-----------|-------|----|-----------|-----------|-------|
| 1  | 3.434     | Isoamyl acetate | 1.98  | 23 | 9.885     | Borneol    | 0.17  |
| 2  | 4.344     | Origanene   | 0.36  | 24 | 10.271    | 1-Terpinen-ol | 0.39 |
| 3  | 4.496     | α-Pinene    | 0.14  | 25 | 10.360    | (Z)-Verbenol | 0.34 |
| 4  | 4.863     | Isocumene   | 0.06  | 26 | 10.624    | α-Terpineol | 0.08  |
| 5  | 5.018     | 1-Ethyl-3-methylbenzen | 0.31 | 27 | 10.787    | Pulegone   | 0.10  |
| 6  | 5.270     | Sabinene    | 0.04  | 28 | 11.038    | Myrtenol   | 0.22  |
| 7  | 5.316     | 1-Octen-3-ol | 0.25  | 29 | 11.577    | Nerol      | 2.15  |
| 8  | 5.502     | Heptenone   | 0.10  | 30 | 11.924    | (Z)-Citral | 5.73  |
| 9  | 5.581     | β-Myrcene   | 0.59  | 31 | 11.999    | Isothymol methyl ether | 0.46 |
| 10 | 5.969     | 1,2,3-Trimethylbenzen | 0.23 | 32 | 12.271    | Geraniol   | 0.28  |
| 11 | 5.928     | α-Phellandrene | 0.09  | 33 | 12.704    | (E)-Citral | 7.23  |
| 12 | 6.202     | α-Terpineol | 0.63  | 34 | 13.252    | Tymol      | 0.17  |
| 13 | 6.383     | p-Cymene    | 6.94  | 35 | 13.545    | Carvacrol  | 47.56 |
| 14 | 6.489     | Limonene    | 0.73  | 36 | 15.729    | α-Bourbonene | 0.07 |
| 15 | 6.564     | Eucalyptol  | 0.14  | 37 | 16.607    | β-Caryophyllene | 2.61 |
| 16 | 7.198     | γ-Terpinene | 5.54  | 38 | 17.447    | α-Bisabolene | 0.13 |
| 17 | 7.428     | Terpinene-4-acetate | 0.09 | 39 | 18.122    | Germacrene D | 1.42 |
| 18 | 8.202     | Linalool    | 0.16  | 40 | 18.498    | Germacrene B | 1.78 |
| 19 | 8.370     | β-Pinene oxide | 0.10 | 41 | 18.735    | (+)-β-Bisabolene | 5.80 |
| 20 | 8.832     | (E)-Sabine hydrate | 0.11 | 42 | 19.535    | α-Caryophyllene | 0.21 |
| 21 | 9.261     | Myrtanal    | 0.19  | 43 | 20.416    | (−)-Spatulenol | 0.36 |
| 22 | 9.533     | 2,2-Dimethylocta-3,4-dienal | 0.42 | 44 | 20.555    | Caryophyllene oxid | 0.40 |

*RT - retention time.
The list of secondary components of essential oils of Moldovan and Croatian origin, correspondingly, is comparable but differs quantitatively and includes: α-pinene, limonene, α-terpinene, thymol, linalool, β-myrcene, limonene, geraniol and others. In contrast with essential oil of Croatian origin, the studied by us oil is characterized by high content of unreported before components like γ-terpinene, (E)- and (Z)-citral, β-bisabolene, nerol, β-caryophyllene, germacrenes (B and D) and others. As a result, biological activity, pharmaceutical and perfumery value of S. subspicata oil of Moldovan origin can be much higher.

Conclusions

The quantitative and qualitative chemical differentiation of essential oil obtained from S. subspicata plants growing in Moldova and Croatia can be correlated with different geographic location and ecological conditions. The high content of the phenolic terpene carvacrol as a main component (47.56%) suggests that S. subspicata plants cultivated in Republic of Moldova belong to a new high yielding carvacrol genotype.

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