Scientific Bulletin. Series F. Biotechnologies, Vol. XXI, 2017
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

DRY AND FRESH HERBA OF *Satureja montana* L.: A COMPARATIVE STUDY REGARDING CHEMICAL COMPOSITION AND ANTIOXIDANT CAPACITY OF VOLATILE OILS

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Abstract

Mountain or winter savory Satureja montana L. is one of the most cultivated aromatic plant from Lamiaceae family. As most of the species from their genus, Satureja montana L. contains essential oils which differ in chemical composition as plants are from different origin or has been dried in different regime. This study has been shown that chemical composition and antioxidant capacity are different for dry and fresh herba and even has been varying if the herba was dried in air or in the oven. It has been demonstrated that the major compound, carvacrol, of volatile oils from dry herba was found to be in higher percent than in volatile oils extracted from fresh herba. In contrast, different sesquiterpenes concentrations were higher for fresh herba. The antioxidant capacity was two times bigger for the oil extracted from dry herba. This surprising feature could be explained by the higher carvacrol concentration and lower thymol concentration determined in volatile oil extracted from dry herba.

Key words*: Satureja montana L., volatile oils, carvacrol, fresh and dry herba.*

INTRODUCTION

Since ancient times, aromatic herbs and spices, rich in volatile oils, have been used as additives because of their ability to improve flavor and food-preservation properties. Today, they are not only valuated for improvement of organoleptic properties, but also for their nutritive and medicinal benefits (Cavar et al., 2008; Vidovic et al., 2014) as well as for their potential in commercial exploitation in various fields such as aroma and flavor enhancers, cosmetics and pharmaceutic products (Mirjana and Nada, 2004).

One of the most important families of medicinal plants is *Lamiaceae*, which consists of over 3000 species (Vladic et al., 2016). The genus *Satureja* belongs to the *Lamiaceae* family, and comprises over 30 herbs, sub-shrubs, and shrubs (Cavar et al., 2008; Garcia-Rellan et al., 2015; Kremer et al., 2015).

Satureja montana L., commonly called winter or mountain savory, is a bushy perennial subshrub with woody stems at the base, small linear leaves, pale pink and white flowers (Chizzola, 2003; Wesolowska et al., 2014). *Satureja* species are used widely as a flavoring agent of food products, and as traditional herbal medicine for the treatment of gastrointestinal disorders (antispasmodic and antidiarrhoeal) (Marin et al., 2012).

Because of its preference for dry climatic conditions, *S. montana* has developed several morphological and physiological adaptations, effecting oil yields and composition (Mirjana and Nada, 2004).

Most of the *Satureja* species contain essential oil in all their aerial botanical parts. The yield of volatile oils obtained by hydro-distillation of aerial parts of those plants species have been found between 0.3 - 5%. Volatile oils are rich in aromatic monoterpenes such as carvacrol, thymol, p-cymene, β-caryophyllene, γ-terpinene and linalool, which are responsible for the characteristic smell and taste (Chizzola, 2003; Ciulei, 1993; Jafari et al., 2016) and give the oil certain biological properties such as antibacterial, fungicidal, antiviral, antioxidant,

antispasmodic and antidiarrhoeal (Mirjana and Nada, 2004). Flavonoids, tannins, acids and exudates are other known compounds of *Satureja* species (Jafari et al., 2016).
The aim of this study was to determine the difference between chemical composition and antioxidant capacity of fresh and dry herba of *S. montana* L. harvested in 2016: summer (July) and late autumn (November).

MATERIALS AND METHODS

Plant material

Plants of *Satureja montana* L. was gathered in July and November 2016, from the Young Naturalists Station 21°13' E longitude, 45°45' N latitude, from Timisoara county.
The aerial parts harvested in July were left to air-dry in a well-ventilated room for at least 15 days before packaging in paper bags, and stored until extraction at room temperature.
Aerial parts harvested in November were divided in two parts: one was processed fresh and the other one was dried at 35°C for 7 days using a drying oven (Model FD23, Binder, Germany).

Essential oil extraction

For the oil extraction, dried leaves and flowers were subjected to hydro-distillation using a 1L Clevenger apparatus. The extraction yields have been 2% for dry leaves and 0.5% for fresh herba.

Gas Chromatography/Mass Spectrometry (GC/MS) analyses of essential oils

The separation and identification of different compounds had been done using a GC-MS system Shimadzu 2010 Plus gas chromatography apparatus (Shimadzu, Kyoto, Japan) and triple quadrupole mass spectrometer (TQ 8040). The column used was a capillary column 1MS Accent (30 m length; 0.25 mm i.d.; 0.25 µm film thickness, Macherey-Nagel, Duren, Germania) with helium as the carrier gas at 0.83 L min^{-1}. The oven temperature was programmed at 70 ^{o}C for 11 min, then 5 ^{o}C/min to 190 and 20 ^{o}C/min to 240 ^{o}C, and then left at 240 ^{o}C for 5 min. The injector temperature and MS source were maintained at a temperature of 250 ^{o}C and 200 ^{o}C, respectively. Identification of different compounds has been done based on their mass spectra using NIST 14 library and Willy 09 library. Retention indices (RI) were calculated for separate compounds relative to C4-C26 *n*-alkanes mixture.

Antioxidant assays

For this assay a spectrophotometric analysis was performed using as reference a relatively stable organic radical DPPH (2.2-diphenyl-1-picrylhydrazyl). This assay is based on the ability of the antioxidant to scavenge the radical cation DPPH. Results were expressed as Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent antioxidant capacity (TEAC) using a Trolox calibration curve (Tuberoso et al., 2007).
Antioxidant capacity was determined for the essential oils. Different quantities of oils were mixed with 0.5 mL of 0.4 mM DPPH solution in methanol as presented in (Arsenijevic et al., 2016).
The spectrophotometric readings were carried out after a 1-hour period of incubation, in the dark, at room temperature, with a ScanDrop Nano-volume Spectrophotometer from AnalytikJena (Germany) at 517 nm using a 10 mm quartz cuvette. A Trolox calibration curve in the range 0.02 – 4.00 mM was prepared, and data were expresses in Trolox equivalent antioxidant capacity (TEAC, mmol/l).

RESULTS AND DISCUSSIONS

Essential oil composition

The essential oil extraction yields differ between fresh herba and dry herba. For fresh herba the yield has been 0.5%, while for dry herba has been 2%.
The chemical composition of volatile oils has been determined using gas-chromatography coupled with mass spectrometry techniques. The chromatogram for volatile oils extracted from dry herba is shown in Figure 1.

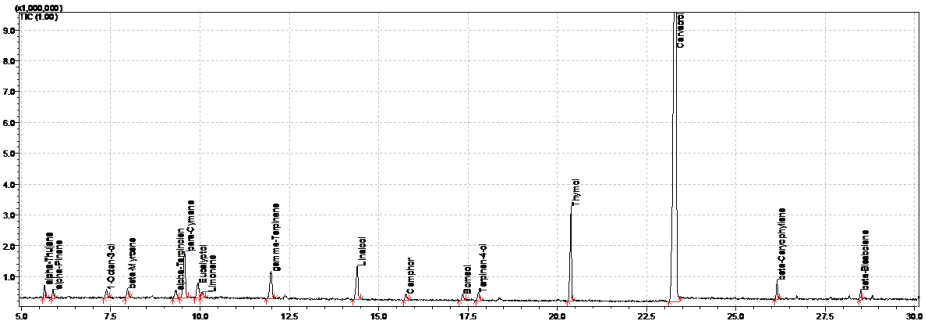

Figure 1. The composition of essential oil from dry herba of *Satureja montana* L.

It has been shown that in the composition of essential oils extracted from dry herba of *Satureja montana* L. the main component is carvacrol (an oxygenated phenolic monoterpenoid) at a level of 72% from all identified compounds (Table 1 for all chemical composition). Usually, the amount of carvacrol in *Satureja montana* L. essential oil varied from 15.19% to 69.99% (Mihajilov-Krstev et al., 2014; Mirjana and Nada, 2004; Skocibusic and Bezic, 2003; Wesolowska et al., 2014).

Table 1. Chemical composition of *Satureja montana* L. volatile oils

Compound	Retention time (s)	Dry herba	Dried in oven herba	Fresh herba
α-Thujene	5.666	0.8	1.32	1.58
α-Pinene	5.899	0.63	0.97	0.63
1-Octen-3-ol	7.391	0.71	1.42	0.78
β-Myrcene	7.994	0.84	0.61	0.94
α-Terpinolen	9.34	1.05	1.04	0.5
para-Cymene	9.6	5.12	15.17	10.57
Eucalyptol	9.923	1.72	0.44	0.04
Limonene	10.006	0.67	0.11	0.17
γ-Terpinene	12.016	3.03	1.68	2.19
Linalool	14.387	3.01	1.05	0.45
Camphor	15.726	0.62	0.07	0.16
Borneol	17.332	0.5	0.33	0.19
Terpinen-4-ol	17.795	0.81	1.13	0.94
Thymol	20.407	6.68	10.23	18.92
Carvacrol	23.286	71.95	61.87	59.46
β-Caryophyllene	26.163	1.24	2.27	2.12
β-Bisabolene	28.501	0.62	0.29	0.36

Such high content of carvacrol has been found in plants harvested in temperate climate. Interesting, the composition of carvacrol was higher in volatile oils obtained from dry herba compared with that obtained from fresh or dried in the oven herba (Table 1). In contrast, other minor components as beta-caryophyllene, thujene or terpinen-4-ol were higher for fresh herba.

Furthermore, the concentration of thymol was 3 times higher in fresh herba than dry herba (Table 1, Figure 2).

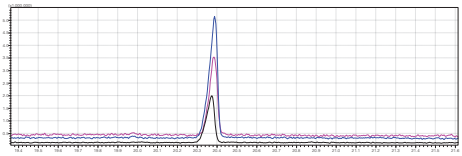

Figure 2. The chromatographic peak for Thymol from essential oils of dry (black), dry in the oven (pink) and fresh (blue) herba of *Satureja montana* L.

Antioxidant capacity

The antioxidant capacity of volatile oils extracted from different herba has been determined as Trolox equivalent antioxidant capacity (TEAC, mmol/l) and relative inhibition to sample without plant extract (Table 2).

Table 2. Antioxidant capacity of volatile oils extracted from *Satureja montana* L.

Antioxidant capacity	Dry herba	Dry herba in oven	Fresh herba
TEAC, mmol/l	0.65	0.78	1.27
% inhibition	18.18	21.82	35.27

All type of oils have quite high antioxidant capacity. The higher antioxidant capacity of all volatile oils has been determined for the oil extracted from dry herba. This surprising effect could be explained by higher concentration of carvacrol which has been shown to have such properties in other plants volatile oils (Ang et

al., 2015). The tymol concentration was lower for dry herba which could be another reason of a better antioxidant capacity exhibited by this oil.

CONCLUSIONS

The present research had shown the difference in chemical composition and antioxidant capacity of volatile oils extracted from dry and fresh herba. The chemical composition of volatile oils is similar but the concentration of the major compound, carvacrol, is higher in dry herba, which determine higher antioxidant capacity.
More studies are needed to understand if really just one of the compound contained in the essential oil is responsible for the antioxidant capacity and which other compound/s could prime or inhibit its activity.

ACKNOWLEDGEMENTS

Funding for this study has been provided by The European Commission and The Romanian Government (project POSCCE 621/2014).

REFERENCES

Ang, L.Z.P., Hashim, R., Sulaiman, S.F., Coulibaly, A.Y., Sulaiman, O., Kawamura, F., Salleh, K.M., 2015. In vitro antioxidant and antidiabetic activites of Gluta torquata. Industrial Crops and Products 76: 755-760.

Arsenijevic, J., Drobac, M., Sostaric, I., Razic, S., Milenkovic, M., Couladis, M., Maksimovic, Z., 2016. Bioactivity of herbal tea of Hungarian thyme based on the composition of volatiles and polyphenolics. Industrial Crops and Products 89: 14-20.

Cavar, S., Maksimovic, M., Solic, M.E., Jerkovic-Mujkic, A., Besta, R., 2008. Chemical composition and antioxidant and antimicrobial activity of two Satureja essential oils. Food Chemistry 111: 648-653.

Chizzola, R., 2003. Volatile oil composition of four populations of Satureja montana L. from Southern France, in: Duzyaman, E., Tuzel, Y. (Eds.), Proceedings of the International Symposium on Sustainable Use of Plant Biodiversity to Promote New Opportunities for Horticultural Production Development, pp 143-147.

Ciulei, I.E., Grigorescu; Ursula, Stănescu, 1993. Plante medicinale, fitochimie și fitoterapie - Tratat de farmacognozie. Editura medicală, Bucureşti.

Garcia-Rellan, D., Blazquez, M.A., Boira, H., 2015. Differential Essential Oil Composition and Morphology between Perennial Satureja species Growing in Spain. Records of Natural Products 9: 623-627.

Jafari, F., Ghavidel, F., Zarshenas, M.M., 2016. A Critical Overview on the Pharmacological and Clinical Aspects of Popular Satureja Species. Journal of Acupuncture and Meridian Studies 9: 118-127.

Kremer, D., Kosir, I.J., Koncic, M.Z., Cerenak, A., Potocnik, T., Srecec, S., Randic, M., Kosalec, I., 2015. Antimicrobial and Antioxidant Properties of Satureja montana L. and S. subspicata Vis. (Lamiaceae). Current Drug Targets 16: 1623-1633.

Marin, M., Ascensao, L., Marin, P.D., 2012. MICROSCOPIC INVESTIGATION OF TRICHOMES OF WILD-GROWING SATUREJA MONTANA L. Archives of Biological Sciences 64: 1599-1605.

Mihajilov-Krstev, T., Radnovic, D., Kitic, D., Jovanovic, V.S., Mitic, V., Stojanovic-Radic, Z., Zlatkovic, B., 2014. Chemical composition, antimicrobial, antioxidative and anticholinesterase activity of Satureja Montana L. ssp montana essential oil. Cent. Eur. J. Biol. 9: 668-677.

Mirjana, S., Nada, B., 2004. Chemical composition and antimicrobial variability of Satureja montana, L. essential oils produced during ontogenesis. Journal of Essential Oil Research 16: 387-391.

Skocibusic, M., Bezic, N., 2003. Chemical composition and antidiarrhoeal activities of winter savory (Satureja montana L.) essential oil. Pharmaceutical Biology 41: 622-626.

Tuberoso, C.I.G., Kowalczyk, A., Sarritzu, E., Cabras, P., 2007. Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use. Food Chemistry 103: 1494-1501.

Vidovic, S., Zekovic, Z., Marosanovic, B., Todorovic, M.P., Vladic, J., 2014. Influence of pre-treatments on yield, chemical composition and antioxidant activity of Satureja montana extracts obtained by supercritical carbon dioxide. Journal of Supercritical Fluids 95: 468-473.

Vladic, J., Zekovic, Z., Jokic, S., Svilovic, S., Kovcevic, S., Vidovic, S., 2016. Winter savory: Supercritical carbon dioxide extraction and mathematical modeling of extraction process. Journal of Supercritical Fluids 117: 89-97.

Wesolowska, A., Grzeszczuk, M., Jadczak, D., 2014. Influence of Harvest Term on the Content of Carvacrol, p-Cymene, gamma-Terpinene and beta-Caryophyllene in the Essential Oil of Satureja montana. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 42: 392-397.