Identification and comparison of essential oil composition and mineral changes in different phenological stages of *Satureja hortensis* L.

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Abstract

Satureja hortensis L., (summer savory) is an annual, herbaceous aromatic and well-known medicinal plant. *S. hortensis* essential oil has a high percentage of carvacrol which is responsible mainly for its biological activity. The biomass production, essential oil composition and mineral content of *Satureja hortensis* are known to be dependent on its growth and development conditions. The aim of this work was, therefore, to evaluate the essential oil composition as well as minerals content at different stages of growth of Satureja *hortensis.* The growth media was 10 cm diameter pots containing perlite watered using Hoagland nutrient solution. Plants were harvested at different stages of growth (before flowering and after flowering period) and dry weights were measured. The essential oils were analyzed by GC-MS. Before flowering stage and in full flowering stage, 29 compounds were characterized. In both stages, carvacrol showed the highest rates of the compounds an increasing trend from before flowing to flowering so that it changed from 47.77% to 49.96% whereas α-terpinene and cymene had a decreasing trend from before flowing to flowering stage. There was a rapid uptake of minerals during early growth and gradual dilution as the plant matured. Phosphorus and magnesium concentrations increased with expansion of growth and development in shoots and roots.

Keywords: Satureja hortensis; essential oil; carvacrol; mineral

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Introduction

The genus *Satureja* L. (savory, saturei) includes more than 30 species belonging to the family Lamiaceae, subfamily *Nepetoideae*, tribe *Mentheae* (Cantino et al., 1992). *Satureja hortensis* L., (summer savory) is an annual, herbaceous aromatic and well-known medicinal plant, native to southern Europe and naturalized in parts of North America (Sefidkon et al., 2006)., which is used as a spice and traditional herb in Iran.The aerial parts of some *Satureja* plants have been widely used in foods as a flavor component and in folk and traditional medicine (Zargari, 1990; Baytop, 1997; Madsen et al., 1998; Hajhashemi et al., 2000). Within the genus *Satureja*, *S. hortensis* has received by far more attention. It has shown antispasmodic,

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antidiarrheal, antioxidant, sedative and antimicrobial properties (Zargari, 1990; Hajhashemi et al., 2000; Gursoy et al., 2009). *S. hortensis* essential oil has a high percentage of carvacrol which is responsible mainly for its biological activity (Deans and Svoboda, 1989; Martini et al., 1996). It is a common understanding that chemical constituents of medicinal plants and therefore their biological activities are influenced by the genetic and environmental factors (Heywood, 2002).

The essential oil of aromatic plants is mostly produced by specialized cell structures and stored in specially formed storage compartments in the plant (Fahn, 1979). Literature review on essential oil composition in *Satureja* species shows it to be rich in phenolic components such as carvacrol, gama-terpinene, thymol, *p*-cymene, β-caryophyllene, linalool and other terpenoids. However, chemical composition and the amount of components show variation between oils of different *Satureja* species (Baher et al., 2002; Baser et al., 2004; Kurcuoglu et al., 2001; Novak et al., 2006; Rojas and Usubillaga, 2000; Sefidkon et al., 2006; Svoboda et al., 2006; Viturro et al., 2000). The biosynthesis of secondary metabolites in medicinal and aromatic plants is strongly influenced by environmental factors and plants growth stage (Stutte, 2006). These conditions cause variations in the fresh and dry weight, as well as active components.

Contents of inorganic elements are very important traits in plants. Some of the elements have causal effects on plant growth. Also, their effects on plant metabolism and therefore quantity of essential oils are important issues. Measurement and determination of elements such as K, Na, Ca, Mg, Mn, Zn and Cu of minerals in plants led to find some critical information about the relationship between plant and medium for application of the plant in medicinal, hygienic and adornment uses (Macrae et al., 1993). The major minerals serve as structural components of tissues, functioning in cellular, basal metabolism, and water and acid-base balance (Macrae et al., 1993; Nielsen, 1984; Smith, 1988). The nutritional and medicinal properties of these plants may be interlinked through photochemical, both nutrient and non-nutrient (Ranhotra et al., 1998). Therefore, quantifying and qualifying medicinal plants oil component and minerals in different growth stages are an important issue. In the present investigation, we identified and compared the composition of *Satureja hortensis* L. essential oil and minerals in two stages of plant growth.

Materials and Methods

Isolation and GC-MS analysis of essential oils

Mature seeds of *S. hortensis* were sterilized in 70% ethanol for 1 min and then by 0.1% mercuric chloride for 5 min, followed by three washes of sterile distilled water. After sterilization, seeds were germinated in pots filled with perlite. The uniform seedlings were fed with modified 10% Hoagland nutrient solution containing: 0.2 mM KH_2PO_4, 0.8 mM $Ca(NO_3)_2.4H_2O$, 1 mM KNO_3, 0.4 mM $MgSO_4.7H_2O$, 15 µM FeEDTA, 10 µM H_3BO_3, 3 µM $MnCl_2.4H_2O$, 0.2 µM $ZnSO_4.7H_2O$, 0.2 µM $CuSO_4.5H_2O$, 0.1 µM $Na_2.MoO_4.2H_2O$. Nutrient solution pH was adjusted daily to 5.8 with 0.1M NaOH or 0.1M HCl. Plants were grown in greenhouse with photo period of 16/8 h light/dark cycles and light intensity approximately at 280 $mmolm^{-2}$ s^{-1}, day/night temperature were adjusted at 26/20 $^{\circ}C$. The nutrient solution was renewed every day. After the seedlings established, the pots were transferred to a growth chamber (Walking Conviron Growth Chamber) with mentioned adjustments and treatments.

The aerial parts of *S. hortensis* cultivated in growth chamber, were collected in two stages of plant growth (before flowering and after full flowering). Air-drying of plant material was performed in a shady isolated sterile place at room temperature for 30 days. Dried aerial parts (50 g) were cut and subjected to hydro-distillation for 3 h, using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia (1993). The resulting essential oil was dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4 $^{\circ}C$ until analysis.

GC analysis was performed using a Thermoquest gas chromatograph with a flame ionization detector (FID). The analysis was carried

out on fused silica capillary DB-5 column (30 m × 0.25 mm i. d.; film thickness 0.25 µm). The injector and detector temperatures were kept at 250 °C and 300 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1.1 ml/min; oven temperature program was 60–250 °C at the rate of 4 °C/min and finally held isothermally for 10 min; split ratio was 1:50.

GC–MS analysis was carried out by use of Thermoquest-Finnigan gas chromatograph equipped with fused silica capillary DB-5 column (60 m × 0.25 mm i. d.; film thickness 0.25 µm) coupled with a TRACE mass (Manchester, UK). Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 200 °C and 250 °C, respectively. Mass range was from 35 to 456 AMU (atomic mass unit). Oven temperature program was the same as mentioned above for the GC.

Determination of mineral contents

The aerial parts and root of *S. hortensis* were collected and air-drying of plant material was performed in a shady place at room temperature for 30 days. About 10 g dried and ground sample was put into a burning cup and sample was incinerated in a MARS 5 Microwave Oven at 550 °C for 2 h (Indian Standard, 1972) then 15 ml pure HNO3 was added. Dissolved ash was diluted to a certain volume with distilled deionized water. Minerals were determined by following methods: calcium and magnesium by complexometry (Walsh, 1971), sodium and potassium by flame photometer (Corning 410) (Qupta, 2000; Tandom, 1995) and phosphorous by spectrophotometer (Tomas 302) (Cottenie, 1980).

Results

From the collected plant material of *S. hortensis* L., 2.05% (v/w) of essential oil was isolated by the process of hydro-distillation. The oil was intensively yellow in color, with a characteristically strong and pleasant odor. The composition of the essential oil constituents isolated from the aerial parts of *S. hortensis* by two stages of plant growth, are presented in Table 1.

Before flowering stage the major components were carvacrol (47.77%), γ-terpinene (17.988%), and cymene (5.038%) and at full flowering stage carvacrol (49.96%), n- hexa decanoic acid (2.596%), cymene (2.537%), β-bisabolene (2.525%) and γ-terpinene (2.396%) were identified as constituents of the oil.

The mineral compositions of *S. hortensis*

Table 1
Comparison of *S. hortensis* essential oil in two stages of plant growth

No.	Constituents	RI[a]	Before flowering (%)	Full flowering (%)
1	α-thujene	924	1.125	0.315
2	α-pinene	932	0.831	0.402
3	Sabinene	969	0.373	0.394
4	β- mycrene	988	1.412	0.770
5	1-phellandrene	1025	0.436	0.256
6	α-Terpinene	1014	3.76	2.396
7	Cymene	1020	5.038	2.537
8	δ-3- careen	1031	0.652	0.425
9	γ- Terpinene	1054	17.988	0.053
10	α-terpinolene	1186	0.116	0.133
11	Carvacrol methyl ether	1241	0.342	1.067
12	Carvacrol	1298	47.77	49.96
13	Eugenol	1356	0.486	1.315
14	Trans Caryophylene	1419	0.541	0.844
15	Aaromandrene	1439	0.283	0.308
16	β- Ionone	1472	0.077	0.187
17	Ledene	-	0.303	0.390
18	β- bisabolene	1504	1.609	2.525
19	α-Bisabolene	1506	0.214	0.291
20	Spathulenol	1577	0.343	0.315
21	Diphenyl methanone	1622	0.228	0.744
22	Isobutyl phthalate	-	0.160	0.470
23	Tricyclene	-	0.418	0.499
24	Methyl phthalate	-	0.283	0.775
25	Dibuthyl phthalate	1974	0.609	0.971
26	n- hexadecanoic acid	1970	1.286	2.596
27	Linolenic acid methyl ester	-	1.692	1.708
28	Neophytadiene	-	0.392	0.371
29	Ethyl linoieolate	-	1.105	1.051

RI[a]: Retention index

Table 2
Mineral composition and organic matter content of *S. hortensis* in two stages of plant growth

Sample	Organic matter (%)	Potassium (mg 100 g^{-1} dw)	Magnesium (mg 100 g^{-1} dw)	Calcium (mg 100 g^{-1} dw)	Phosphorus (mg 100 g^{-1} dw)
Aerial before Flowering	85.2	302.54	72.5	112.5	297.31
Aerial Flowering	86.3	253.71	68	94	243.7
Root before Flowering	87.7	228.29	94	50	291.202
Root Flowering	87.5	224.69	85	30	296.25

which were measured in two stages of plant growth are shown in Table 2. Potassium content at all stages of maturity was higher than 200 mg 100 g^{-1} dw. The shoot fraction of *S. hortensis* had generally higher content of potassium, and with growth and development this decreased from 302.5 to 253.7 mg 100 g^{-1} dw.

In the second developmental stage, phosphorus content in aerial part was higher than the first stage (before flowering) but in roots, content of phosphorus increased from 291.2 (the first stage) to 296.25 mg 100 g^{-1} dw (the second stage).

Calcium plays a very important role in plant growth and nutrition, as well as in cell wall deposition and increasing mechanical strength of the plant. Much higher content of calcium (from 94 to 112.5 mg 100 g^{-1} dw) were found in the aerial parts than in the roots (the highest value 50 mg 100 g^{-1} dw in the first, and the lowest 30 mg 100 g^{-1} dw in the second stage of development).

Discussion

The results of this study showed that the oils obtained from the different phonological stages had similar compositions and the main compounds were carvacrol (47.77%), γ-terpinene (17.988%), and cymene (5.038%). The time of harvesting of this plant materials did not have a major effect on chemical composition of the essential oil although time has important effects on the essential oil content of the plant and the flowering stage is generally the best time for harvesting the plant and obtaining the essential oil because at this stage the plants contain highest percent of the essential oil.

Variation in essential oil content and composition of *S. hortensis* L. and some other native plants of Kurdistan province from different origins has been reported by several authors (Baser et al., 2004: Ghasempour et al., 2007; Taran et al., 2011 and Zebarjadi et al., 2011). Therefore, thymol (29-43%) was the major component of wild accessions of *Satureja hortensis* L. and oil content of the accessions varied between 1.28% and 4.75%. In case of cultivated accessions, carvacrol (42.0-63.0%) was the major component and oil content ranged from 1.30% to 2.67% (Baser et al., 2004).

The percentage of carvacrol in 34 *S. hortensis* populations investigated by Pank et al., (2004) ranged between 54.2% and 88.3% and the oil content varied between 1.28% and 3.5%. Galambosi et al., (2002) reported carvacrol content of 59.3-65.1% for the essential oil *S. hortensis* cv.

Contents of inorganic elements are very important in plants. Furthermore, different stages of plant maturity affect the concentration of a number of minerals in *S. hortensis*. The aerial part tissues contained higher concentrations of elements, except magnesium. In both investigated plant anatomical fraction, the highest content of elements were in the first stage of growth. Also, potassium and calcium decreased markedly with increasing maturity, while phosphorus did not greatly alter by stage of maturity.

The main role of potassium is to provide the appropriate ionic environment for metabolic processes in the cytosol, and as such functions as a regulator of various processes including growth regulation. Plants require potassium ions for protein synthesis and for the opening and closing of stomata, which is regulated by proton pumps to make surrounding guard cells either turgid or

flaccid (Öborn et al., 2003). Therefore, potassium tends to be stored in the aerial parts.

Phosphorus is vital to plant growth and is found in every living plant cell. It is involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to the next (White et al., 2003).

The calcium concentration in *S. hortensis* decreased with growth and development. This data are in agreement with those reported by Ignjatović et al., (1998). Although shoots are generally high in calcium, the availability of calcium in some shoots may be low because of the presence of calcium oxalate. As in alfalfa, 20 to 33 % of the calcium was present as insoluble calcium (Martz et al., 1990).

Magnesium is closely associated with calcium and phosphorus, and the magnesium content of forage crops varies widely. Magnesium is an important part of chlorophyll, a critical plant pigment important in photosynthesis. Also, it is important in the production of ATP through its role as an enzyme cofactor (White et al., 2003). The concentration of magnesium declined with growth and development in *S. hortensis* shoots and roots. The highest magnesium content was in the first stage of development (72 and 94 mg 100 g^{-1} in shoot and root dry weights, respectively).

Calcium plays a very important role in plant growth and nutrition, as well as in cell wall deposition and increasing mechanical strength of the plant. Therefore, much higher content of calcium (from 94 to 112.5 mg 100 g^{-1} dw) were found in the aerial parts than in the roots (the highest value 50 mg 100 g^{-1} dw in the first, and the lowest 30 mg 100 g^{-1} dw in the second stage of development).

References

Baher, Z. F., M. Mirza., M. Ghorbanli and **M. Rezaii.** 2002. 'The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis* L.'**.** *Flavour and Fragrance Journal*, 17 (4): 275-277.

Baser, K. H. C., T. Ozek., N. Kirimer and **G. Tumen.** 2001. 'A comparative study of the essential oils of wild and cultivated *Satureja hortensis* L. *Journal of Essential Oil Research*, 16(5): 422-424.

British Pharmacopoeia. 1993. vol. 2, HMSO, London, pp. 137-138.

Cantino, P. D., R. M. Harley and **S. J. Wagstaff.** 1992. *Genera of Labiatae status and classification, In: Harley RM, Reynolds T (eds) Advances in Labiatae science.* Royal Botanic Gardens, Kew, 511-522.

Cottenie, A., 1980. 'Soil and plant testing as a basis of fertilizer recommendation'. *FAO Soil Bulletin*, 38, 2, Rome.

Fahn, A., 1979. *Secretory Tissues in Plants*. Academic Press, London.

Galambosi, B., Z. Galambosi., R. Pessala., I. Hupila., A. Aflatuni., K. P. Svoboda and **M. Repcak.** 2002. 'Yield and quality of selected herb cultivars in FINLAND'. *Acta Horticulture,* 276: 139-149.

Gursoy, U. K., M. Gursoy., O. V. Gursoy., L. E. Cakmakci and **V. J. Uitto.** 2009. 'Anti- biofilm properties of *Satureja hortensis* L. Essential oil against periodontal pathogens'. *Anaerobe*, 15: 164-167.

Hajhashemi, V., H. Sadraei., A. R. Ghannadi and M. Mohseni. 2000. Antispasmodic and anti-diarrhoeal effect of *Satureja hortensis* L. essential oil. *Journal of Ethnopharmacology*, 71: 187-192.

Hey wood, V. H., 2002. 'The conservation of genetic and chemical diversity in medicinal and aromatic plants'. In: *Sener*, B. (Ed.), *Biodiversity*: *Biomolecular Aspects of Biodiversity and Innovative Utilization*. Kluwer Academic/Plenum Publishers, New York, 13-22.

Ignjatović, S., B. Dinić, D. Kolarski and **B. Urošević.** 1998. 'Chemical composition of first and second cut of lucerne (*Medicago sativa* L.) at different stages of maturity'. Proc. 17th General Meeting EGF, may 18-21, Debrecen, Hungary.

Indian Standard, 1972. 'specefication for acacia (arabic) gum, foodgrade', 6795. appendix a.

Kurcuoglu, M., G. Tumen and **K. H. C. Baser.** 2001. 'Essential oil constituents of *Satureja*

biossieri from Turkey'. *Khimiya Prirodnykh Soedinenii*, 37(4): 280–281.

Macrae, R., R. K. Robinson and **M. J. Sadler.** 1993. *Encyclopaedia of food science, food technology and nutrition*. Eds. vol. 5. San Diego, CA: Academic Press INC.

Madsen, H. L., B. Sorensen, L. H. Skibsted and **G. Bertelsen.** 1998. 'The antioxidative activity of summer savory (*Satureja hortensis* L.) and rosemary (*Rosmarinus officinalis* L.) in dressing stored exposed to light or in darkness'. *Food Chemistry*, 63: 173-180.

Martini, H., M. Weidenborner, S. Adams and **B. Kuntz.** 1996. 'Eugenol and carvacrol, the main fungicide components in clove and savory, Ital'. *Journal Food Science*, 8(1): 63-67.

Martz, F. A., A. T. Belo., M. F. Weiss., R. L. Belzea and **J. P. Goff.** 1999. 'True absorption of calcium and phosphorus from alfalfa and corn silage when fed to lactating cows'. *Journal Dairy Science*, 73: 1288-1295.

Nielsen, F. H., 1984. 'Ultratrace elements in nutrition'. *Annual Review of Nutrition*, 4: 21–41.

Novak, J., Bahoo, L., U. Mitteregger and **C. H. Franz.** 2006. 'Composition of individual essential oil glands of savory (*Satureja hortensis* L., Lamiaceae) from Syria'. *Flavonoid and Fragment Journal*, 21: 731-734.

Öborn, I., A. C. E. Edwards., Witter., O. Oenema., K. Ivarsson., P. J. A. Whithers., S. I. Nilsson and **A. Richert Stinzing.** 2003. 'Element balances as a tool for sustainable nutrient management: a critical appraisal of their merits and limitations within an agronomic and environmental context'. *European Journal of Agronomy*, 20: 211-225.

Pank, F., A. Pfefferkorn and **H. Kruger.** 2004. 'Evalution of a Summer Savory (*Satureja hortensis* L.) collection with regard to morphology, precocity, yield components and essential oil and carvacrol content'. *Z. naturforsch*, 9: 72-79.

P. J. White and **P. H. Brovn.** 2010. 'Plant nutrition for sustainable development and global health'. *Annual of Boatany,* 105: 1073-1080.

Qupta, P. K., 2000, *Soil, Plant, Water and Fertilizer Analysis*. Agrobios (India).

Ranhotra, G. S. J. A., S. D. Leinen., M. A. Vinas and **K. J. Lorenz.** 1998. 'Nutritional profile of some edible plants from Mexico'. *Journal of Food Composition and Anlaysis*, 11: 298–304.

Rojas, L. B. and **A. Usubillaga.** 2000. 'Composition of the essential oil of *Satureja brownei* (sw) Briq From Venezuela'. *Flavour and Fragrance Journal*, 15(1): 21-22.

Sefidkon, F., K. Abbasi and **G. Bakhshi Khaniki.** 2006. 'Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Satureja hortensis*'. *Food Chemistry,* 99: 19-23.

S. G. Deans and **K. P. Svoboda.** 1989. 'Antibacterial activity of Summer Savory (*Satureja hortensis* L.*)* essential oil and its constituents'. *Journal of Horticultural Science*, 64(2): 205-210.

Shiyab, S., M. Shatnawi., R. Shibli., M. Al-zweiri., M. Akash and **T. Aburijai.** 2012. 'Influence of developmental stage on yield and composition of *Origanum syriacum* L. oil by multivariate analysis'. *Journal of Medicinal Plants Research*, Vol. 6(15), pp. 2985-2994,

Smith, K. T., 1988. *Trace minerals in foods*. New York: Marcel Dekker.

Stutte, G. W., 2006. 'Process and product recirculation hydroponics and bioactive compounds in controlled environment'. *Horticulture Science*, 41: 526- 530.

Svoboda, K. P., R. K. M. Hay and **P. G. Waterman.** 2006. 'Growing summer savory (*Satureja hortensis*) in Scotland: Quantitative and qualitative analysis of the volatile oil and factors influencing oil production'. *Journal of Science and Food Agriculture,* 53: 193-202.

Viturro, C. I., A. Molina., I. Guy., B. Charles., H. Guinaudeau and **A. Fournet.** 2000. 'Essential oils of *Satureja boliviana* and *S. pavifolia* growing in the region of Jujuy'. *Argentina, Flavour and Fragrance Journal*, 15(6): 377–382.

Walsh, L. M., 1971. 'Instrumental methods for analysis of soils and plant tissue. So society of America'. *Inc. Madison, W* USA, 222.

Zargari, A., 1990. *Medicinal Plants*; fourth ed. Tehran University Publications, Tehran, 42-45.