



Quantitative Monitoring of the Volatiles from the Aerial Parts of *Satureja hortensis* by the use of HS-SPME-GC-MS Approach

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ABSTRACT

The volatile fractions isolated from the ground aerial parts of *Satureja hortensis* were trapped using the headspace solid phase microextraction (HS-SPME) strategy and subsequently monitored using gas chromatography in combination with mass spectrometry (GC-MS) instrumentation. Eleven constituents were totally recognized in the analyzed volatile parts, representing entire of the corresponding profile. Among the natural groups present in the chemical profile, monoterpene hydrocarbons (99.5%) were found as the major constituents with the dominant compounds being γ -terpinene (46.0%), *p*-cymene (11.8%), α -terpinene (11.1%), α -pinene (10.4%) and α -thujene (6.8%). However, lower contents of some natural compounds including myrcene (5.2%), β -pinene (3.9%), *o*-cymene (2.6%), α -phellandrene (1.1%), camphene (0.6%) and thymol (0.5%) were identified. Accordingly, only 0.5% of the profile included oxygenated monoterpenes.

Keywords: *Satureja hortensis*, volatile compounds, γ -terpinene, *p*-cymene, α -terpinene, GC/MS, monoterpene hydrocarbons.

INTRODUCTION

In the plant sciences, the Lamiaceae family has been known in detail of which many species can produce adequate essential oils having versatile pharmaceutical characteristics. In the literature, it has been pointed out that the *Satureja* genus involves

twelve species with promising phytochemical and medicinal properties. The endemic species of this genus in Iran include *S. atropatana*, *S. bachtiarica*, *S. edmondi*, *S. intermedia*, *S. isophylla*, *S. kallarica*, *S. khuzistanica* and *S. sahendica*. It should be also noted that the other famous species of the *Satureja*, namely *S. boissieri*, *S. macrantha*, *S. mutica* and *S.*

spicigera are mainly found in some parts of Iraq, Turkey, Ghafghaz (Northern and Southern regions) as well as Turkmenistan¹.

In the Iranian traditional and folk medicine, *Satureja hortensis* L. which is called as Marzeh is widely used as an effective remedy to treat some disorders and malfunctions of digestions in the human body. The extracts of this herbal plant serve as potent sedative agents and can also relief cramps or address nausea along with diarrhea. Moreover, in the cookery this plant is frequently used as a flavoring agent².

From morphological point of view, its average height falls within the 10-35 cm range. The flowers of *S. hortensis* appear in white or lilac colors. This valuable plant could be found in different situations including wet, dried and moderate climatic conditions³.

A brief literature survey reveals that some species of the *Satureja* genus could be considered as proper medical remedies in view of their potential anti-inflammatory⁴⁻⁶, antioxidant⁷⁻¹⁰, antinociceptive^{4, 5}, antimicrobial⁹⁻¹⁵, antigenotoxic¹⁶, antispasmodic and anti-diarrheal¹⁷, anti-fungal¹⁸, insecticidal^{19, 20} and antibacterial¹¹ activities.

The relaxant effect of an aqueous-methanol extract of *S. hortensis* has been tested on guinea pig trachea²¹. According to the findings of this report, *S. hortensis* serves as an excellent relaxant and its influence is comparable with that of theophylline. In addition, according to some of the previous studies, this plant is a rich source of some useful phenolic, antioxidant and flavonoids²²⁻²⁴. Furthermore, an important oxygenated monoterpene, namely thymol has been measured in the extracts of *S. hortensis* by using the HPTLC technique²⁵.

Regarding a broad spectrum of phytochemical activities related to the different members of the *Satureja* genus, it is so rational to characterize the composition of the secondary metabolites or volatiles and separate bioactive compounds in each profile.

Over the recent decades, the combination of headspace analysis with solid phase microextraction

standpoint, namely abbreviated as HS-SPME has gained a unique interest in different fields of analytical sciences. The HS-SPME technique is inherently free of solvent and can be directly used to analyze diverse matrices like plants, foods, fragrances and biological samples²⁶⁻³³. In a very recently report, we have described the potential capability of this technique using the least amounts of plant samples when using four types of organic fibers³⁴.

In the present report, we intend to describe the results of quantitative and qualitative analyses of the volatiles obtained from the aerial parts of *S. hortensis* using the HS-SPME-GC-MS approach. In the literature, some sporadic studies are found relating to the characterization of the water-distilled essential oils of this plant^{11, 13, 20, 35}. However, there is no report concerning profiling of the volatiles of *S. hortensis* using the aforementioned approach in Semnan Province, Iran up to present.

EXPERIMENTAL

Reagents and plant authentication

To calculate the Kovats indexes, a complete range of normal paraffins (C₉-C₂₄) were provided from Fluka Company. Additionally, all the carrier gases utilized in gas chromatographic-based separations were ultrapure. On 15 April 2015, fresh aerial parts of *S. hortensis* were sampled from a small village (Foroomad, 1230 msl) located at 150 km far from Shahrood. According to our global positioning system (GPS), the geographic coordinates of the sampling area were found to be north latitude 36°.30' and east longitude 56°.45'. Moreover, to authenticate the botanical name of the plant sample, a voucher specimen has been deposited at a reliable herbarium belonging to Research Institute of Forests and Rangelands (RIFR), Tehran, Iran.

General characteristic of the proposed HS-SPME approach

To trap the volatiles from the aerial parts of *S. hortensis*, a complete SPME set-up was provided from Supelco, Bellefonte, USA. This assembly involves a miniaturized microtubing which its surface has been coated with a proper organic polymer namely PDMS-CAR. To perform the respective analysis, as it is customary in such sorts of studies, the fiber should be initially conditioned

at a temperature of 250°C for about 30 minutes in the injector of the GC-MS apparatus. Immediately after, minimal amounts of the dried and powdered plant sample (about 1.0 g) in the especial vials were situated under a temperature of 70°C for about fifteen minutes. This results in the release of volatile fractions from the plant material. After piercing the septum by the needle of the SPME, its fiber was allowed to stand in the headspace medium formed above the sample vials. In the next step, the fiber retracted to the needle and the needle was directly placed in the injection port of the GC-MS instrument. Finally, the temperature was regulated at 250°C when the injection port was set at the splitless mode for about five minutes.

Chemical profile identification

The main patterns to finalize identification of the chemical profile related to the volatile fractions from the aerial parts of *S. hortensis* using the HS-

SPME-GC-MS technique were as follows.

1. Matching of numerical Kovats indices with those tabulated in the standard references³⁶⁻³⁹,
2. Consistency of mass spectral fragmentation with reliable references,
3. The resemblance reports given by the library of the GC-MS apparatus (>90%),
4. Regarding the findings of our previous reports^{27-30, 33, 40-71}.

GC and GC-MS quantifications

Both gas chromatography (GC, Shimadzu 15A: FID detector) and gas chromatography-mass spectrometry (GC-MS, Hewlett-Packard 5973) determinations were performed under the identical temperature programmings. However, the column types in these techniques were respectively a DB-5 with general dimensions of 50 m×0.2 mm, film thickness 0.32 μm as well as an HP-5MS

Table 1: Volatile composition from the aerial parts of *S. hortensis* obtained by using the HS-SPME-GC-MS method^a

| No. | Compound | Class | R _t ^d | RI (Lit.) ^e | RI (Cal.) ^f | Percentage |
|-----|----------------|-----------------|-----------------------------|------------------------|------------------------|------------|
| 1 | α-Thujene | MH ^b | 6.4 | 931 | 923.3 | 6.8 |
| 2 | α-Pinene | MH | 6.5 | 939 | 929.9 | 10.4 |
| 3 | Camphene | MH | 6.8 | 953 | 945 | 0.6 |
| 4 | β-Pinene | MH | 7.5 | 980 | 973.8 | 3.9 |
| 5 | Myrcene | MH | 7.8 | 991 | 988.9 | 5.2 |
| 6 | α-Phellandrene | MH | 8.1 | 1005 | 1002.3 | 1.1 |
| 7 | α-Terpinene | MH | 8.4 | 1018 | 1015.2 | 11.1 |
| 8 | p-Cymene | MH | 8.5 | 1020 | 1023.8 | 11.8 |
| 9 | o-Cymene | MH | 8.6 | 1022 | 1027.6 | 2.6 |
| 10 | γ-Terpinene | MH | 9.3 | 1062 | 1061.3 | 46.0 |
| 11 | Thymol | OM ^c | 14.1 | 1290 | 1313.2 | 0.5 |
| | | MH | | Number | | 10 |
| | | | | Percentage | | 99.5 |
| | | OM | | Number | | 1 |
| | | | | Percentage | | 0.5 |
| | | Total | | | | 100 |

^a The compounds have been sorted according to their retention indices on an HP-5 MS capillary column

^b Monoterpene hydrocarbons

^c Oxygenated monoterpene

^d Retention time (Min.)

^e Kovatz retention indices given in the literature

^f Calculated Kovatz retention indices

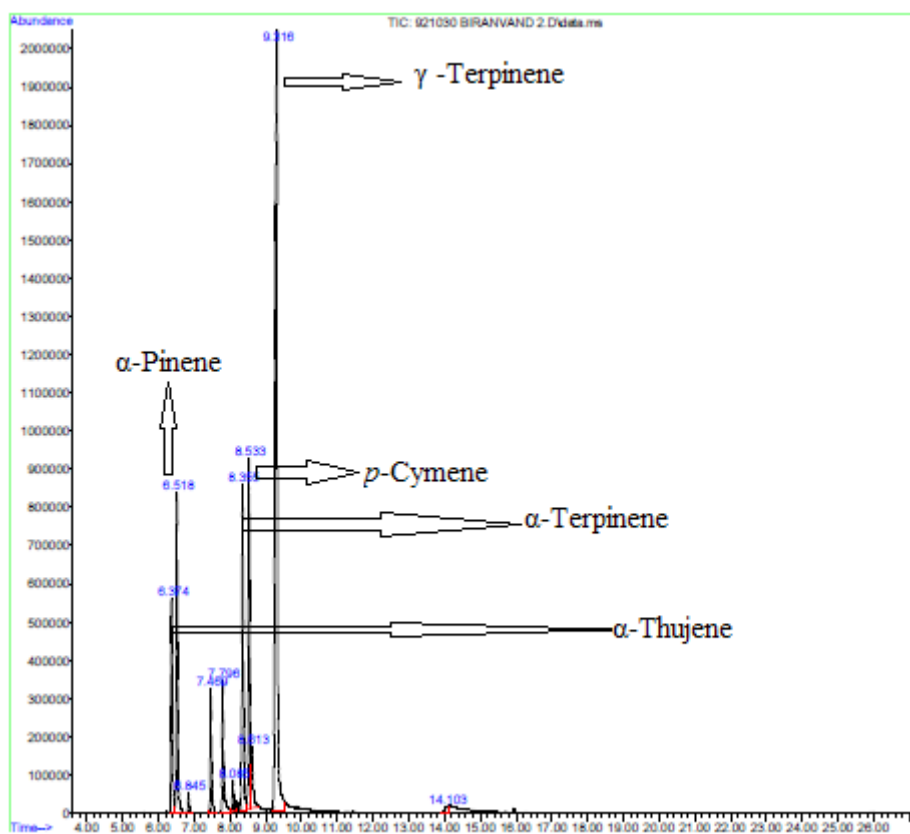


Fig. 1: GC-MS chromatogram for the volatile fraction from the aerial parts of *S. hortensis*

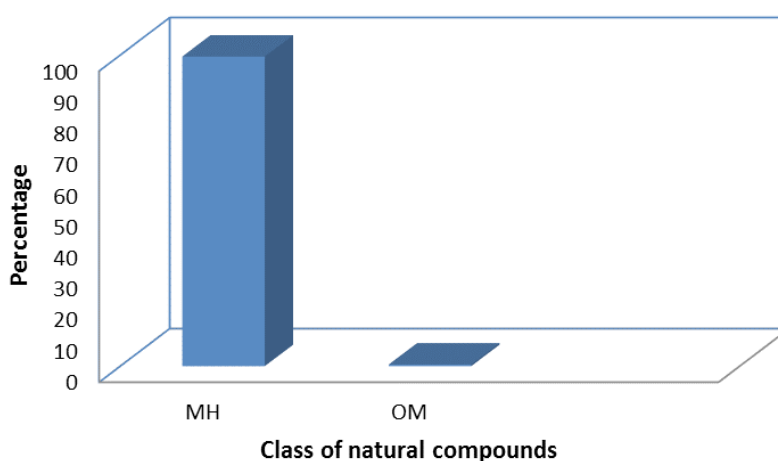


Fig. 2: Percentages of the classes of natural compounds found in the volatiles from aerial parts of *S. hortensis* using the HS-SPME-GC-MS method (MH= monoterpene hydrocarbon; OM= oxygenated monoterpene)

column having 50 m×0.2 mm, film thickness 0.32 µm. In all of our determinations, the temperature of each column was first set at the temperature of 60°C for three minutes and then gradually increased to a final temperature of 230°C by applying a rate of 5°C/min. The carrier gases used in the GC and GC-MS approaches were respectively nitrogen and helium all having a flow-rate of 1.0 ml/min. The ionization energy in the GC-MS instrumentation was 70 eV within the mass range of 30-350 amu.

RESULTS AND DISCUSSION

Composition of the *S. hortensis* volatile profile

Characterization of the chemical compositions of the essential oils separated from *S. hortensis* has been reported in some of the previous studies in the literature^{12,13,72}. However, regardless of some similarities, we identified a rather new profile relating to the volatiles from the aerial parts of this species. According to our identification, totally eleven compounds contributed to the volatile profile of this plant. The names, groups, retention times, retention indexes (calculated and literature tabulated) have been shown in Table 1. The closeness between the Kovats values confirms the authenticity of the profile identification.

Screening of the general composition of the volatiles from the aerial parts of *S. hortensis* resulted in recognition of ten monoterpene hydrocarbons (MH) together with one oxygenated monoterpene (OM) respectively accounting for 99.5% and 0.5% of the total profile (see Table 1 and Figure 1). In this regard, γ -terpinene (46.0%), *p*-cymene (11.8%), α -terpinene (11.1%), α -pinene (10.4%), α -thujene (6.8%) and myrcene (5.2%) were found as the most abundant constituents of the profile. Furthermore, β -pinene (3.9%), *o*-cymene (2.6%), α -phellandrene (1.1%), camphene (0.6%) and thymol (0.5%) were detected among the other components contributing to the profile.

Meanwhile, the schematic representation of the natural compound groups in this profile has been represented in Figure 2. As can be seen, most of the chemical composition from the volatiles of *S. hortensis* consists of monoterpene hydrocarbons.

Comparison of the chemical profiles of *S. hortensis* oils

According to the literature, profiling of the chemical compositions of the water-distilled essential oils from different parts of *S. hortensis* has been the subjects of some earlier works. In the work of Adiguzel and coworkers, the major constituent of *S. hortensis* oil (dried fruits) has been reported as thymol (40.54%) followed by terpinene (18.56%), carvacrol (13.98%) and *p*-cymene (8.97%)³. Baser *et al.*⁷² have also reported components of the essential oils of twenty wild and cultivated samples in different regions of Turkey. In accordance with this study, in all of the cultivated profiles carvacrol was identified as the main constituent, whereas another oxygenated monoterpene, namely thymol constituted most of the essential oils from the wild samples profiles⁷². It should be also mentioned that carvacrol has been identified as the main constituent component in a variety of similar works being focused on the compositions of the essential oils of *Satureja* species^{11,13,20,35,74-76}.

Khajeh *et al* have identified high quantities of γ -terpinene, thymol and carvacrol in the hydro-distilled essential oil and SFE-extracts (CO₂ fluid) of *S. hortensis*⁷³. The quantitative and qualitative influence of growth stage on compositions of the oils of *S. hortensis* revealed dominance of γ -terpinene in all cases with the exception of ripened fruit stage involving carvacrol as the major component⁷⁴. Using a combination of traditional and advanced separation techniques to isolate the essential oils from *S. hortensis* before an HPTLC analysis resulted in identifying some oxygenated monoterpenes (thymol, carvacrol, linalool, citronellol, and geraniol) together with two monoterpene hydrocarbons (α -pinene: mixture and limonene)²⁵, as well. However, the findings of the present report are similar to our recently published work⁷⁵. In fact, the simple comparison shows that both profiles mainly consist of monoterpene hydrocarbons.

CONCLUSION

The main goal of this study was to establish a deeper insight into the capability of the HS-SPME technique coupled to GC-MS analysis to evaluate composition of the volatile fractions from the aerial parts of *S. hortensis* as a proper medicinal

plant. Our study led to whole identification of the corresponding chemical profile consisting of eleven natural compounds of which ten were monoterpene hydrocarbons (99.5%) and only one (0.5%) was an oxygenated monoterpene. The most abundant compounds present in the volatile profile were found to be γ -terpinene (46.0%), *p*-cymene (11.8%), α -terpinene (11.1%), α -pinene (10.4%), α -thujene (6.8%) and myrcene (5.2%) followed by β -pinene (3.9%), *o*-cymene (2.6%), α -phellandrene (1.1%),

camphene (0.6%) and thymol (0.5%) with lower percentages.

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