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Determination of volatile aroma compounds in fresh *Origanum vulgare* and *Hyssopus officinalis*: Headspace GC/FID/MS profile

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Abstract. Origanum vulgare and Hyssopus officinalis are important aromatic and medicinal plants. The chemical composition of the volatile aroma compounds was defined in the fresh aerial part of Origanum vulgare (f-O) and Hyssopus officinalis (f-H), collected in Valbona (Albania). The analysis were made by gas chromatography – mass spectrometry (GC/FID/MS) on HP5-ms column and equipped with automated headspace (HS) system. 0,3 g fresh plant material (homogenized samples from flower, leaf and stem) was put in sealed vials, heated (80 °C) and the gas phase was investigated. Total of 21 individual volatile aroma compounds were identified in f-O sample, 14 monoterpenes (78.35%) and 7 sesquiterpenes (3.03%). Dominant components were monoterpene hydrocarbons: sabinene (55.05%), trans-β-ocimene (5.19%) and γ-terpinene (4.05%), followed by cis-sabinene hydrate, α-terpinene and β-pinene. In f-H sample were identified 16 individual volatile aroma components, 11 monoterpenes (84.51%) and 5 sesquiterpenes (0.29%). The major components were monoterpene hydrocarbons: β-pinene (48.66%) and cis-pinocamphone (29.77%), followed by α-pinene and α-thujene.
Keywords: Origanum vulgare, Hyssopus officinalis, aroma compounds, Headspace GC/FID/MS.

Introduction

Oregano (*Origanum vulgare* L.) is an important aromatic plant widely produced in many countries for flavoring food. Oregano leaves and its essential oil were used in the food, drink, perfume, and cosmetics industry due to its pleasant and highly desirable aroma. Historically, the composition of oregano leaves has been studied using leaves oils obtained from hydrodistillates of the leaves. This approach produced samples with high volatile concentrations which were necessary for extraction and identification technologies available at that time. However, the heat applied during this process and long extraction time produced oils whose smell bore only limited resemblance to the original leaves [1]. Numerous studies have shown that *Origanum vulgare* subs.*vulgare* is poor in sources of volatiles and rich in acyclic compounds and sesquiterpenoids. Its principal constituents are Sabinene, (Z)-β-Ocimene, β-Caryophyllene and Germacrene D, while the phenols Thymol and Carvacrol are nearly absent. The essential oils of *O. vulgare* subsp. *vulgare* have a high antimicrobial activity against bacteria, fungi and yeast [2]. In recent years, many of its therapeutic properties have been demonstrated: antioxidant, antimicrobial, antiinflammatory, antiviral, antispasmodic, antiproliferative, and neuroprotective, among others [3].

Hyssop (*Hyssopus officinalis*, synonym *Hyssopus decumbens*) is a herbaceous plant of the genus Hyssopus native to Southern Europe, the Middle East, and the region surrounding the Caspian Sea [4]. A literature review on the chemical and biological aspects of the plant indicates that the main constituents of *H. officinalis* include several polyphenolic compounds, primarily the flavonoids apigenin, quercetin, diosmin, luteolin and their glucosides followed by other phenolic compounds chlorogenic, protocatechuic, ferulic, syringic, p-hydroxybenzoic and caffeic acids. Reports on the essential oils extracted from aerial parts of *H. officinalis* revealed several principal components, including terpenoids pinocamphone, isopinocamphone and β-pinene. Hyssop has moderate antioxidant and antimicrobial activity against Gram positive and negative bacteria activities together with antifungal and insecticidal antiviral properties *in vitro*. Animal model studies indicate myorelaxant, antiplatelet and α-glucosidase inhibitory activities for this plant [5]. The specific and particular aroma is maybe the most important reason for the wide use of the fresh and dried aerial parts of *Origanum vulgare and Hyssopus officinalis*. Chemical composition of *O.vulgare* and *H. officinalis* extracts as well as essential oil has been studied, but still very little is known about its aroma components. There are not data about the volatile aroma compounds in the fresh aerial parts. To analyze these compounds a refined method of headspace sampling hyphenated with GC/FID/MS analysis can be utilized [6].

The aim of this work was the determination of the volatile aroma compounds in the fresh aerial parts of Orignanum vulgare and Hyssopus officinalis using a headspace (HS) method with gas chromatography – mass spectrometry **(**GC/FID/MS).

Material and method

Plant material: The fresh aerial parts of Origanum vulgare (f-O) and Hyssopus officinalis (f-H) were collected in Valbona (Albania).
Analyses of aroma compounds:
GC and GC-MS analyses: 0.3 g of fresh aerial parts (homogenized samples from flower, leaf and stem) of f-O and f-H was put in sealed vials, warmed for 5 minutes and the gas phase (highly volatile compounds) was investigated on Agilent 7890A Gas Chromatography system equipped with flame ionization detector (FID) and Agilent 5975C Mass Quadrupole detector as well as capillary flow technology which enable simultaneous analysis of the sample on both detectors. HP-5ms (30 m x 0.25 mm, film thickness 0.25 µm) capillary column was used. Operating conditions were as follows: oven temperature 60°C, 20°C/min to 280°C; helium as carrier gas at a flow rate of 1 mL/min; injector temperature 260°C and FID temperature 270°C. 1000 µL of gas phase was injected at split ratio 1:1. The mass spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230°C, transfer line temperature 280°C and mass range from 50-500 Da. The MS was operated in scan mode.

Headspace method: Incubation temperature 80˚C; incubation time 5 min; syringe temperature 85˚C; agitator speed 500 rpm.

Identification of components: Identification of the components was made by comparing the mass spectra of components with those from NIST, Wiley and Adams mass libraries, by AMDIS (Automated Mass Spectral Deconvolution and Identification System) and by comparing literature and estimated Kovat's (retention) indices that were determined using a mixture of a homologous series of normal alkanes from C_9 to C_{25} in *n*-hexane, under the same conditions. The percentage ratio of the components was computed by the normalization method of the GC/FID peak areas and average values were taken.

Results and discussion

Table 1 shows the relative percentage of the volatile components identified in the fresh aerial parts of *O. vulgare* and *H. officinalis*; compounds are listed according to their linear retention indices.

Table 1. Chemical composition of volatile aroma compounds in the fresh aerial parts of *Origanum vulgare* (f-O) and *Hyssopus officinalis* (f-H) (%)

N.	Components	KIL	Fresh *Origanum vulgare* (f-O) (%)	Fresh *Hyssopus officinalis* (f-H) (%)
1.	*α*-Thujene	931	2.25	1.14
2.	*α*-Pinene	939	1.90	3.12
3.	Camphene	953	0.20	0.51
4.	Sabinene	976	55.05	-
5.	*β*-Pinene	980	2.38	48.66
6.	*α*-Phellandrene	1005	0.37	-
7.	*α*-Terpinene	1018	2.40	-
8.	*β*-Phellandrene	1031	-	0.11

9.	*trans-β*-Ocimene	1040	5.19	0.69
10.	*γ*-Terpinene	1062	4.05	0.06
11.	*cis*-Sabinene hydrate	1068	2.96	-
12.	Terpinolene	1088	0.56	0.10
13.	*cis*-Thujone	1102	-	0.17
14.	*allo*-Ocimene	1129	-	0.17
15.	Isoborneol	1156	0.12	-
16.	*cis*-Pinocamphone	1173	-	29.77
17.	*α*-Terpineol	1189	0.80	-
18.	Thymol, methyl ether	1235	0.15	-
19.	*α*-Copaene	1376	-	0.01
20.	*β*-bourbonene	1384	0.14	-
21.	*α*-Gurjunene	1409	-	0.01
22.	*trans*- Caryophyllene	1418	1.01	-
23.	*α*-Guaiene	1439	-	0.02
24.	*α*-Humulene	1454	0.14	-
25.	*trans*-9-*epi*-Caryophyllene	1467	0.14	-
26.	Germacrene D	1480	1.28	0.10
27.	Bicyclogermacrene	1494	0.25	0.15
28.	*γ*-Cadinene	1513	0.06	-
	Total (%):		81.41	84.80

KIL- Kovats (retention) index- literature data [7]; (-) did not identify.

Total of 21 individual volatile aroma compounds were identified in fresh aerial parts of *Origanum vulgare* (f-O), representing 81.41 % of the total content (Table 1). Data analysis of the chemical composition revealed three different classes of components: monoterpene hydrocarbons (MH) (9 compounds) 73.79%, oxygen containing monoterpenes (OM) (5 compounds) 4.59% and sesquiterpene hydrocarbons (SH) (7 compounds) 3.03%. The abundant components (> 1%) in f-O sample were MH: sabinene (55.05%), *trans-β*-ocimene (5.19%), *γ*-terpinene (4.05%), α-terpinene (2.40%), β-pinene (2.38%) and α-thujene (2.25%), followed by OM: *cis*-sabinene hydrate (2.96%) as and SH: germacrene D and *trans*-caryophyllene.

In fresh aerial parts of *Hyssopus officinalis* (f-H) were identified total of 16 individual volatile aroma compounds, representing 84.80% of the total content (Table 1). Data analysis of the chemical composition revealed three different classes of components: monoterpene hydrocarbons (MH) (8 compounds) 54.46%, oxygen containing monoterpenes (OM) (3 compounds) 30.04% and sesquiterpene hydrocarbons (SH) (5 compounds) 0.29%. The major components (> 1%) were monoterpene hydrocarbons: *β*-pinene (48.66%), *α*-pinene (3.12%) and α-thujene (1.14%), as and OM *cis*-pinocamphone (29.77%).

Hatipi *et al.* analyzed the chemical composition of the essential oil of wild *Origanum vulgare* collected from different locations in Kosovo using GC/MS method. Main identified individual compounds were sabinene (1.81-12.34 %), 1,8-cineole (1.31-13.54 %), caryophyllene oxide (0.18-38.05 %),(E)-β-caryophyllene (0.48-14.0%), p-cymene (1.27-19.62 %), α-terpineol (1.05-19.23 %), and germacrene D (0.35-16.09 %) [8]. Compared with these data, in the HS GC-FID/MS profile of our sample of fresh aerial part of *O. vulgare*, sabinene was dominant component (55.5%) as and germacrene D exceeded 1% (1.28 %).

Moghtader reported *iso* pinocamphone (38.47%), pinocomphone (13.32%), n-decane (8.67%), pinocarvone (5.34%) and β-pinene (1.54 %) as dominant components of the essential oil of the fresh leaves as and *iso* pinocamphone (40.25%), pinocomphone (14.92%), n-decane (8.63%), pinocarvone (6.76%) and β-pinene (1.78 %) as dominant components of the essential oil of the fresh flowers of *Hyssopus officinalis*, collected in Iran, analysed by GC and GC/MS [4]. In our tested sample of fresh aerial part of *H. officinalis*, only *cis*-pinocamphone (29.77 %) and β-pinene (48.66 %) were the dominant compounds. The other compounds were not identified in our tested sample.

Conclusion

Responsible compounds of the specific and particular aroma of the fresh aerial part of *O. vulgare* and *H. officinalis*, collected in Valbona (Albania) and analyzed by the HS-GC / FID / MS method belong to the group of mono and sesquiterpenes. The dominant individual compounds in both analyzed samples were monoterpenes: sabinene in the f-O sample as and *β*-pinene and *cis*-pinocamphone in the f-H sample.

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