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Volatile Oil Composition From Flowers and Leaves of *H.officinalis* L. Grown in Esfahan Headsapce Gas Chromatography/Mass Spectrometry

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ABSTRACT

The aim of this study was to analyze the chemical composition of essential oils from flowers and leaves of *Hyssopus officinalis* L. from Esfahan province, Iran. The volatile compounds were investigated using Headspace Gas Chromatography/Mass Spectrometry in flowering stage. Twenty nine and twenty seven compounds constituting 99.84% and 99.70 of the oil were identified in the essential oil of flowers and leaves of *Hyssopus officinalis* L. in flowering stage, respectively. Our results demonstrated that in the flowering stage of *H. officinalis* L. the major constituents grown in Isafahan of essential oil from the flowers and leaves respectively were Beta-pinene (23.38%– and 31.2%), trans-Pinocamphone (19.34% and, 15.5%), Cis-pinocamphone (27.49% and, 28.1%), myrcene (6.01% and, 6.0 %) and Sabinene (5.89% and, 6.4%)

Keywords: *Hyssopus officinalis* L., Volatile compounds, Headspace GC–MS, Beta-pinene, trans-pinocamphone, Cis-pinocamphone, Myrcene , Sabinene.

INTRODUCTION

Plants are important sources for the discovery of new products of medicinal compounds for drug development and plants secondary metabolites are unique sources for pharmaceuticals food additives, flavors and others industrial uses either as part of a final product or as a raw material (Zhao *et al*., 2005). One of the most frequently consumed herbal remedies available today is the hyssop preparations prepared from *Hyssopus officinalis* (L.) which is gaining increased importance as a minty flavor, condiment and spices in food industries as well (Dragland *et al*., 2003; Jung *et al*., 2004; Lugasi *et al*., 2006; Wesołowska *et al*., 2010). Hyssop (*Hyssopus officinalis* L.) belongs to the Lamiaceae family and is a native plant from central and Southern Europe, Western Asia, and North Africa (Mitić and Đorđević, 2000;

Lawless, 2002). The plant is a typical xerophyte and is well adapted to drought and low input conditions (Hornok, 1992). Medicinal herb, hyssop has a long history of medicinal use as viral infections such as colds, coughs, sore throats, bronchitis and asthma. The oil is antimicrobial, mildly spasmolytic and exhibit strong antiviral activity against HIV (Gollapudi *et al*., 1995). Antibacterial (Michalczyk *et al*., 2012), anti-fungal (Fraternale *et al*., 2004) and antioxidant property of hyssop has been attributed to the presence of pinocamphone, iso-pinocamphone and β-pinene. Antiviral activity has probably been attributed to the presence of caffeic acid and tannins (Gollapudi *et al*., 1995; Grzeszczuk and Jadczak, 2009). Pinocamphone (trans-Pinocamphone) and iso-pinocamphone (cis-pinocamphone) are generally known as the main characteristic components of the oils of *Hyssopus officinalis* (Mazzant *et al*., 1998) contributing approximately 36 to 41% of the total extract. Furthermore, the recommended levels of β-pinene cis-pinocamphone and the second isomer of pinocamphone (trans-pinocamphone) are 13.5- 23% , 34.5 – 50% and 5.5 – 17.5 %, respectively (Mazzant *et al*., 1998). In some studies, iso-pinocamphone, pinocamphone, β-pinene and pinocarvone were reported to be the most abundant components in hyssop oil (Kizil *et al*., 2008; De Martino *et al*., 2009; Veres *et al*, 1997; Shah *et al*., 1986; Garg *et al*., 1999). Generally, Essential oils are complex mixtures of volatile, lipophilic and odiferous substances from the secondary metabolism of plants. They are mainly composed of monoterpenes, sesquiterpenes and their oxygenated derivatives.

Salma (2002) identified *H. officinalis* as a new source of essential oil in Egypt that was characterized by high content of β-pinene (19.60%), pinocamphone (19.20%) and camphor (16.3%). The highest yield of oil production was determined at the flowering stage of growth in July (Salma. 2002). Different compounds have been identified as the main component in hyssop oil by other researchers. Furthermore, the presence of aliphatic fatty acids, such as palmitic acid (15.60%), stearic acid (10.73%), linolenic acid (63.98%), arachidic acid (2.64%) and eicosadienoic acid 0.68% in the Romanian hyssop oil was determined (Benedec *et al*., 2002). Özer *et al*. (2005) analyzed the essential oil of *Hyssopus officinalis L.* subsp. angustifolius (Bieb.). The essential oil of this plant demonstrated the presence of many monoterpenes that were identified by gas chromatography; about thirty-four components were characterized, representing 91.0% of the total components detected. The main components were identified as pinocarvone, pinocamphone, β-pinene, 1,8-cineole and isopinocamphone (Hold and Sirisoma, 2002; Ozer *et al*., 2005). *H. officinalis* var. angustifolius (Persian name: "Zoofa") is grown and cultivated in some parts of Iran. The aerial parts of hyssop are used in Iranian folk medicine for their asthma, bronchitis, ulcers and wounds, carminative, antiseptic and antimicrobial (Zargari 1990; Ghasemi Pirbalouti, 2009).

Kazazi *et al*. (2007) reported that the main components of the extracts under different SFE conditions from *H. officinalis* cultivated in Iran were sabinene (4.2–17.1%), iso-pinocamphene (0.9–16.5%) and pinocamphene (0.7–13.6%). Detailed examination of the SFE of the hyssop oil was undertaken by Kazazi *et al*. (2007) at various pressures, temperatures, extraction (dynamic and static), times and modifier (methanol) concentrations. Considering the impacts of different factors during the extraction, it was shown that the composition of the extracted oils was significantly influenced by the operating conditions. Major components of

the extracts under different SFE conditions were sabinene (4.2 to 17.1%, w/w), iso-pinocamphene (0.9 to 16.5%) and pinocamphene (0.7 to 13.6%). On the whole, Volatile oil composition varies in dependence on variety, growth stage on the date of collection, climatic conditions and also is affected by extraction and isolation method, and agrotechnical factors (Benhammou *et al*., 2008; Ghalem and Mohamed, 2009; Xu *et al*., 2011). Additionally, many plants have various chemotypes that differ in their both quantitative and qualitative diversity in the composition of essential oils obtained (Varga *et al*., 1998). This work was concluted the chemical composition of essential oils from flowers and leaves of *H. officinalis* L. at flowering stage using Headspace Gas Chromatography/Mass Spectrometry.

MATERIALS AND METHODS

Plant Material

H. officinalis seeds obtained from the Pakan Seed Company, Isfahan, Iran. Were grown on 26th March, 2017, in plastic greenhouse conditions. Six seeds were sown in each plastic pot filled with clay-loamy soil and after six weeks were thinned to two healthy seedlings per pot. On May 14, 2017, the pots transferred to the Research Field of Islamic Azad University, Branch, Isfahan (Khorasgan), Iran. On July 8, 2017, healthy leaves and flowers were collected from 10 different plants for analysis of essential oils**.**

Identification of the oil components

Headspace GC/MS technique was used to analyze volatile compounds. A fresh sample was placed in a closed sampling vessel, heated using a known temperature profile, and the vapor in the vessel was sampled for analysis.

GC–MS Analysis

An Agilent model 7890 GC interfaced to a 5975C mass selective detector was used for mass spectral identification of the components of the oils. HP-5MS capillary columns (60 m × 0.25 mm × 0.25 μm film thick-ness) were used for GC. The oven temperature was maintained at 40°C for 3 min then programmed to 290°C at 5° C min−1 and remind for 5 min. The carrier gas was helium, at a flow rate of 1.3 mL min−1, and the injection volume was 250 μL. In mass spectrometry electron-impact ionization was performed at electron energy of 70 eV. MS interface temperature was 280°C, and the mode was EI. Detector voltage, mass range scan speed and interval 0.01 min (20 Hz) were, 1.66 Kv, 30 to 550 u, 2.86 scans/s, respectively.

Identification of components

The constituents of the volatile oils were also identified by comparing their GC retention indices. A mixture of aliphatic hydrocarbons (C8–C24) in hexane (Sigma–Aldrich, St. Louis,

USA) was injected under the above-mentioned temperature programmed to calculate the retention indices. Compound identification was based on the comparison of retention indices using a MS library. The NIST and Wiley spectrometer data bank was used to determine the percentage composition of the compounds.

Table1. Chemical composition (%) of essential oil in flowers and leaves.of *Hyssopus officinalis L.*

	Compound*	Molecular formula	Class	Ki Cal	%flower	% leaf
1	alpha-thujene	C10H16	MH	930	1.25	1.2
2	1R-alpha-pinene	C10H16	MH	939	1.46	2.0
3	camphene	C10H16	MH	958	0.37	0.5
4	Sabinene	C10H16	MH	981	5.89	6.4
5	beta-pinene	C10H16	MH	989	23.38	31.2
6	myrcene	C10H16	MH	994	6.01	6.0
7	α-Phellandrene	C10H16	MH	1016	0.11	0.1
8	alpha-terpinene	C10H16	MH	1026	0.98	0.3
9	limonene	C10H16	MH	1040	2.04	2.0
10	beta-phellandrene	C10H16	MH	1043	1.90	2.2
11	β-Ocimene	C10H16	MH	1053	4.74	1.7
12	gamma-terpinene	C10H16	MH	1068	1.38	0.4
13	cis-beta-terpineol	$C_{10}H_{18}O$	MH	1089	0.26	0.1
14	alpha-terpinolene	C10H16	MH	1095	0.29	0.1
15	Linalool	C10H18O	MH	1116	0.28	0.2
16	β-Thujone	C10H16O	OM	1120	0.24	0.1
17	E,E-alloocimene	C10H16	MH	1137	0.34	0.1
18	Myrtenyl methyl ether	$C_{11}H_{18}O$	MH	1166	0.33	0.5
19	trans-Pinocamphone	$C_{10}H_{16}O$	OM	1181	19.34	15.5
20	isopinocamphone	$C_{10}H_{16}O$	OM	1196	27.49	28.1
21	terpinen-4-ol	C10H18O	MH	1202	0.41	0.2
22	Myrtenol	C15H24	SH	1221	0.26	0.2
23	2,5-Dimethyl-3-methylene-hepta-1,5-diene	C10H16	MH	1342	0.24	0.1
24	α-Gurjunene	C15H24	SH	1424	0.05	-
25	β-Gurjunene	C15H24	SH	1435	0.10	0.2
26	beta-caryophyllene	$C_{15}H_{24}$	SH	1442	0.09	0.1
27	Germacrene D	C15H24	SH	1450	0.12	-
28	α-Humulene	C15H24	SH	1518	0.14	0.1
29	Oleic Acid	C18H34O2	Fatty Acyls	2077	0.33	0.2
	Total percentage				99.84	99.70

*The compounds have been arranged according to retention indices relative to (C8-C24) hexane on an HP-5MS capillary column. Ki: Kovatz retention indices given in the literature, MH: Monoterpene hydrocarbons, OM: Oxygenated monoterpene, SH; Sesquiterpene hydrocarbons

RESULTS AND DISCUSSION

The constituents of the obtained essential oils of *Hyssopus officinalis* L. are presented in Table 1. The Headspace GC/MS analysis method revealed several monoterpenoid hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpenoid hydrocarbons (SH). Twenty nine constituents were identified in the essential oil of flowers. The major components were beta-pinene (23.38%), trans-Pinocamphone (19.34%), Cis-pinocamphone (27.49%), myrcene (6.01%) and Sabinene (5.89%). Twenty seven constituents were identified in the essential oil of the leaves and the major components were the same as of the flowers (31.2; 15.5; 28.1; 6.0 and 6.4%, respectively). It seems that the geographical origin of *H. officinalis* L. greatly influences the oil quality. The essential oil of *H. officinalis* L. plant has been widely studied in Iran and other countries but the chemical composition of the essential oil of *H. officinalis* grown in Esfahan province is yet to be determined.

The results of present study showed that the major oil constituents of the flowers and leaves of *H. officinalis* L. from Esfahan province, Iran were beta-pinene, trans-Pinocamphone and Cis-pinocamphone. In 1997, Veres *et al*. found that the oils from nine collections of *H. officinalis* grown from seed of different sources could be categorized depending upon their percentage composition of beta-pinene, limonene, pinocamphone and isopinocamphone. The oils were rich in isopinocamphone (5-50%), pinocamhone (3-50%) or contained beta-pinene and limonene (1-60%) as major the components. According to the ISO 9847/1991 standard, commercial oil should contain 40-67.5 % monoterpene ketones and 13.5-23.0 % β-pinene (Mazzanti *et al*. 1998). In this study, the values obtained for flowers were 46.83 % and 23.38 % for monoterpene ketones β-pinene in flowers and 43.6 % and 31.2 % β-pinene in leaves respectively. Figueredo *et al*. (2012) revealed that the major constituents of *Hyssopus officinalis* grown in Turkey were pinocarvone (29.2 %), trans-Pinocamphone (27.2 %), β-pinene (17.6 %), cis-pinocamphone (4.7 %) and myrcene (2.92 %). The literature data show that cispinocamphone compound can be predominate in hyssop oil, simultaneously with a low content of trans-pinocamphone (Mazzanti *et al*. 1998, Baj *et al*. 2010, Wesołowska *et al*. 2010). According to this, the examined sample belong to oils rich in β-pinene, cis-pinocamphone and transpinocamphone. Our results were in accordance with the most of the previously published. Cis-pinocamphone and transpinocamphone were the dominant constituents in hyssop oil in the studies of Mitić and Dordević (2000), Fraternale *et al*. (2004), Rosłon *et al*. (2002), Rey *et al*. (2004), and Zheljazkov *et al*. (2012). Mitić and Dordević (2000) showed that the content of cispinocamphone was at a level of 44.7%, whereas the content of trans–pinocamphone were lower (14.1%) than the present study. In this study, a higher amounts of β-pinene was found in flowers and leaves (23.38% and 31.2%, respectively). The oils from hyssop plants grown in Italy (Mazzanti *et al*. 1998, Fraternale *et al*., 2004), India (Garg *et al*., 1999), Egypt (Salma., 2002) and Hungary (Rey *et al*., 2004) were rich in β-pinene.

The other ther important compounds which were indentied in our study were β-ocimene, limonene, α-phellandrene, gamma-terpinene. The components with small amount were β-

gurjunene, myrtenol, α-terpinolene, β-caryophyllene, linalool and camphene. Furthermore, 0.33% and 0.2% of in the were determined oleic acid flowers and leaves, respectively.

CONCLUSION

Our results demonstrated that in the flowering stage of officinalis L. of the major constituents grown in Isafahan essential oil from the flowers and leaves, respectively were β-pinene (23.38% and, 31.2%), trans-Pinocamphone (19.34%, 15.5%), cis-pinocamphone (27.49%, 28.1%), myrcene (6.01%, 6.0 %) and Sabinene (5.89%, 6.4%).

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