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Essential Oil Composition of *Hyssopus officinalis* L. Cultivated in Egypt

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Abstract

Hyssopus officinalis L. belonging to the family Lamiaceaeis a perennial herb known as a culinary and medicinal herb. The chemical constituents of essential oil by hydro distillation from the aerial parts of *Hyssopus officinalis* L. from Egypt were identified by gas chromatograph-mass spectrometer (GC-MS) demonstrated the presence of 33 compounds. The major constituents of *H. officinalis* oil were cis-pinocamphone (26.85 %), β-pinene (20.43 %), trans-pinocamphone (15.97%), α-elemol (7.96 %), durenol (3.11%), β-phellandrene (2.41%), caryophyllene (2.34%), (E)-2,6-dimethyl-1,3,5,7-octatetraene (2.27%), 3(10)-caren-4-ol,acetoacetic acid ester (2.14%), bicyclogermacrene (1.83%), myrtenol (1.73%), germacrene-D (1.68%), limonene (1.56%), γ-eudesmol (1.36%) and linalool (1.08%).

Keywords

Hyssopus officinalis, Essential Oil, Cis-Pinocamphone, β-Pinene, Trans-Pinocamphone

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1. Introduction

Hyssopus officinalis L. is an important medicinal plant native to central and Southern Europe, Western Asia, and North Africa. The herb is an evergreen perennial plant with small, linear leaves and purplish-blue flowers (Lawless, 2002). It is commonly used in folk medicine. Hyssop oil may be found as flavour ingredient in many food products. The oil is a fragrance component in soaps, cosmetics and perfumes (Wesołowska et al., 2010). Hyssop, rich in volatile oil, flavonoids, tannins, and marrubin, has been used as a healing herb to alleviate digestive disorders, cure laryngitis, or accelerate wound healing in Turkish folk medicine. It relaxes peripheral blood vessels and promotes sweating. It is also used as an expectorant, carminative, anti-inflammatory, anticatarrhal, and antispasmodic in traditional medicine in many parts of the world (Kizil et al., 2008). It is further reported that certain fractions of hyssop (one being a polysaccharide designated as MAR-10) may inhibit the activity of the human immunodeficiency virus (HIV) (Kreis et al., 1990; Gollapudi et al., 1995).

As a medicinal herb, hyssop is used in viral infections such as colds, coughs, sore throats, bronchitis and asthma. A tea made from the herb is effective in nervous disorders and toothache (Wesołowska et al., 2010). The oil is antimicrobial, mildly spasmolytic and exhibit strong antiviral activity against HIV (Kaplan et al., 1990). Antibacterial, antifungal 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 (Kaplan et al., 1990;

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Letessier et al., 2001; Ozer et al., 2006).Moreover, fresh herb of hyssop is also characterized by a high content of vitamin C (Grzeszczuk and Jadczak, 2009). Hyssop has soothing, expectorant, and cough suppressant properties. The plant also includes the chemicals thujone and phenol, which give it antiseptic properties. Antimicrobial, antifungal, antiprotozoal and anticancer effects of Hyssop extract have been also reported (Kermanjani et al., 2015).

Several essential oils have been used as therapeutic agents since ancient times, and some of them have been scientifically proven to possess medicinal properties, including anti-inflammatory (Koudou et al., 2005), antiviral (Loizzoa et al., 2008), antitumor (M'Barek et al., 2007), cytotoxic (Zarai et al., 2011) and antimicrobial activities (Alviano and Alviano, 2009). 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 (alcohols, aldehydes, esters, ketones, phenols and oxides).The bicyclic monoterpene ketones pinocamphone (trans-pinocamphone) and iso-pinocamphone (cis-pinocamphone) are generally known as the main characteristic components of the oils of *Hyssopusofficinalis* (Mazzant et al., 1998) contributing approximately 36 to 41% of the total extract, which have to betaken into account due to their toxicity problems. Furthermore, the level of cis-pinocamphone recommends 34.5-50%, 5.5-17.5% of of the second isomer of pinocamphone (trans-pinocamphone) and 13.5-23% of β-pinene (Mazzant et al., 1998). Pinenes and bicyclic terpenes can be found in the essential oils of coniferous trees (pine), rosemary, lavender, and turpentine (da Silva et al., 2012). These compounds may exhibit differences in toxicity and biological activity (Tabanca et al., 2007; Yang et al., 2011). Pinenes have two active constitutional isomers: α- and β-pinene. The racemic mixture is present in some essential oils, such as eucalyptus oil (da Silva et al., 2012; Tabanca et al., 2007; Yang et al., 2011). Pinenes show fungicidal activity and have been used for centuries to produce flavors and fragrances. Several biological activities are associated with pinenes, including use as a natural insecticide and antimicrobial activity (da Silva et al., 2012).

Garg et al. (1999) found that the main volatile constituents of *H. officinalis* essential oil from a variety of locations included β-pinene, limonene, β-phellandrene, 1,8-cineole, pinocamphone,iso-pinocamphone, pinocarvone, germacrene-D and methyleugenol. They also found that the oils extracted from different subspecies or plant populations of varying origin or morphology differed in percentage composition of the major volatile constituents. Similarly, Kizil et al. (2008) found that the main compounds of the hyssop oil were iso-pinocamphone, β-pinene, 4-carvomenthenol, γ-terpinene, carvacrol, and pinocarvone, and these six components together constituted 75-81% of total essential oil. It is well-known that most spices possess a wide range of biological and pharmacological activities. These volatile compounds are widely used in cosmetics as fragrance components, in the food industry to improve the aroma and the organoleptic properties of different types of foods, and in a variety of household products. In addition to their particular aroma, many essential oils and their isolated components also exhibit muscle relaxant, antibacterial and antifungal activities (De Martino et al., 2009).

In some studies, the major components were iso-pinocamphone (57.27%), β-pinene (7.23%), terpinene-4-ol (7.13%), pinocarvone (6.49%), carvacrole (3.02%), p-cymene (2.81%) and pinocamphone (2.59%). These seven components constitute 86.54% of total oil (Kizil et al., 2010). In the literature, 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). The composition of the essential oil of *H. officinalis* has been examined previously by Svoboda et al. (1993), Veres et al. (1997), Vallejo et al. (1995), Mazzanti et al. (1998), Garg et al. (1999), Jankovsky and Landa (2002), Mitic and Dordevic (2000), Ozer et al. (2005) and Kizil et al. (2008). Veres et al. (1997) reported that hyssop essential oil could be categorised depending upon their percentage composition of β-pinene, limonene, pinocamphone, and iso-pinocamphone. These results are in agreement with some of the previously published data. Different compounds have been identified as the main component in hyssop oil by other researchers: methyleugenol (38%) by Gorunovic et al. (1995), 1,8-cineole (53%) by Vallejo et al. (1995), pinocamphone (49.1%) by Garg et al. (1999), iso pinocamphone by Mitic and Dordevic (2000), and pinocarvone (36.3%) by Ozer et al. (2005).

The aim of this paper was to determine the composition of the oil of *Hyssopus officinalis* cultivated in Egypt andcompare our results to those previously published.

2. Materials and Methods

2.1. Plant Material

Seeds of *Hyssopus officinalis* L. were obtained from the HEM ZADEN B.V - P.O. Box 4 - 1606 ZG Venhuizen - The Netherlands. Seeds were sown in the nursery on 25th November, 2013. On February 10, 2014, uniform seedlings were transplanted into the experimental farm of the Faculty of Pharmacy, Cairo University, Giza, Egypt, which represents clay-loamy soil. The fresh herb was collected at the end of July.

2.2. Gas Chromatography/Mass Spectrometry

Extraction of essential oil: Fresh herb (100 g) was extracted by water distillation using Clevenger apparatus for 2 h according to Guenther (1961).

GC/MS Analytical Condition: The volatile oil analysis was carried out using gas chromatography-mass spectrometry instrument stands at the Central Laboratories, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer).The GC/MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 40 ^{o}C for 1 min; rising at 4.0 ^{o}C/min to 160 ^{o}C and held for 6 min; rising at 6 ^{o}C/min to 210 ^{o}C and held for 1min. The injector and detector were held at 210 ^{o}C. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using two different analytical methods: relative retention time and mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

3. Results and Discussions

The essential oil of *Hyssopus officinalis* L. growing in Egypt was subjected to detailed GC/MS analysis. Exactly 33 compounds, mostly aromatic, were identified, representing 99.99 % of the total oil. The major compounds were cis-pinocamphone 26.85%, β-pinene 20.43% and trans-pinocamphone (15.97%). Other important compounds were α-elemol 7.96%, trans-pinocamphone 5.99%, durenol 3.11%, β-phellandrene 2.41%, caryophyllene 2.34%, (E)-2,6-dimethyl1,3,5,7-octatetraene 2.27%, 3(10)-caren-4-ol,acetoacetic acid ester 2.14%, bicyclogermacrene 1.83%, myrtenol (α-pinene-10-ol) 1.73%, germacrene-D 1.68%, limonene 1.56%, γ-eudesmol 1.36% and linalool 1.08% (Table 1). In Poland, iso-pinocamphone was the dominating component (40.07–45.45%) in the all analysed oil samples (Wesołowska et al., 2010). Also in Turkey, iso-pinocamphone was the dominating component (47.9–51.4%) in the all analysed oil samples (Kizil et al., 2008). In Serbia and Montenegro, Glamoclijaet al.(2005) found thatthe most abundant components in oil are iso-pinocamphone (43.29%), pinocamphone (16.79%) and β-pinene (16.31%). Another study in Poland, (Zawiślak, 2013) reported that main components of hyssop essential oil were: cis-pinocamphone (33.52-37.13%), trans-pinocamphone (23.43-28.67%), β-pinene (7.89-8.12%), elemol (5.86-8.95%), germacrene-D (3.23-4.65%), E-caryophyllene, (2.67%), β-phellandrene (2.17-2.54%), sabinene (2.08%), β-myrtenol (2.11%). 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 %).

Table 1. Principal constituents of *Hyssopus officinalis* essential oil

Compound	%	Compound	%
β-pinene	20.43	nerol	0.20
limonene	1.56	myrtenol	1.73
β-phellandrene	2.41	geraniol	0.28
β-ocimene	0.89	citral	0.13
3(10)-caren-4-ol, acetoacetic acid ester	2.14	guaia-1(10),11-diene	0.12
(E)-2,6-dimethyl-1,3,5,7-octatetraene	2.27	methyleugenol	0.90
1,5,5-trimethyl-6-methylene-cyclohexene	0.83	α-elemol	7.96
α-gurjinene	0.23	caryophyllene oxide	0.39
linalool	1.08	durenol	3.11
caryophyllene	2.34	spathulenol	0.27
trans-pinocamphone	15.97	(+)-epi bicyclosesquiphellandrene	0.41
cis-pinocamphone	26.85	γ-eudesmol	1.36
α-humulene	0.48	α-cadinol	0.16
α-terpineol	0.66	α-eudesmol	0.41
germacrene D	1.68	β-eudesmol	0.61
bicyclogermacrene	1.83	α-gurjenene	0.30
		Total Identified Compounds	99.99

4. Conclusion

It can be concluded that the major constituents of *Hyssopus officinalis* L. essential oil grown in Egypt from the aerial parts were cis-pinocamphone, β-pinene, and trans-pinocamphone.

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