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Comparative evaluation of the essential oil composition from the leaves and flowers of *Hyssopus officinalis* L.

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The chemical constituents of essential oil were obtained from the fresh leaves and flowers of *Hyssopus officinalis* L. (Lamiaceae) by hydro distillation method analyzed by gas chromatography and gas chromatography mass spectrometry. Hydro distillation method was used to extract the essential oil. Thirty-five compounds were identified, accounting for 92.13% of the total oil with 0.75% (v/w) oil yield in the essential oil of the leaves. The main constituents of the essential oil were Iso pinocamphone (38.47%), Pinocomphone (13.32%), n-decane (8.67%) and Pinocarvone (5.34%). Thirty-six compounds were identified, accounting for 98.68% of the total oil with 1.38% (v/w) oil yield in the essential oil of the flowers. The main constituents of the essential oil were Iso pinocamphone (40.25%), Pinocomphone (14.92%), n-decane (8.63%) and Pinocarvone (6.76%).

Key words: *Hyssopus officinalis* L., iso pinocamphone, volatile oil, gas chromatography mass spectrometry (GC/MS).

INTRODUCTION

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. *H. officinalis* L. is an evergreen Shrub growing to 0.6 m (2ft) by 0.6 m (2ft in). This plant can be grown for ground cover when spaced about 45 cm apart each way. It is hardy to zone 7. It is in leaf 12-January. It is the in flower of July to September, and its seeds ripen from August to October. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Bees. Hyssop is a brightly colored shrub or subshrub that ranges from 30 to 60 cm in height. The stem is woody at the base, a number of straight branches are grown from it. Its leaves are lanceolate, dark green in color, and from 2 to 2.5 cm long. During the summer, the plant produces bunches of pink, blue, or, more rarely, white fragrant flowers. These give rise to small oblong achenes. The species as a whole is resistant to drought, and tolerant of chalky, sandy soils. It thrives in full sun and warm climates. The plant is commonly used by beekeepers to produce a rich and aromatic honey. Herb hyssop leaves are used as an aromatic condiment. The leaves have a lightly bitter taste due to its tannins, and an intense minty aroma. It is used moderately in cooking due to its intensity. The herb is also used to flavor liqueur, and is part of the official formulation of Chartreuse. The plant also includes the chemicals thujone and phenol, which give it antiseptic properties (Van Wyk and Michael, 2004). A strongly aromatic flavor, somewhat like a cross between sage and mint, it has fallen out of flavor in the recent years. It has a positive effect when used to treat bronchitis and respiratory infections, especially where there is excessive mucous production (Chevallier, 2001). Hyssop can irritate the mucous membranes, so it is the best given after an infection has peaked, when the herb's tonic action

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encourages a general recovery (Chiej, 1984). The leaves and flowering tops are antiseptic, antitussive, astringent, carminative, diaphoretic, emmenagogue, expectorant, pectoral, sedative, stimulant, stomachic, tonic and vasodilator. It is commonly used as an aromatic herb and medicinal plant (Mills and Bone, 2005). The plant can be harvested when in full flower and dried for later use. A tea made from the leaves is used in the treatment of flatulence, stomach-aches, upper respiratory tract infections, coughs in children etc. A poultice made from the fresh herb is used to heal wounds. The essential oil is used in aromatherapy. This oil should not be used on the people who are highly strung as it can cause epileptic symptoms. The essential oil should not be used internally except under professional supervision.

Hyssop can be grown as a dwarf hedge, it responds well to trimming in the spring. The growing plant attracts cabbage white butterflies away from brassicas. Another report says that hyssop attracts cabbage white butterflies and should not be grown near cabbages. An essential oil from the leaves is antiseptic and also used in perfumery and as a food flavoring (Huxley, 1992). It has a particularly fine odor and is much valued by perfumers. Average yield of the oil is about 0.6%. Yields from the blue-flowered variety are circa 1 to 1.5% of essential oil, the red-flowered variety yields about 0.8%, whilst the white-flowered form yields 0.5% of essential oil. The plant was formerly used as a strewing herb (Huxley, 1992) and is also used in pot-pourri. A tea made from the leaves is useful for controlling bacterial plant diseases. The essential oil of hyssop is widely used for food, pharmaceutical and cosmetic industries throughout the world. Therefore, it is very important to know the chemical characteristics of the oil for economic use and enhanced performance of the end products. This study was carried out to determine the essential oil of *H. officinalis* (L.) (Lamiaceae) collected from wild in the Khalkhal, Southeast Ardabil Province in Iran. The essential oil of *H. officinalis* plant has been studied in Iran and other countries but the chemical composition of the essential oil of *H. officinalis* has not been determined in Khalkhal, Iran. In the present work we have analyzed the chemical composition of the leaves and the flowers of *H. officinalis* L. that grow in Khalkhal and then the results were compared with various origins in other countries.

MATERIALS AND METHODS

Plant material collection and isolation of their essential oil

The leaves and flowers of *H. officinalis* were obtained from Khalkhal in Ardabil Province from 1843 m height, at full flowering stage in June 2012. The samples were cleaned in the shade condition to prevent volatility of the plant material constituents and to keep the natural color of the sample fixed. Then they were air-dried and powdered using a milling machine and kept in a cool dry place until ready for extraction of the essential oil. Afterwards, essential oil was extracted from 150 g of t he powdered sample using hydro-distillation method with the help of Clevenger set for three hours. Following, the oil samples were dried using anhydrous sodium sulfate and kept in sterile sample tubes in refrigerator. The oil yields of leaves and flowers were calculated.

Analysis of essential oil

Gas chromatography

GC analysis was performed using a model of HP-439 gas chromatograph equipped with column CP Sil. 5CB in 25 meters length, internal diameter of 0.25 mm and film thickness 0.39 µm. Oven temperature was between 60 and 220°C at a rate of 7°C slope per minute. The injector temperature was 280°C and detector (FID) temperature was 270°C while the carrier gas was helium.

Gas chromatography/mass mass spectrometry

The chromatograph gas set was attached to a Mass Spectrometry to analyze and identify the combinations forming the essential oil, Model Hewlett Packard-5973 was used for this purpose. The conditions of analysis and specifications of the GC/MC set were as follows: Capillary column HP 5MS in 60 meters length, internal diameter of 0.25 mm and layer thickness of 0.25 µm, thermal program of oven (3 min) in 60°C, then 60 to 220°C with a 6°C slope per minute, then 3 min in 220°C, the temperature of place of injection 280°C, gas conveying helium, the speed of gas move 1.0 milliliter per minute, the ratio of fission 1 to 43, the rate of injection 0.1 µl, temperature of the reservoir of ionization 230°C, ionization mode EI, with an Ionization energy of 70eV. The series of normal Alkans C8-C17 were also injected to the set under the same condition with that of essential oil injection to calculate Restrictive Index (RI) of components of essential oil. The RI of components of the sample was calculated by using a computerized program. Finally, the components of essential oil were identified by comparing the mass spectrums obtained using the existing standard mass spectrums at electronic library of Wiley 2000 existing in Absolution software of GC/Ms set and calculation of standard RI in accordance with C_8-C_{17} Alkanes and by comparing them with the existing standard figures in references (Adams, 2001).

RESULTS AND DISCUSSION

The identified combinations of essential oil, RI, and quantitative percentage of the compounds from seeds and flowers are listed in Table 1. The study of the analysis of *H. officinalis* L. essential oil under investigation showed that thirty-five compounds, accounting for 92.13% of the total oil with 0.75% (v/w) oil yield were identified in the essential oil of the leaves. The main constituents of the essential oil were *Iso* pinocamphone (38.47%), Pinocomphone (13.32%), n-decane (8.67%) and Pinocarvone (5.34%) with 65.8% constituting the highest percentage of essential oil. Also from thirty-six compounds, accounting for 98.68% of the total oil was identified in the essential oil of the flowers with 1.38% (v/w) oil yields. The main constituents of the essential oil were *Iso* pinocamphone (40.25%), Pinocomphone (14.92%), n-decane (8.63%) and Pinocarvone (6.76%) with 70.56% constituting the highest

Table 1. Combinations identified in the essential oil of *Hyssopus officinalis* L.

Compound name	(RI)	Leave (%)	Flower (%)
α-thujene	928	0.32	0.59
α-pinene	935	1.14	1.27
Sabinene	978	0.97	0.28
β-pinene	987	1.78	1.64
Myrcene	992	-	0.61
n-decane	997	8.67	8.63
δ-3-carene	1012	0.85	1.23
P-cymene	1029	0.45	0.68
Limonene	1035	0.47	0.33
1,8-cineole	1038	0.56	1.69
(Z)-β-ocimene	1045	1.27	0.95
(E)-β-ocimene	1054	0.75	0.48
Linalool	1107	0.53	0.67
Camphor	1136	0.65	0.42
Pinocomphone	1158	13.32	14.92
Pinocarvone	1165	5.34	6.76
Iso pinocamphone	1178	38.47	40.25
Terpinen-4-ol	1192	1.78	1.89
α-terpineol	1203	0.25	0.34
Myrtenol	1212	1.75	1.65
Carvon	1236	0.27	0.44
Cumin aldehyde	1245	0.68	0.45
Piperitone	1263	1.65	1.34
Thymol	1289	0.45	0.89
Carvacrol	1306	2.54	2.97
Eugenol	1348	0.89	0.54
β-bourbonene	1375	1.63	1.27
Methyl eugenol	1396	0.21	0.46
α-gurjunene	1417	0.39	0.34
β-caryophyllene	1425	0.32	0.41
α-humulene	1466	0.18	-
Allo aromadendrene	1467	0.67	0.24
Germacrene D	1482	0.57	0.68
Elemol	1537	0.53	0.78
Spathulenol	1568	-	0.16
Caryophyllene oxide	1582	0.48	0.67
α-bisabolol	1675	1.35	1.76
Total		92.13	98.68

The indexes of restrictive have been calculated by injecting the mixture of normal hydrocarbons (C8-C17) to HP-5MS column.

percentage of essential oil. The quality and quantity of the materials forming *H. officinalis* L. essential oil had some differences and similarities with the cases reported in other regions. The studies of essential oils ingredients for botanical populations with ecological and genetic differences can be of great importance in identifying the variety of essential oils inside the population of species. 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 Ardabil province is yet to be determined. The present study, results showed the major oil constituents of the leaves and the flowers of *H. officinalis* L. from Ardabil province, Iran were *Iso* pinocamphone and Pinocomphone. In a study they were the chemical compositions of essential oil of aerial part of *H. officinalis* obtained from the hydrodistillation. The chemical composition of essential oil was determined using gas chromatography/mass spectrometry (GC/ MS).

Thirty nine components were identified in *H. officinalis* oil that include thymol (18.95%), fl-bisabolol (10.62%), carvacrol (7.73%), n–Dodecan (5.23%), caryophyllene (4.96%), ortho–acetanisol (4.72%), camphor (3.47%), cumin aldehyde (3.22%) and spathulenol (3.02%) as major components in essential oil (Dehghanzadeh et al., 2012). In a research the plants of *H. officinalis* ssp. *officinalis* genotype raised through seeds sown in early December 1997 flowered in May 1998. The essential oil yields obtained upon hydrodistillation of above ground parts, harvested in May, were 0.25% on fresh herbage weight basis and 1.18% on dry herbage weight basis.

The GC and GC–MS analysis of the essential oil led to the identification of 21 compounds representing 95.6% of the oil, having seven monoterpene hydrocarbons (32.3%), five oxygenated monoterpenes (60.5%) one phenol (0.2%) and six sesquiterpene hydrocarbons (0.35%). The major constituents of the camphorous odoured oil were pinocamphone (49.1%) >β-pinene (18.4%) >isopinocamphone (9.7%) (Garg et al., 1999). The oil obtained by hydrodistillation from the aerial parts of *H. officinalis* L. subsp. *angustifolius* (Bieb.) Arcangeli from Turkey was analyzed by GC–MS. Thirty-four components were characterized, representing 91.0% of the total components detected. The major constituents were identified as pinocarvone (36.3%), pinocamphone (19.6%), *β*-pinene (10.6%), 1,8-cineole (7.2%) and isopinocamphone (5.3%) (Ozer et al., 2005). The essential oil from *H. officinalis* grown in Spain was examined by GC and GC/MS. The oil was characterized by a high content of 1,8-cineole (52.89%) and β-pinene (16.82%) as the main components (Vallejo et al., 1995).

In a comparison study of Hyssop (*H. officinalis* L.), native to the Caucasus, North Western Iran, Turkish North Eastern Black Sea region, and Southern Anatolia, it is a highly valued medicinal plant. The experiment was conducted to find the effect of harvesting at different blooming stages of the plant on fresh and dry herbage yield, dry leaf yield, essential oil content, and essential oil components.

In total, twenty-nine components were identified in hyssop essential oil by GC/MS. Isopinocamphone was the dominating component (47.9 to 51.4%) in all analyzed oil samples. The results clearly demonstrated that oil contents are seriously affected by the environmental conditions and stage of blooming, with the highest oil yield and oil contents at the post-blooming stage (Kizil et al., 2008). In a study the essential oil was obtained (0.66%) from the aerial plant parts of wild *H. officinalis* L., collected around Petnjica (Montenegro). It was examined by a combination of GC and GC/MS. Fifty-seven constituents were found, out of which the major ones were methyl eugenol (38.3%), limonene (37.4%) and β-pinene (9.6%) (Gorunovic et al., 1995). In a research the essential oils were obtained from wild growing *H. officinalis* L. ssp. *aristatus* (Godr.) Briq. At two stages of development, they are found to be very similar in composition with 1,8-cineole (48.2 and 39.6%), isopinocamphone (16.3 and 29.2%) and β-pinene (11.4 and 39.6%) as major constituents. The essential oil obtained commercially from cultivated *H. officinalis* contains larger amounts of isopinocamphone (40.2%), pinocamphone (10.3%), and β-pinene (14.2%), but no traces of 1,8-cineole (Tsankova et al., 1993). The essential oils from different parts of hyssop (*H. officinalis* L.) were investigated by means of GC and GC-MS at three developmental stages of plant. 15 other terpenes were detected besides the main components pinocamphone, camphor and β-pinene, that included isopinocamphone, α and β-phellandrene, germacrene D, and some derivatives of myrtenol. The sesquiterpene alcohol hedycaryol was found to be converted to elemol during GC and MS analysis. As compared with the essential oil content (0.03 to 0.16% of the fresh plant material), the glycosidic bound volatiles were present in lower concentrations (0.01 to 0.06%).

The glycosidic fraction was hydrolysed by means of Pectinol C and β-glucosidase yielding among others octan-3-ol, linalol, cis-nerolidol, benzyl alcohol, phenylethanol, eugenol and o-vanillin. The bicyclic terpenes myrtenol and verbenol could only be detected in small amounts as glycosides of the leaves. This fact gives reason for doubt about a direct connection between the glycosidic bound volatiles and the biogenesis of the essential oil components in hyssop (Schulz and Stahl-Biskup, 1991). Three forms of hyssop *H. officinalis* L., f. *cyaneus* Alef. f. *ruber* Mill, and f. *albus* Alef. exist in the wild of Yugoslavia that were multiplied and cultivated. The *cyaneus* form, characterized by its blue flowers, yielded between 4.9 and 5.8 tonnes of fresh plant material per hectare, and essential oil in yields ranging from 0.65 to 0.75%. The pink-flowered *ruber* form and the white flowered *albus*, respectively, yielded 3.9 to 5.1 tonnes/ha and 4.5 to 6.5 tonnes/ha of fresh plant material in yields of 0.7 to 1.1% and 0.6 to 1.0%. The chemical analysis of different batches of oils produced shows that they are mainly composed of *cis* and *trans*-pinocamphone, and pinocarvone, together with lesser amounts of germacrene D, bicyclogermacrene, elemol and spathulenol (Chalchat et al., 2001). Three Italian strains of hyssop wildly grown in different natural habitats of the Abruzzi region (Central Italy) and classified as *H. officinalis* L. subsp. *aristatus*, were characterized on the basis of their essential oil composition. The oils were obtained by steam distillation of the fresh aerial parts of the plants and analyzed by GC and GC/MS. Thirty-three compounds were identified in this connection. Relevant differences in the quantitative composition were observed among the oils so that the existence of three different chemotypes could be realized.

In particular, one of the strains was characterized by high contents of myrtenol (32.6%) and β-pinene (193%), another contained β-pinene (24.7%) and 1,8-cineole (231%) as the main components, while the third was very rich of methyl eugenol (439%) and limonene (15.9%).

Whereas the latter two strains were similar in composition of oils from Spain and Montenegro, respectively, the former possessed a very unusual oil composition (Piccaglia et al., 1999).

In a study the leaves gathered near Khandiza (Uzbek SSR) in 1986 yielded 0.34% essential oil, that is, only half of the quantity expected by literature. Its composition was investigated by GC and chromato-mass spectrometry, and is tabulated in comparison with that of Zotov (1974). The main constituents were identified as pinocamphone (71%), β-pinene (8.6%) and 1,8-cineole (6.4%). Sabinene content was only 1.3%, with no limonene or isopinocamphone (respectively 16.3, 13.8 and 12.1% according to Zotov) (Dzhumaev et al., 1990). The chemical composition of *H. officinalis* (Lamiaceae) essential oil grown in southeastern Spain was analyzed by GC-MS.

The study is focused on chemical heterogeneity of different oil batches and their extraction yield due to the high relevance of this species in the world market, cultivated under irrigation and non-irrigation conditions and with different harvesting dates.

All essential oil samples have two main terpene compounds which are pinocamphone and *iso*-pinocamphone, accounting for approximately 35 to 40% of the total oil contents. Other relevant compounds were identified, with *β*-pinene, which accounted for 10 to 17% contribution to the total composition, standing out. Significant differences between their volatile compositions have been observed between treatments, being limonene, (E)-*β*-ocimene, pinocarveol, *α*-pinene and *β*-phellandrene the compounds that contributed most to discrimination.

It was also observed that the irrigation period is the most favorable for the cultivation of hyssop in this region, especially for batch 7 which gives the highest extraction yield and the best EO quality (Moro et al., 2011). A study was carried out to determine the essential oil of *H. officinalis* (L.) (*Lamiaceae*) collected from the wild of Southeast Anatolian, Turkey.

Chemical compositions of hydrodistilled essential oils obtained from hyssop leaves were analyzed by GC-MS. It was determined that hyssop essential oil contained *iso*pinocamphone (57.27%), (-)-β-pinene (7.23%), (-)-terpinen-4-ol (7.13%), pinocarvone (6.49%), carvacrol (3.02%), *p*-cymene (2.81%) and myrtenal (2.32%) as its major components (Kizil et al., 2010). The composition of *H. officinalis* L. essential oil was analyzed by GC and GC/MS. The main components of the oil were cis-pinocamphone (42.9%), trans-pinocamphone (14.1%), germacrene-D-11-ol (5.7%) and elemol (5.6%) (Mitic and Dordevic, 2000).

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