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# DETERMINATION AND SEASONAL VARIATION OF CHEMICAL CONSTITUENTS OF ESSENTIAL OIL OF *Hyssopus officinalis* GROWING IN KASHMIR VALLEY AS INCORPORATED SPECIES OF WESTERN HIMALAYA

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*Hyssopus* (Labiatae) is a small genus of undershrubs, distributed mainly in Central Asia, South Europe, and North Africa. It comprises about 15 species of which only *Hyssopus officinalis* L. occurs in India; in Kashmir it exists as exotic species. *Hyssopus officinalis* is an aromatic evergreen, shrubby and perennial. It belongs to the family Labiatae and grows to about 2 ft high; it is found in the Himalayas from Kashmir to Kumaon at an altitude of 8.000–11.000 ft.

The essential oil is a component of many liqueurs. It is also used as an ingredient in the production of many brands of colognes and perfumes. Hyssop oil finds its greatest use in flavoring alcoholic beverages, meat products, and seasonings [1, 2]. It also possesses antibacterial and antimycotic activity against fungal species of *Candida* genus and antiviral properties against herpes simplex virus [3–5]. The essential oil of *H. officinalis* was confirmed to have anthelmintic and antituberculosis activity [6], and also muscle-relaxing activity [7]. There are studies that show that some fractions isolated from *H. officinalis* can inhibit the human immunodeficiency virus (HIV) [8]. Some constituents of hyssop exhibit high antioxidant activity [9–11]. $\alpha$-Glucosidase inhibitory activity has been found in aqueous methanol extracts of dried hyssop leaves [12]. The essential oil of *hyssopus* has an influence on the chemical constituents of certain fungi spp. [13, 14], and it has been found to have antimicrobial and insecticidal activities [15].

Hydrodistillation of the aerial parts of a composite sample of *Hyssopus officinalis* gave a pleasant-smelling oil [0.4% (v/w)] on a fresh weight basis. Qualitative and quantitative analyses of this oil were performed by GC and GC/MS, which enabled identification of 33 constituents, out of which 89.10% were monoterpenoids and 8.46% were sesquiterpenoids. Out of the 89.10% of monoterpenoids, 23.83% were monoterpene hydrocarbons and 65.27% were oxygenated monoterpenoids, and out of the 8.46% of sesquiterpenoids, 3.83% were sesquiterpene hydrocarbons and 4.63% were oxygenated sesquiterpenoids. One phenyl propanoid derivative, methyl eugenol, was found in trace amount. Pinocamphone was the major compound (53.54%), followed by $\beta$-pinene (9.91%) and limonene (7.19%).

Although all the compounds were identified by GC and GC-MS, the structure of pinocamphone was further confirmed by $^1$H NMR spectrum. Pinocamphone is in great demand in perfumery, especially in liqueurs, because of its warm camphoraceous slightly spicy odor of moderate to weak tenacity. The chemical composition and relative concentrations of the volatile components of the oil sample are presented in Table 1.

Fresh samples of the aerial parts of *Hyssopus officinalis* cultivated in IIIM-Srinagar were subjected to hydrodistillation in a conventional Clevenger-type apparatus for 3 hours. The oil obtained was dried over anhydrous sodium sulfate and stored at 4°C prior to analysis.

The essential oil was obtained by the hydrodistillation of fresh plant material in a Clevenger-type apparatus for 4 h. The sample afforded a white viscous oil with a characteristic floral woody scent (yield 0.4%). The oil was dried over anhydrous $Na_2SO_4$ and was placed at low temperature in the refrigerator until analysis.

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TABLE 1. Chemical Composition and Relative Concentrations of the Essential Oil of *H. officinalis*

| Compound | RI ($RT_x$-5) | % | Compound | RI ($RT_x$-5) | % |
|---|---|---|---|---|---|
| $\alpha$-Thujene | 931.4 | 0.28 | Pinocamphone | 1162.0 | 53.54 |
| $\alpha$-Pinene | 936.0 | 0.46 | (*Z*)-Pinocamphone | 1175.2 | 2.55 |
| Camphene | 946.0 | 0.07 | Terpinen-4-ol | 1177.0 | 2.55 |
| Sabinene | 969.8 | 1.45 | $\alpha$-Terpineol | 1186.5 | 0.26 |
| $\beta$-Pinene | 974.0 | 9.91 | Myrtenol | 1194.0 | 2.59 |
| Myrcene | 988.0 | 2.24 | Methyl eugenol | 1403.0 | 0.39 |
| $\alpha$-Phellandrene | 1003.3 | 0.06 | (*E*)-$\beta$-Caryophyllene | 1417.4 | 0.39 |
| $\alpha$-Terpinene | 1014.9 | 0.45 | $\alpha$-Humulene | 1453.0 | 0.08 |
| *p*-Cymene | 1023.4 | 0.07 | *allo*-Aromadendrene | 1461.2 | 0.50 |
| Limonene | 1027.4 | 7.19 | Germacrene-D | 1484.0 | 1.61 |
| 1,8-Cineole | 1031.5 | 0.40 | Bicyclogermacrene | 1499.5 | 1.20 |
| (*E*)-$\beta$-Ocimene | 1042.8 | 0.37 | $\gamma$-Cadinene | 1515.2 | 0.05 |
| $\gamma$-Terpinene | 1054.3 | 1.03 | Elemol | 1550.0 | 3.43 |
| (*Z*)-Sabinene hydrate | 1066.6 | 2.02 | Spathulenol | 1574.9 | 0.08 |
| Terpinolene | 1082.6 | 0.25 | 10-*epi*-$\gamma$-Eudesmol | 1619.7 | 0.31 |
| Linalool | 1095.0 | 1.04 | $\alpha$-Cadinol | 1637.5 | 0.81 |
| (*E*)-Thujone | 1115.0 | 0.32 | Total | | 97.56 |

Method of Identification – MS, RI.

Gas chromatographic analysis of the oil sample was carried out on a Perkin–Elmer Auto system XL gas chromatograph 8500 series with head space analyzer and flame ionization detector (FID) using a fused silica capillary column (30 m × 0.32 mm, film thickness 0.25 μm) coated with 5% diphenyl and 95% dimethyl polysiloxane ($RT_x$-5). Oven temperature was programmed from 60 to 230°C with injector temperature 230°C and detector temperature 250°C. Carrier gas was nitrogen at 8 psi with split ratio 1:80. The compounds were identified by comparing the Kovat retention indices (relative to C-8 to C-20 alkanes) with literature values [16]. Computer matching was made against the library spectra built using pure substances and by peak enrichment on co-injection with standard samples whenever possible.

GC-MS analysis was carried on a Varian GC-3800 capillary column VF-5 ms (60 m × 0.25 mm, film thickness 0.25 μm) coupled with a 4000 series mass detector under the following conditions: injection volume 1 μL with split ratio 1:60, helium as carrier gas at 1 mL/min constant flow mode, injector temperature 230°C, and oven temperature 40°C to 250°C at 3°C/min. Mass spectra ($EI^+$ mode, 70 eV) were recorded over the 50–500 amu range with ion source temperature 250°C. Spectrometric electronic libraries (Wiley and NIST) and mass finder library were used for identification of compounds.

The essential oil composition of *Hyssopus officinalis* from Kashmir has not been investigated earlier. The compounds in Hyssop oil growing in Kashmir that are present in noticeable amounts are myrcene (2.24%), $\gamma$-terpinene (1.03%), (*Z*)-sabinene hydrate (2.02%), linalool (1.04%), terpinen-4-ol (2.55%), germacrene-D (1.61%), and elemol (3.43%). The major constituents of the oil reported previously are pinocamphone, isopinocamphone, $\beta$-pinene, 1,8-cineole, myrtenol, and pinocarvone. On comparing our results with those reported earlier, it is evident that the composition of our sample was qualitatively somewhat similar but showed flexible quantitative differences. The studies that have been tabulated revealed that the variation in chemical composition of essential oils exists according to environment, location, elevation, and harvesting period.

Study of the seasonal variation in the essential oil content of *Hyssopus officinalis* was also carried out from March to October. The essential oil yield was affected by the harvesting stage, such that young plants at the juvenile stage of pre-blooming showed lesser oil yield relative to older plants at full blooming and post-blooming stages. Results showed that Hyssop oil has pinocamphone as the major component, and it increases gradually through the post-blooming stage. The other main component, $\beta$-pinene, first increased and then decreased after the full blooming stage. The relative variation of all chemical constituents found in the oil is shown in Table 2.

In conclusion, the harvesting stage has a significant effect on quantity and quality of the essential oil. This points to the importance of choosing a suitable harvesting stage to achieve the highest quality and quantity of essential oil.

TABLE 2. Seasonal Variation of Oil Content and Chemical Profile

| Compound | Area, % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | March (0.02) | April (0.05) | May (0.14) | June (0.1) | July (0.4) | August (0.4) | September (0.26) | October (0.22) |
| α-Thujene | 0.18 | 0.01 | 0.21 | 0.10 | 0.28 | 0.33 | 0.34 | 0.52 |
| α-Pinene | 0.44 | 1.58 | 0.97 | 0.21 | 0.46 | 0.37 | 0.46 | 0.52 |
| Camphene | 0.08 | 0.01 | – | – | 0.07 | 0.07 | 0.08 | 0.12 |
| Sabinene | 1.00 | – | – | 0.82 | 1.45 | 1.69 | 1.66 | 2.38 |
| β-Pinene | 7.18 | 32.38 | 25.77 | 6.29 | 9.91 | 7.91 | 7.69 | 6.91 |
| Myrcene | 0.75 | 2.78 | 2.55 | 1.58 | 2.24 | 2.21 | 1.95 | 2.90 |
| α-Phellandrene | – | 0.05 | 0.06 | – | 0.06 | 0.06 | 0.05 | – |
| α-Terpinene | – | – | 0.37 | 0.22 | 0.45 | 0.55 | 0.35 | 0.70 |
| *p*-Cymene | 1.81 | 0.14 | 0.09 | 0.13 | 0.07 | 0.11 | 0.18 | 0.12 |
| Limonene | 0.89 | 0.01 | 9.81 | 5.98 | 7.19 | 6.60 | 5.46 | 7.69 |
| 1,8-Cineole | – | 11.85 | 0.28 | 0.33 | 0.40 | 0.33 | 0.31 | 0.42 |
| (*E*)-β-Ocimene | – | 0.82 | 0.21 | 0.20 | 0.37 | – | – | 0.40 |
| γ-Terpinene | – | 0.33 | 0.78 | 0.82 | 1.03 | 1.19 | 0.86 | 1.39 |
| (*Z*)-Sabinene hydrate | – | 0.24 | 1.07 | 3.76 | 2.02 | 3.45 | 5.41 | 2.18 |
| Terpinolene | – | 0.13 | 0.21 | 0.18 | 0.25 | 0.28 | 0.20 | 0.33 |
| Linalool | 0.05 | – | 1.52 | 0.97 | 1.04 | 0.60 | 0.58 | 0.48 |
| (*E*)-Thujone | 0.25 | 0.01 | 0.11 | 0.31 | 0.32 | 0.38 | 0.36 | 0.45 |
| Pinocamphone | 64.88 | 10.90 | 29.59 | 50.94 | 53.54 | 55.02 | 55.16 | 60.88 |
| (*Z*)-Pinocamphone | – | 0.49 | – | 2.35 | 2.55 | – | – | – |
| Terpinen-4-ol | 8.15 | 0.71 | 6.31 | 2.66 | 2.55 | 7.71 | 8.33 | 3.87 |
| α-Terpineol | 1.16 | 0.14 | 0.24 | 0.26 | 0.26 | 0.31 | 0.29 | 0.20 |
| Myrtenol | – | – | 1.34 | 2.46 | 2.59 | 2.51 | 2.45 | 1.69 |
| Methyl eugenol | 0.15 | 0.53 | 0.39 | 0.55 | 0.39 | 0.30 | 0.26 | – |
| (*E*)-β-Caryophyllene | 0.22 | 2.81 | 1.03 | 0.65 | 0.39 | 0.20 | 0.17 | 0.09 |
| α-Humulene | 0.70 | 0.64 | 0.22 | 0.14 | 0.08 | – | – | – |
| *allo*-Aromadendrene | – | 0.98 | 0.87 | 0.63 | 0.50 | 0.49 | 0.35 | 0.18 |
| Germacrene-D | – | 11.27 | 4.50 | 2.48 | 1.61 | 0.89 | 0.71 | 0.50 |
| Bicyclogermacrene | – | 3.77 | 1.89 | 1.46 | 1.20 | 1.04 | 0.83 | 0.44 |
| γ-Cadinene | – | – | 0.12 | – | 0.05 | 0.05 | – | – |
| Elemol | – | 7.28 | 4.96 | 7.53 | 3.43 | 1.78 | 2.40 | 1.56 |
| Spathulenol | 0.51 | 0.53 | 0.04 | 0.15 | 0.08 | 0.10 | 0.16 | – |
| 10-*epi*-γ-Eudesmol | – | 0.54 | 0.57 | 0.31 | 0.31 | 0.11 | 0.08 | – |
| α-Cadinol | – | 0.63 | – | 0.30 | 0.81 | 0.11 | – | – |


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