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Compositional variation in the leaf, flower and stem essential oils of Hyssop (*Hyssopus officinalis* L.) from Western-Himalaya

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ABSTRACT

Hyssop (*Hyssopus officinalis* L.), family Lamiaceae is an important perennial culinary and medicinal plant cultivated in temperate regions of Asia, Europe and America. The hydrodistilled volatile oils derived from leaf, flower and stem of *H. officinalis*, collected from Chamoli, Uttarakhand, India (Western-Himalaya) were investigated by gas chromatography (GC–FID) and GC–mass spectrometry (GC–MS). Essential oil yield varied from 0.22% to 4.4% in the different parts of the plant. Fifty-seven constituents, representing 99.8% of the leaf oil composition; 44 constituents, representing 99.4% of the flower oil composition and 57 constituents, comprising 88.4% of the stem oil composition were identified. Major constituents of the oils were *cis*-pinocamphone (49.7–57.7%), pinocarvone (5.5–24.9%), β-pinene (5.7–9.3%), 1,8-cineole (2.9–8.0%), β-phellandrene (1.8–3.2%), myrtenyl methyl ether (2.7–3.0%), sabinene (0.8–1.9%), isopimara-9(11),15-diene (<0.05–1.9%), myrtenol (1.4–1.7%), myrcene (0.5–1.3%), and *trans*-pinocamphone (<0.05–1.3%). The comparative results clearly indicated that the leaf and stem oil compositions were quite similar in terms of *cis*-pinocamphone and pinocarvone content. However, the flower oil composition could be differentiated from the leaf and stem oils by the presence of a higher amount of pinocarvone.

1. Introduction

Hyssopus officinalis L. (Lamiaceae), popularly known as 'Hyssop', is a perennial shrub, native to southern Europe, the Mediterranean and temperate regions of Asia. In India, this species occurs from Kashmir to Kumaon at an altitude of 2440–3350 m (Hooker, 1885). The aerial parts of the plant, known as 'Zufah Yabis' in the Unani system of medicine, act as a stimulative, carminative, expectorant and antispaspodic and are used for the treatment of cough, cold, and asthma (Chopra et al., 1956). *H. officinalis* has a rich aromatic odour and strong flavour. Its extracts and oil are used in many food products such as condiments and beverages including bitters and liqueurs. In the fragrance industry the essential oil is used in soaps, cosmetics and perfumes (Wesolowska et al., 2010). Leaf extracts/essential oils of Hyssop are antimicrobial, mildly spasmolytic, antioxidant and exhibit strong antiviral

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activity against HIV (Kreis et al., 1990; Letessier et al., 2001; Ozer et al., 2006). Antibacterial and antifungal properties of Hyssop have been attributed to the presence of pinocamphone, isopinocamphone and β-pinene. Antiviral activity of the plant is probably due to the presence of caffeic acid, tannins, and unidentified high molecular weight compounds (Kreis et al., 1990; Letessier et al., 2001).

The essential oil compositions of *H. officinalis* have been investigated from different parts of the world (Schultz and Stahl-Biskup, 1991; Shah, 1991; Galambosi et al., 1993; Tsankova et al., 1993; Kerrola et al., 1994; Gorunovic et al., 1995; Garcia-Vallejo et al., 1995; Veres et al., 1997; Garg et al., 1999; Kizil et al., 2008; Wesolowska et al., 2010). Hyssop oils from different phenotypes or from different areas show considerable variability in chemical composition (Lawrence, 1992a; Piccaglia et al., 1999). In some cases, constituents such as β-phellandrene (Lawrence, 1992a), 1,8-cineole (Garcia-Vallejo et al., 1995), limonene and methyl eugenol (Gorunovic et al., 1995) have been reported to be the main constituents of the oils instead of pinocamphone and isopinocamphone.

The aim of this study was to determine and compare the compositions of the leaf, flower and stem oils of *H. officinalis* growing wild in Western Himalaya (India).

2. Materials and methods

2.1. Plant materials

The plant material of *H. officinalis* was collected from a wild population growing in Malari, district Chamoli; Garhwal region of Uttarakhand (79.829 N; 30.643 E; altitude 2761 m) during the third week of August, 2012 when the plants were in bloom. The sampling was done from five plants and combined. The plant material was authenticated at the Botany Department of the CSIR-CIMAP, Research Centre Pantnagar India. A voucher specimen is stored in the Departmental Herbarium (CIMPANT-353). Leaf, flower, and stem of the herb were separated, shade dried and used for the study.

2.2. Isolation of essential oil

The essential oils of dried leaf, flower, and stem (100 g each) of *H. officinalis* were extracted separately by hydrodistillation for three hrs using a Clevenger's apparatus. The essential oil content (%, v/w) was estimated on a dry weight basis. The oil samples obtained were dehydrated over anhydrous sodium sulfate and kept in a cool and dark place before analyses.

2.3. Gas chromatography (GC/FID)

GC analysis of the essential oils was carried out on a PerkinElmer AutoSystem XL gas chromatograph, equipped with DB-5 capillary column (60 m × 0.32 mm i.d., film thickness 0.25 μm) and flame ionization detector (FID). The oven column temperature ranged from 70 to 250 °C, programmed at 3 °C/min, with initial and final hold time of 2 min, using H_2 as carrier gas at 10 psi constant pressure, a split ratio of 1:35, an injection size of 0.03 μL neat, and injector and detector temperatures were maintained at 250 °C and 280 °C, respectively.

2.4. Gas chromatography–mass spectrometry (GC/MS)

GC/MS analysis of the essential oil samples was carried out on a Clarus 680 GC interfaced with a Clarus SQ 8C mass spectrometer of PerkinElmer fitted with Elite-5 MS fused-silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature programme was from 60 to 240 °C, at 3 °C/min, and programmed to 270 °C at 5 °C/min; injector temperature was 250 °C; transfer line and source temperatures were 220 °C; injection size 0.03 μL neat; split ratio 1:50; carrier gas He at 1.0 mL/min; ionization energy 70 eV; mass scan range 40–450 amu.

2.5. Identification of essential oil constituents

Identification of the essential oil constituents was done on the basis of retention time (t_R) and retention index (RI) using a homologous series of *n*-alkanes (C_8–C_{30}, Supelco Bellefonte, PA, USA) under identical experimental conditions, co-injection with standards or known essential oil constituents, mass spectra library search (NIST/EPA/NIH, version 2.0 g, and Wiley registry of mass spectral data 9th edition) and by comparing the mass spectral and retention data with the literature (Adams, 2007). The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

3. Results and discussion

The yield of essential oils observed in shade dried leaf, flowers, and stem of *H. officinalis* were 4.2%, 4.4%, and 0.22%, respectively. A total of 57 constituents, representing 99.8% of the leaf oil; 44 constituents, representing 99.4% of the flower oil and 57 constituents, forming 88.4% of the stem oil compositions were identified using GC/FID and GC/MS analyses. The detailed results are summarized in Table 1. Total Ion Current chromatograms (TIC) of essential oils of the different parts of *H. officinalis* are shown in Fig. 1. The main constituents found in the oils were characterized as *cis*-pinocamphone (=isopinocamphone; 49.7–57.7%), pinocarvone (5.5–24.9%), β-pinene (5.7–9.3%), 1,8-cineole (2.9–8.0%), β-phellandrene (1.8–3.2%), myrtenyl methyl ether (2.7–3.0%), sabinene (0.8–1.9%), isopimara-9(11),15-diene (<0.05–1.9%), myrtenol (1.4–1.7%), myrcene (0.5–1.3%), and *trans*-pinocamphone (=pinocamphone; <0.05–1.3%).

In general, the major constituents identified in the leaf, flower and stem essential oils of Hyssop were similar. Nevertheless, the highest amount of *cis*-pinocamphone was noticed in the essential oil obtained from the leaves (57.7%), followed by the stem (57.3%) and flower (49.7%). Other constituents (amount >1.0%), viz. β-pinene (9.3%), 1,8-cineole (8.0%), β-phellandrene (3.2%), sabinene (1.9%), myrcene (1.3%), and *trans*-pinocamphone (1.3%) were also found to be higher in leaf essential oil. However, pinocarvone (24.9%), myrtenyl methyl ether (3.0%), and myrtenol (1.7%) were recorded higher in the flower oil. In addition, the diterpene content was relatively higher in the stem essential oil as compared to leaf or flower oils. Thus, the comparative results clearly indicated that the leaf and stem oil compositions were quite similar in terms