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Harvesting season and plant part dependent variations in the essential oil composition of *Salvia officinalis* L. grown in northern India

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ABSTRACT

The essential oil composition of *Salvia officinalis* L. (Lamiaceae) grown in northern India was investigated using gas chromatography (GC/FID) and GC–mass spectrometry (GC/MS). The essential oil yield was found to vary from 0.22% to 0.43% (whole aerial parts) and 0.15% to 0.60% (individual plant parts) depending on the season of harvesting and plant parts processed, respectively. Altogether, sixty constituents, corresponding to 95.5–99.2% of the oil compositions were identified. Major constituents of the oil were *cis*-thujone (19.8–42.5%), (*E*)-caryophyllene (1.2–16.1%), manool (3.6–15.1%), viridiflorol (3.1–12.8%), 1,8-cineole (2.8–13.8%), camphor (1.4–22.1%), borneol (0.9–4.8%), α-humulene (1.5–4.5%), β-pinene (0.7–4.1%), and *trans*-thujone (1.4–3.7%). Comparative results showed considerable variations in the essential oil composition dependent upon the season of harvesting and plant parts processed. Thujones and manool were highest in the stem oil; camphor was highest in leaf oil, while (*E*)-caryophyllene and viridiflorol were shown to be highest in the inflorescence oil. The essential oils of the leaf and herb (aerial parts) of *S. officinalis* matched well with the ISO standard.

1. Introduction

The genus *Salvia* that belongs to the family Lamiaceae comprises nearly 900 species spread throughout the world (Hedge, 1992). Many *Salvia* spp. are used as herbal tea and for food flavouring, as well as in cosmetics, perfumery and the pharmaceutical industry (Demirci et al., 2003). Garden sage (*Salvia officinalis* L.) is a perennial hardy sub-shrub native to Mediterranean regions. Today, due to its medicinal, aromatic, and culinary properties, it is cultivated in temperate regions all around the world. It can be added to foodstuffs providing that the concentration of thujones present in the final product does not exceed 0.5 mg/kg. In the United States, sage is listed as "generally recognized as safe", 'GRAS' (US-FDA). The Commission E Monograph for *S. officinalis* gives a warning that the pure essential oil should not be used internally during pregnancy and also that prolonged ingestion may cause seizures, but otherwise a daily dose range of 100–300 mg (Blumenthal et al., 2000). *S. officinalis* is included in the Pharmacopoeias

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of a number of countries including Austria, Czechoslovakia, Germany, Hungary, Yugoslavia, Netherlands, Poland, Portugal, Romania, Russia and Switzerland. Traditionally, it is used in the treatment of fever, indigestion, sexual debility, rheumatism, wound healing, nervous and mental conditions. It is also used for skin and hair care (Dweck, 2000). It serves many purposes, being an insecticidal and antimicrobial, and a source of essential oil of pharmaceutical importance (Tucakov and Mihajlov, 1977; Todorov et al., 1984; Daniela, 1993; Perry et al., 1999; Baricevic et al., 2001). *S. officinalis* is a rich source of potent antioxidants with compounds such as rosmarinic acid and carnosic acid and their derivatives being the best known examples (Cuvelier et al., 1996; Wang et al., 1998; Lu et al., 2002). Experimental studies on sage extracts or essential oil showed hypotensive, central nervous system-depressant actions, and antispasmodic activities. Furthermore, sage is externally used for the treatment of insect bites (Barnes et al., 2002).

Several studies have analyzed the essential oil composition of *S. officinalis* of different origins. Considerable variability has been found in the compositional characteristics due to the influence of several extrinsic and intrinsic factors (Tucker and Maciarello, 1990; Mockute et al., 2003; Bernotiene et al., 2007; Raina et al., 2013). *cis*-Thujone is observed as a major constituent of the volatile oil from garden sage produced in Portugal, Reunion Island, Albania and New Zealand (Perry et al., 1999; Vera et al., 1999; Santos-Gomes and Fernandes-Ferreira, 2001; Mockute et al., 2003). The camphor rich essential oil of this plant is reported from Croatia and Italy (Baratta et al., 1998; Mastelic, 2001; Giamperi et al., 2002). Viridiflorol and manool rich essential oil is reported from Cuba (Lawrence, 2006). In addition to this, germacrene D, β-caryophyllene and caryophyllene oxide chemotype of *S. officinalis* is also reported from Cuba (Pino et al., 2002). Further, variations in the essential oil composition due to soil mineral fertilization (Piccaglia and Marotti, 1993), light intensity (Li et al., 1996), organ age and organ type (Langer et al., 1993; Santos-Gomes and Fernandes-Ferreira, 2001), season (Putievsky et al., 1986), and culture site (Perry et al., 1999) have also been reported.

However, to the best of our knowledge no previous attempt has been made to explore the variations in the essential oil composition of *S. officinalis* due to the season of harvesting and different plant parts processed from India. Therefore, in the present research, essential oils derived from leaf, flower, stem, and whole herb (aerial parts) of *S. officinalis* have been analyzed and compared using GC/FID and GC/MS.

2. Materials and methods

2.1. Plant material

S. officinalis plant material was obtained from a local nursery, Dehradun (Uttarakhand) and grown at the experimental field of the CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre Purara (Uttarakhand) for further study. The plant material was authenticated at the Botany Department of this Centre (voucher specimen: CIMPANT-212) by one of the authors (AC). To investigate the seasonal variations in the essential oil yield and chemical composition of *S. officinalis*, the herb (whole aerial parts) was harvested in different seasons, viz. spring, summer, rainy and autumn seasons. Moreover, leaves, inflorescence and stem of the plant were also collected in spring during the flowering stage for comparing their essential oil yields and chemical compositions. The experimental site is located in the Kattyur valley in western-Himalaya. Climatologically, it is categorized as a sub-temperate zone.

2.2. Isolation of essential oil

The essential oil from the fresh herb (whole aerial parts) harvested over different seasons and from different plant parts, viz. leaves, inflorescence and stem collected in spring were extracted separately using hydrodistillation in a Clevenger's type apparatus for 3 h. The extraction of the essential oil was performed immediately after harvesting the plant material(s). The essential oil content (%, v/w) was estimated on a fresh weight basis. The oil samples obtained were dehydrated over anhydrous sodium sulphate and kept in a cool and dark place until further analyses.

2.3. Gas chromatography (GC-FID)

GC analysis of the oil samples was carried out on a Nucon Gas Chromatograph model 5765 equipped with DB-5 (coated with 5% phenyl polysiloxane, 30 m length × 0.32 mm internal diameter; 0.25 μm film coating) fused silica capillary column and flame ionization detector (FID). Hydrogen was the carrier gas at 1.0 mL min^{-1}. The temperature programming was done from 60 °C to 210 °C at 3 °C min^{-1} with final hold time of 10 min. Injector and detector temperatures were 210 °C and 220 °C, respectively. Injection size was 0.02 μL neat (syringe: Hamilton 1.0 μL capacity, Alltech USA) and split ratio was 1:40.

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

GC–MS analysis of the essential oil sample was carried out on a PerkinElmer AutoSystem XL GC interfaced with a Turbomass Quadrupole mass spectrometer fitted with a DB-5 fused-silica capillary column (60 m × 0.32 mm i.d., film thickness 0.25 μm). The oven temperature programme was from 70 °C to 250 °C, at 3 °C min^{-1}, with initial and final hold time of 2 min; injector, transfer line and source temperatures were 250 °C; injection size 0.03 μL neat; split ratio 1:30; carrier gas He at 1.0 mL min^{-1}; ionization energy 70 eV; mass scan range 40–450 amu.

2.5. Identification of essential oil constituents

Identification of the essential oil constituents was carried out on the basis of retention index (RI), determined 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 2.1 and Wiley registry of mass spectral data 7th edition) and by comparing the mass spectral and retention data with literature (Adams, 2007). The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.