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RESEARCH COMMUNICATION

Chemical composition of the essential oil of *Ocimum tenuiflorum* L. (Krishna Tulsi) from North West Karnataka, India

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Abstract

The chemical composition of the essential oil of flowering aerial parts of *Ocimum tenuiflorum* L. growing in the North West Karnataka, India, was investigated. The hydro-distilled essential oil was analyzed by gas chromatography equipped with flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC/MS). Results demonstrated that the oil was found to be rich in phenyl derivative compounds (83.8%). The major compound was identified as methyl eugenol (82.9%) among twenty-six compounds, comprising 98.9% of the total oil.

Keywords: *Ocimum tenuiflorum*; Lamiaceae; essential oil composition; methyl eugenol; GC/MS.

Introduction

Ocimum tenuiflorum L. (syn. *Ocimum sanctum* L.) of the family Lamiaceae is an erect, softly hairy, aromatic herb. The plant is commonly cultivated in temple premises and households as a sacred plant (Yadav & Sardesai, 2002). Two types of *O. tenuiflorum* are met within cultivation: (i) with green leaves known as Sri or Lakshmi Tulsi and (ii) with purple leaves known as Krishna Tulsi (Pandey, 1990). In Ayurveda, this plant has been well documented for its therapeutic potentials and described as Dashemani Shwasaharni (antiasthmatic) and antikaphic drugs (Kaphaghna) (Sirkar, 1989). Anticancer (Kathiresan, Guanasekan, Rammurthy, & Govidswami, 1999), radioprotective, anticarcinogenic (Devi, 2001), antioxidant (Devi, 2001; Joshi, 2013a), chemopreventive (Prashar, Kumar, Banerjee, & Rao, 1994; Karthikeyan, Ravichadran, & Govindasamy, 1999), immunotherapeutic (Mukherjee, Das, & Ram, 2005), antimicrobial (Singh, Malhotra, & Majumdar, 2005; Joshi, 2013a), anti-inflammatory (Godhwani, Godhwani, & Vyas, 1987; Singh & Majumdar, 1997), analgesic, antipyretic (Godhwani *et al*., 1987), antispermatogenic (Seth, Johri, & Sundaram, 1981) and antistress (Bhargava & Singh, 1981) activities of this plant have also been reported. The essential oils of *O. tenuiflorum* have been reported to possess methyl eugenol (Joshi, 2013a), methyl eugenol, *β*-caryophyllene (Bhattacharya, Kaul, & Rajeswara Rao, 1996; Kothari, Bhattacharya, Ramesh, Garg, & Khanuja, 2005), methyl eugenol, (E)-caryophyllene, eugenol and, *β*-elemene (Awasthi & Dixit, 2007), methyl chavicol, and linalool (Khan *et al*., 2010) from India; *β*-bisabolene, 1,8-cineole and methyl chavicol (Kicel, Kurowska, & Kalemba, 2005) from Poland; methyl eugenol and isocaryophyllene (Gbolade & Lockwood, 2008) from Nigeria; eugenol, *β*-caryophyllene and caryophyllene oxide (Machado, Silva, Matos, Craveiro, & Alencar, 1999) from Northeastern Brazil; eugenol, *β*-elemene and *β*-caryophyllene (Pino, Rosado, Rodriguez, & Garcia, 1998) from Cuba; methyl chavicol, camphor and *β*-caryophyllene (Brophy, Goldsack, & Clarkson, 1993) from Australia. The aim of the present study was to investigate the essential oil composition of *O. tenuiflorum* (Krishna Tulsi) growing in North West Karnataka, India, using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analyses.

Materials and Methods

Plant Material

The plant was collected in the month of January 2011 from

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Sankeshwar (N 16°27'470"; E 74°49'081") of district Belgaum, Karnataka, India, at an elevation of ~653 m. The plant was identified by Dr. H. V. Hegde, Taxonomist, Regional Medical Research Centre (RMRC), Belgaum (voucher specimen No. RMRC-1251).

Isolation of essential oil

The flowering aerial parts (100 g) were chopped into small pieces and subjected to hydro-distillation (1500 mL distilled water + 100 g plant material in 3000 mL round bottom flask) using a Clevenger type apparatus for 3h (Joshi, 2013b; Joshi & Badakar, 2012). The oil was trapped by adding of *n*-hexane and dried over anhydrous Na_2SO_4 and kept in a sealed vial at -4°C until analysis. The yield of oil was 0.2%, w/w.

GC and GC-MS analysis

The analysis of oil was achieved using Varian 450 Gas Chromatograph (GC) equipped with a fused silica CP-Sil 8 CB capillary column (30 m × 0.25 mm; 0.25 m film thickness) and flame ionization detector. The carrier gas was nitrogen at 1.0 mL/min flow rate. The initial oven temperature was 60°C which was raised to 220°C with 3°C/min ramp rate and was held at that temperature for 5 min. The injector and detector temperatures were 230 and 240°C, respectively. The injection volume of the sample was 1.0 µL diluted in *n*-hexane. The sample was injected using a split ratio of 1:50. The Gas Chromatography/Mass Spectrometry (GC-MS) analysis of the oil was carried out in Thermo Scientific Trace Ultra GC interfaced with a Thermo Scientific ITQ 1100 Mass Spectrometer fitted with TG-5 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) using above stated oven temperature program. The carrier gas was helium at 1.0 mL/min. The injector temperature was 230°C and the injection volume 0.1 µL prepared in *n*-hexane. The sample was injected using a split ratio of 1:50. MS were taken at 70 eV with mass scan range of 40-450 amu. The GC and GC/MS parameters were those reported earlier (Joshi, 2011; Joshi, Badakar, & Kholkute, 2011a; Joshi, Badakar, Kholkute, & Khatib, 2011b; Joshi & Sharma, 2014).

Identification of the compounds

Identification of constituents were done on the basis of Retention Index (RI, determined with reference to homologous series of *n*-alkanes C_8-C_{25} under identical experimental conditions), MS library search NIST 08 MS Library (Version 2.0 f; Thermo Fisher Scientific Austria) and WILEY MS 9th Edition (Thermo Fisher Scientific Austria), and by comparing with the MS literature data (Adams, 2007) and co-injection of available authenticated samples purchased from Sigma-Aldrich, India (⩾98% purity). The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

Results and Discussion

Twenty-six compounds were characterized and identified according to their mass spectra and their relative retention indices determined on a non-polar stationary phase capillary column, comprising 98.9% of the total oil constituents. The compounds identified are listed in Table 1 in the order of their elution from the TG-5 column (Fig. 1), along with the percentage composition of each component and its retention index.

Table 1. Chemical composition of the essential oil of *Ocimum tenuiflorum*

Compound	RI	%	Identification
α-Pinene	918	0.2	RI, MS
Camphene	929	0.1	RI, MS
Sabinene	947	t	RI, MS
β-Pinene	951	0.1	RI, MS
p-Cymene	992	t	RI, MS
Limonene	996	0.2	RI, MS
Linalool	1069	0.5	RI, MS
Camphor	1123	0.1	RI, MS, CI
Borneol	1147	2.4	RI, MS, CI
Terpin-4-ol	1160	0.1	RI, MS
α-Terpineol	1177	t	RI, MS
Methyl chavicol	1185	t	RI, MS, CI
α-Cubebene	1368	t	RI, MS
Eugenol	1379	0.9	RI, MS, CI
α-Copaene	1399	1.9	RI, MS
β-Bourbonene	1410	0.2	RI, MS
β-Cubebene	1417	0.1	RI, MS
α-Elemene	1419	0.5	RI, MS
Methyl eugenol	1442	82.9	RI, MS, CI
β-Caryophyllene	1453	4.1	RI, MS, CI
β-Gurjunene	1464	t	RI, MS
α-Humulene	1493	0.2	RI, MS
Germacrene D	1525	2.3	RI, MS
Germacrene A	1553	0.7	RI, MS
Cubebol	1564	0.3	RI, MS
δ-Cadinene	1574	1.1	RI, MS
Monoterpene hydrocarbons		0.6	
Oxygenated monoterpenes		3.1	
Sesquiterpene hydrocarbons		11.1	
Oxygenated sesquiterpene		0.3	
Phenyl derivatives		83.8	
Total identified		98.9	

RI=Retention index relative to C_8-C_{25} *n*-alkanes on TG-5 column, MS=NIST and Wiley library and the literature, t=trace (<0.1%), CI=Co-injection of authentic samples.

The main constituent was identified as methyl eugenol (82.9%). The other minor constituents were *β*-caryophyllene (4.1%), borneol (2.4%), germacrene D (2.3%) and *α*-copaene (1.9%). Phenyl derivative (83.8%) constituents were the prominent group of compounds followed by sesquiterpene hydrocarbons (11.1%), oxygenated monoterpenes, (3.1%), monoterpene

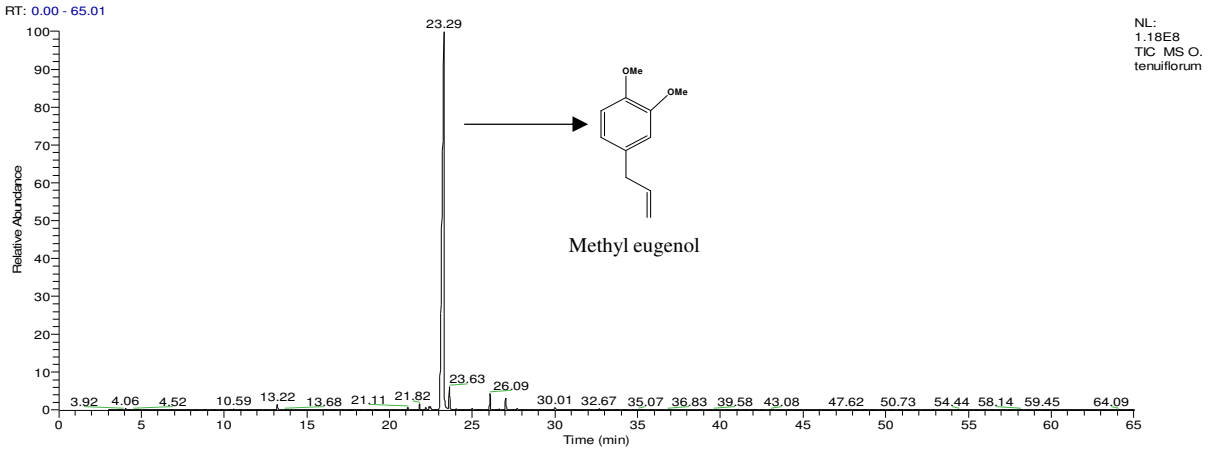

Fig. 1 GC-TIC chromatogram of the essential oil of *Ocimum tenuiflorum*

hydrocarbons (0.6%) and oxygenated sesquiterpene (0.3%).

The compound methyl eugenol has been reported in varying amounts along with diverse chemotypes from different regions (Bhattacharya *et al*., 1996; Kothari *et al*., 2005; Joshi, 2013a; Awasthi & Dixit, 2007; Gbolade & Lockwood, 2008). It is interesting to note that chemotypes containing other compounds have also been reported (Khan *et al*., 2010; Kicel *et al*., 2005; Machado *et al*., 1999; Brophy *et al*., 1993; Pino *et al*., 1998).

This report presents low amount of methyl eugenol as compared to the earlier report (Joshi, 2013a) from the essential oil of *O. tenuiflorum* collected from North West Karnataka, India. The quantitative differences in the major constituents of plant could be due to the season, climate or soil conditions.

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