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RESEARCH COMMUNICATION

cis-Ocimenone chemotype essential oil of green mint (*Mentha viridis* L.) from Western Ghats region of North West Karnataka, India

R. K. Joshi & A. K. Sharma

Abstract

The hydro-distilled essential oil of the leaves of *Mentha viridis* L. was analyzed by gas chromatography equipped with flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC/MS). A total of fifty constituents were identified, accounting 95.4% of the total oil. The major compounds identified were *cis*-ocimenone (61.7%), limonene (10.5%) and *trans*-carveol (5.0%). The essential oil consists mainly of oxygenated monoterpenes (73.1%), followed by monoterpene hydrocarbons (14.2%), sesquiterpene hydrocarbons (5.2%), phenyl derivatives (1.5%), and oxygenated sesquiterpenes (1.4%). This study revealed that the leaves of *M. viridis* from Western Ghats of North West Karnataka, India, produced *cis*-ocimenone chemotype essential oil.

Key words: *Mentha viridis* L.; Lamiaceae; *cis*-ocimenone; essential oil composition; GC/MS.

Introduction

Mint is one of the most widely used and most famous of all herbs. It is easy to cultivate and has a wide variety of culinary and medicinal uses. *Mentha viridis,* commonly known as garden or green mint, is originally a native of the Mediterranean region (Grieve, 2013). The boiled leaf extract of green mint has anti-infectious, anti-flatulence and anti-inflammatory properties and also used in viral hepatitis, colitis, gastric acidity, aerophagia, and also stimulates the digestion. (Saleem *et al.,* 2000; Kouhila *et al.,* 2001; Kumar & Chattopadhyay, 2007). The essential oil composition of specified plant *M. viridis* has been reported from some other countries. The essential oil composition of *M. viridis* reported from Tunisie were carvone, 1,8-cineole, and limonene (Mkaddem *et al.,* 2009), while that from Morocco contained high pulegone content (Talbaoui *et al.,* 2012). This communication presents essential oil composition of *M. viridis* collected from Western Ghats region of North West Karnataka, India with an aim to delineate the terpenoid composition.

Materials and methods

Plant material

The leaves of *M. viridis* were collected from medicinal garden of Regional Medical Research Centre (RMRC) Belgaum (N 15.88668; E 74.52353) Karnataka, India, at an elevation of 800 m in March 2011. The plant was identified and authenticated by Taxonomist of RMRC, Belgaum, where a voucher specimen (No. RMRC-570) has been deposited.

Isolation of essential oil

The fresh plant material (200 g) was hydro-distilled for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous sodium sulfate and stored at -4°C until analysis. The yield of oil was 0.19 % (v/w).

Gas chromatography (GC)

The GC analysis of the oil was carried out on Varian 450 gas chromatograph equipped with FID, using stationary phase CP Sil-8-CB (30m × 0.25mm i.d., 0.25 µm film thickness) column under the experimental conditions reported earlier (Joshi, 2013a). Nitrogen was a carrier gas at 1.0 mL/min flow rate. Temperature programming was

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set to 60-220°C at 3°C/min and the injector and detector temperatures were 230 and 240°C, respectively. The injection volume was 1.0 mL of 1% solution diluted in n-hexane; split ratio was 1: 50.

Gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis of the oil was carried out on Thermo Scientific Trace Ultra GC (Thermo Fisher Scientific Austria, Vienna, Austria) interfaced with a Thermo Scientific ITQ 1100 Mass Spectrometer (Thermo Fisher Scientific Austria) fitted with TG-5 (30m × 0.25mm i.d., 0.25 µm film thickness) column under the experimental conditions reported earlier (Joshi, 2011a). The oven temperature was programmed from 60 to 220°C at 3°C/min using helium as a carrier gas at 1.0 mL/min. The injector temperature was 230°C, injection volume was 0.1mL of 1% solution prepared in n-hexane; split ratio was 1 : 50. MS were taken at 70 eV with mass scan range of 40–450 amu. All the experimental parameters were applied from those reported earlier (Joshi, 2013c,d).

Identification of the components

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 condition), MS library search (NIST and WILEY) and by comparison with MS literature data (Adams, 2007). The relative amount of individual components was calculated based on GC peak area (FID response) without using correction factor.

Results and discussion

Fifty compounds were characterized and identified by GC-MS, comprising 95.4 % of the total oil. The compounds identified are listed in Table 1 in elution order from the TG-5 MS column along with the percentage composition of each component and its retention index.

The major compounds identified were *cis*-ocimenone (61.7%), limonene (10.5%), and *trans*-carveol (5%). The other minor constituents were *α*-selinene (1.7%), *iso*-dihydrocarveol acetate (1.5%), *Z*-jasmone (1.3%), 1,8-cineole (1.2%), and *cis*-carveol (1.0%). The essential oil consists mainly of oxygenated monoterpenes (73.1%), followed by monoterpene hydrocarbons (14.2%), sesquiterpene hydrocarbons (5.2%), phenyl derivatives (1.5%), and oxygenated sesquiterpenes (1.4%). The earlier report on the essential oil of *M. viridis* from Tunisie revealed the presence of carvone, 1,8-cineole, and limonene ((Mkaddem *et al.,* 2009), while the study from Morocco showed that the oil contains high pulegone content (Talbaoui *et al*., 2012). Contrary, this report found that the oil have high content of *cis*-ocimenone (61.7%).

Table 1. Chemical composition of the essential oil of *M. viridis*

Compound	RI	%	Identification
Tricyclene	898	t	RI, MS
α-Thujene	902	0.3	RI, MS
α-Pinene	907	0.4	RI, MS
Camphene	953	t	RI, MS
Sabinene	976	t	RI, MS
β-Pinene	940	0.3	RI, MS
Myrcene	991	0.5	RI, MS
α-Terpinene	972	0.7	RI, MS
Limonene	985	10.5	RI, MS
1,8-Cineole	987	1.2	RI, MS
Z-*β*-Ocimene	991	0.5	RI, MS
E-*β*-Ocimene	1001	0.7	RI, MS
γ-Terpinene	1013	0.2	RI, MS
Terpinolene	1043	0.1	RI, MS
Linalool	1056	t	RI, MS
cis-Thujone	1060	0.2	RI, MS
cis-Verbenol	1099	0.1	RI, MS
Borneol	1131	0.1	RI, MS
Terpin-4-ol	1177	0.4	RI, MS
α-Terpineol	1189	0.3	RI, MS
Dihydrocarveol	1165	0.1	RI, MS
cis-Dihydrocarveol	1167	0.5	RI, MS
trans-Carveol	1195	5.0	RI, MS
cis-Carveol	1209	1.0	RI, MS
cis-Ocimenone	1230	61.7	RI, MS
Dihydroedulan I	1283	0.2	RI, MS
neo-Dihydrocarveol acetate	1301	0.2	RI, MS
iso-dihydrocarveol acetate	1325	1.5	RI, MS
trans-Carvyl acetate	1337	0.3	RI, MS
cis-Carvyl acetate	1367	0.2	RI, MS
β-Bourbonene	1392	0.3	RI, MS
β-elemene	1401	t	RI, MS
Z-Jasmone	1411	1.3	RI, MS
α-Gurjunene	1422	0.2	RI, MS
β-Caryophyllene	1433	0.6	RI, MS
β-Gurjunene	1444	0.9	RI, MS
α-Humulene	1473	0.2	RI, MS
cis-Muurola-4 (14), 5-diene	1484	0.3	RI, MS
Germacrene D	1505	0.4	RI, MS
α-Selinene	1524	1.7	RI, MS
Germacrene A	1533	0.3	RI, MS
γ-Cadinene	1544	0.3	RI, MS
cis-Calamenene	1553	0.2	RI, MS
α-Cadinene	1570	0.1	RI, MS
Spathulenol	1615	t	RI, MS
Caryophyllene oxide	1621	0.1	RI, MS
Guaiol	1631	0.4	RI, MS
1, 10-di-*epi*-Cubenol	1656	t	RI, MS
epi-*α*-Cadinol	1684	0.2	RI, MS
α-Cadinol	1699	0.7	RI, MS
Monoterpene hydrocarbons		14.2	
Oxygenated monoterpenes		73.1	
Sesquiterpene hydrocarbons		5.2	
Oxygenated sesquiterpenes		1.4	
Phenyl derivatives		1.5	
Total Identified		95.4	

RI= Retention index on TG-5 column; MS= (GC/MS); t trace<0.1

Limonene and 1,8-cineole were found in low quantity, while pulegone and carvone were not identified. The terpenoid profiles reported from other parts of world (Mkaddem *et al*., 2009; Talbaoui *et al*., 2012) in terms of minor constituents are somehow similar to this study, but different in term of the major compound. This study revealed that the essential oil of *M. viridis* growing in the Western Ghats region of North West Karnataka has *cis*-ocimenone chemotype plant. The quantitative and qualitative divergence may be due to the geographical, climatic, and soil conditions, which in turn may affect the composition and other secondary metabolites of the plants (Joshi *et al.,* 2011b; Joshi, 2012; 2013b). Essential oil formation in the plants is highly dependent on climatic conditions, especially day length, irradiance, temperature, and water supply. Tropical species follow in their vegetation cycle the dry and rainy seasons, while species of the temperate zones react more on day length, the more distant from the equator their natural distribution area is located (Franz & Novak, 2010). Moreover, oils chemical composition was extremely variable, and individual constituents were not affected by intra plant location of the leaves, plant age, or geographic site (Kelsey *et al.,* 1983). This limits their taxonomic value, but possibly enhances their ecological significance as a defense adaptation to herbivores (Rhoades, 1979). Nevertheless, there are an almost uncountable number of single substances and a tremendous variation in the composition of essential oils. Apart from the phytochemical group of substances typical for a taxon, the chemical outfit depends on the specific genotype, the stage of plant development, influence of environmental factors and the part of the plant (Franz & Novak, 2010).

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