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Comparative chemotype determination of *Lamiaceae* plants by means of GC–MS, FT-IR, and dispersive-Raman spectroscopic techniques and GC-FID quantification

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ABSTRACT

Three different techniques: the classical gas chromatography–mass spectrometry (GC–MS) and two "green" alternative techniques to the classical chromatography, the spectroscopic techniques Fourier transform infrared (FT-IR), and dispersive-Raman were employed to characterize the main chemotypes of different essential oils from plants of the *Lamiaceae* family and to compare between techniques. Gas chromatography-flame ionization detector (GC-FID) was also employed to quantify the main compounds present in essential oils isolated by hydrodistillation (HD) and semi-quantify essential oil composition isolated by HD and simultaneous steam distillation – solvent extraction (SDE). While GC cannot differentiate between pure and mixed chemotypes of a compound, FT-IR, and Raman methods allow the creation of libraries, through which chemotype determination is feasible even for mixed chemotypes, thus combining robustness with being rapid and non-destructive techniques.

1. Introduction

Individual plants species of the same genus present distinct chemical profiles called chemotypes. Chemotypes are defined as organisms categorized under the same species, subspecies or varieties having differences in quantity and quality of their component(s) in their whole chemical fingerprint that is related to genome or gene expression differences. These chemotypes can be classified into two types: "pure chemotypes" (only one oil component, the major one, defines "the pure chemical race" and accounts for over 50% of the total essential oil) and "mixed chemotypes" (where there are 2–3 main components, each accounting for less than 50% of the essential oil, which, as an entity, define the chemical composition) (Holopainen et al., 1987; Polatoglu, 2013).

The knowledge of the chemotype of an essential oil is important as numerous species of the *Lamiaceae* family present chemical polymorphism, *i.e.* individual plants have various genotypes which code the production of different dominant terpenes in their essential oil (Keefover-Ring et al., 2009). The determination of chemotype is essential in order to understand the regulatory pathways of secondary metabolism (Yamazaki and Saito, 2011). Furthermore, each chemotype, as determined by genotype, environment, agronomic treatments and their interactions, presents distinct biological activity of its essential oil (Rota et al., 2008).

Essential oils can be isolated from plant tissues using various techniques. Hydrodistillation (HD) and simultaneous steam distillation – solvent extraction (SDE) using Likens-Nickerson apparatus are two classical techniques, well known to yield a rich profile of the essential oil (Daferera et al., 2002a, 2002b; Viljoen et al., 2006). However, these techniques present some disadvantages, the main being thermal transformation of molecules and loss of hydrophilic compounds (Prosen et al., 2010).

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Chemotype determination of plant essential oils has been achieved using various techniques, such as gas chromatography–mass spectrometry (GC–MS) (Stashenko et al., 2010), Fourier transform infrared (FT-IR) spectroscopy (Kanakis et al., 2011), Fourier Transform-Raman (FT-Raman) spectroscopy (Daferera et al., 2002a), dispersive Raman, attenuated total reflectance-infrared (ATR-IR) (Schulz et al., 2003a), *etc.* Spectroscopic techniques are considered an attractive alternative to chromatography, as the latter is time-consuming, destructive and laborious and often requires the use of pollutant solvents. On the contrary, spectroscopic techniques do not require the use of pollutant solvents, allow working with intact samples, thus reducing time and cost of sample treatment, and deliver final results rapidly (Baeten et al., 1996). Recently, several works were focused on the differentiation among samples by means of FT-IR based techniques coupled with spectral libraries (Pappas et al., 2003; Tarantilis et al., 2008).

The present work aimed at the determination of main chemotypes in *Lamiaceae* plants using FT-IR, and dispersive-Raman spectroscopic techniques. The characterization was enabled by the creation of spectral libraries, one for each technique, while the results were compared to the essential oil volatile profile as delivered by means of GC–MS. Furthermore, in order to investigate the effect of essential oil isolation techniques on chemotype, hydrodistillation (HD) and simultaneous steam distillation – solvent extraction (SDE) using Likens-Nickerson apparatus were employed and their comparison was based on quantitative analysis of the resulting essential oil using gas chromatography-flame ionization detector (GC-FID).

2. Materials and methods

2.1. Plant material

Plant samples were obtained from various areas around Greece. Table 1 shows the areas and dates of collection, the part of the plant used to extract the essential oil and indicates whether the plant was wild or cultivated. The last four samples concern essential oils characterized as commercially distilled and were kindly provided by the company Bioparnon (Astros, Arcadia, Peloponnesus, Greece).

2.2. Reagents

Reagents used were: diethyl ether (DEE) stabilized with butylated hydroxytoluene (BHT) (purity ≥ 99.8, Carlo Erba Reagenti SpA; Radano, MI, Italy), acetone (purity ≥ 99.8, Carlo Erba Reagenti SpA; Radano, MI, Italy), anhydrous magnesium sulfate in powder form (purity 99%, Acros Organics, Morris Plains, NJ, USA), 5-isopropyl-2-methylphenol (carvacrol) (purity 98%; Sigma–Aldrich Co., St. Louis, MO, USA), 2-isopropyl-5-methylphenol (thymol) (purity 95%; Sigma–Aldrich Co., St. Louis, MO, USA) and (R)-(+)-pulegone (purity 98%; Sigma–Aldrich Co., St. Louis, MO, USA).

2.3. Hydrodistillation (HD) method

Different quantities of each sample (depending on the initial amount of sample provided) were used for the distillation using Clevenger apparatus to obtain the essential oil. Distillation lasted 3 h and the essential oil obtained was treated as described by Petrakis et al. (2009).

2.4. Simultaneous steam distillation – solvent extraction (SDE) method

SDE of the essential oil was performed using Likens-Nickerson. 5.5 mL of diethyl ether (1.5 mL in the main body of the apparatus

Table 1
Description of essential oil samples under study.

Plant name	Origin	Geographical coordinates	Altitude (m)	Date of collection	Plant part used for distillation	Type
Satureja hortensis	Evros prefecture, Ptelea, (Greece)	41°42′01″ N 26°14′16″ E	110	August 2012	Leaves and flowers	Wild
Satureja pilosa	Evros prefecture, Brysika, (Greece)	41°23′52″ N 26°19′57″ E	65	October 2013	Leaves and flowers	Wild
Mentha pulegium	Evros prefecture, Roussa (Greece)	41°18′41″ N 25°59′50″ E	550	October 2013	Leaves and flowers	Wild
Thymus longicaulis subsp. *chaubardii*	Evia Island, foothills of Mt. Ochi, (Greece)	38°04′45″ N 24°56′02″ E	790	May 2013	Leaves and flowers	Wild
Satureja hortensis	Aitoloakarnania prefecture, Rigani (Greece)	38°34′51″ N 21°14′11″ E	100	October 2010	Leaves	Cultivated
Thymus vulgaris	Aitoloakarnania prefecture, Xiromero (Greece)	38°34′51″ N 21°14′11″ E	270	October 2010	Leaves	Cultivated
Thymus vulgaris	Aitoloakarnania prefecture, Xiromero (Greece)	38°38′01″ N 21°08′29″ E	270	July 2011	Leaves and flowers	Cultivated
Thymus sp.	Bioparnon (Astros, Arcadia, Peloponnesus, Greece)			October 2013	Leaves	Commercially distilled essential oil
Origanum vulgare subsp. *hirtum*	Bioparnon (Astros, Arcadia, Peloponnesus, Greece)	Not applicable	Not applicable	October 2013	Leaves	Commercially distilled essential oil
Origanum onites	Bioparnon (Astros, Arcadia, Peloponnesus, Greece)			October 2013	Leaves	Commercially distilled essential oil
Satureja thymba	Bioparnon (Astros, Arcadia, Peloponnesus, Greece)			October 2013	Leaves	Commercially distilled essential oil