JOURNAL OF FOOD AND DRUG ANALYSIS XXX (2017) 1–16

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Thyme essential oils from Spain: Aromatic profile ascertained by GC–MS, and their antioxidant, anti-lipoxygenase and antimicrobial activities

Ana-Belen Cutillas [a,*], *Alejandro Carrasco* [a], *Ramiro Martinez-Gutierrez* [b], *Virginia Tomas* [c], *Jose Tudela* [a]

[a] *GENZ-Group of Research on Enzymology, Department of Biochemistry and Molecular Biology-A, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Spain*[1]
[b] *Novozymes Spain S.A., Madrid, Spain*[2]
[c] *Department of Analytical Chemistry, University of Murcia, Spain*

ARTICLE INFO

Article history:
Received 23 March 2017
Received in revised form
17 May 2017
Accepted 22 May 2017
Available online xxx

Keywords:
Antioxidants
Lipoxygenase inhibitors
Microbial sensitivity tests
Oils
Volatile
Thymus plant

ABSTRACT

Six samples of red thyme (*Thymus zygis*) and two samples of winter thyme (*Thymus hyemalis*) essential oils (EOs) were obtained from plants cultivated in south-eastern Spain and extracted by steam distillation. Analysis by gas chromatography coupled with mass spectrometry detection provided the relative (%) and absolute (mM) concentrations. Thymol (30–54%), p-cymene (14–27%) and γ-terpinene (8–28%) were the most abundant components of *T. zygis* EO, while 1,8-Cineole (3–37%), p-cymene (1–29%), linalool (8–13%) and thymol (0–19%) were the most abundant components in the case of *T. hyemalis* EO. Enantioselective gas chromatography identified (−)-linalool, (−)-borneol and (+)-limonene as the main enantiomers. Several methods to evaluate antioxidant capacities were applied to the EOs, concluding that their activities were mainly due to thymol and linalool. The inhibition of lipoxygenase activity, mainly due to thymol, p-cymene and linalool, suggested their possible use as anti-inflammatories. The high antibacterial and antifungal activities determined for the EOs means that they can be used as natural preservatives. The results support the potential use of *Thymus* sp. EOs as natural food, cosmetic and pharmaceutical ingredients.

1. Introduction

The genus *Thymus*, predominantly found in the Mediterranean region, Asia, Southern Europe and North Africa, is constituted by more than three hundred species [1]. There are several ecotypes, which differ in their morphological characteristics and in the composition of their essential oils (EOs), although all of them are characterized by a moderate odor and sometimes a very pronounced balsamic and spicy flavor [2].

* *Corresponding author*. Campus de Espinardo, University of Murcia, PC: 30100, Spain. Fax: +34 868 88 41 47.
E-mail address: anabelen.cutillas@um.es (A.-B. Cutillas).
[1] www.um.es/genz.
[2] www.novozymes.com.

http://dx.doi.org/10.1016/j.jfda.2017.05.004

Thymus zygis Loefl. ex L., also known as red thyme, is a widespread endemic plant in the Iberian Peninsula [3]. *Thymus hyemalis* Lange, winter thyme, is mainly found in the south-east of Spain (Alicante, Murcia and Almeria provinces) [4]. The EOs obtained from both *Thymus* species show a high degree of variability, depending on seasonal, phenological or edaphoclimatic conditions [5–10].

Essential oils are increasingly studied for use in the chemical, cosmetic, food, fragrance and pharmaceutical industries due to their potential bioactivities [11]. This is particularly the case with the EOs from *Thymus* species due to the presence of bioactive compounds [12,13]. Indeed, thyme EO is among the world's ten most commonly used EOs as a food preservative [14].

Gas chromatography, coupled with mass spectrometry or flame ionization detection, provides a detailed description of the compounds of EOs, expressed as percentages of total area, and is a useful technique for comparing the composition of EOs – for example, those studied here with those obtained from plants growing in different areas or conditions [8,15]. Moreover, using calibration curves of commercially available terpenes, the absolute concentration of each compound can be determined, which is useful for determining the quality of EOs and also whether they have been adulterated by dilution with volatile solvents.

Furthermore, analysis of the chiral distribution of EO components provides information about the origin and quality of EOs by differentiating between natural and adulterated EOs. Also, the prevalence of different enantiomers could show differences in bioactivity and organoleptic properties [16,17]. There are few chiral studies of some biomolecules on the EOs of *Thymus* sp. [18,19].

Several antioxidant assays serve as models for the preliminary evaluation of potential preservative and pharmacological activities. EOs may diminish oxidative processes in food and cosmetic products and so be used to replace synthetic antioxidants, increasing consumer acceptance of the products [20]. In the same way, they have a potential for use in human health care since they may reduce the oxidative stress that often enhances disease development [21,22].

Lipoxygenase (LOX) catalyzes the biosynthesis of leukotrienes from arachidonic acid and its hyperactivity has been related with inflammatory, tumoral, ischemic, skin and Alzheimer's diseases and also diabetes [23,24]. The inhibition of soybean lipoxygenase can be used as an *in vitro* model to assay human lipoxygenase bioactivities [25].

The use of natural ingredients to prevent the growth of microorganisms is gaining interest [26]. Particularly, organic food cannot include chemical additives [2]. For this reason, several *Thymus* EO species have been proposed as natural antimicrobial alternative [15,27–31].

In this study we describe the composition of *Thymus* EO (relative and absolute concentrations) grown in the province of Murcia (S.E. Spain) and the enantiomeric distribution of some of its compounds. Moreover, antioxidant activity, LOX inhibition and antimicrobial activity are determined. The study will serve to characterize thyme EOs from Murcia, in depth for the first time in the literature, to enhance their potential biotechnological applications.

2. Materials and methods

2.1. Plant material

Six samples of *T. zygis* and two samples of *T. hyemalis* were taken from plants grown in Murcia (Spain). Their EOs were obtained by steam distillation in a Clevenger-type apparatus for 3 h, dried over anhydrous sodium sulfate and stored at 4 °C until use. Samples Tzt1, Tzt2, Tzt3 and Tzt4 are EOs of *T. zygis* thymol chemotype and samples Tzl1, Tzl2, Th3 and Th4 are EOs of *T. zygis* linalool chemotype and *T. hyemalis*, respectively. The plants yielding Tzt1 and Tzt4 were grown in a Lower Meso-Mediterranean bioclimatic zone, Tzt3, Tzl1 and Th2 were grown in an Upper Meso-Mediterranean bioclimatic zone and Tzt2, Tzl2 and Th1 were grown in a Supra-Mediterranean bioclimatic zone. The characteristics of the bioclimatic zones of Spain have been described previously [32]. Plant species were identified in the Plant Biology Department of Murcia University by Dr. Pedro Sanchez-Gomez. The Department of Biochemistry and Molecular Biology-A storage the voucher specimens.

2.2. Chemicals

All the compounds used in this work were of analytical grade, with a purity higher than 95%. The standard substances for GC identification and determination, the chemicals for the antioxidant capacity assays, and the reagents for soybean lipoxygenase inhibition were purchased from Sigma–Aldrich, Spain. The following culture media for bacteria and yeasts were provided by VWR Chemicals, Spain: Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), Roswell Park Memorial Institute (RPMI-1640), Sabouraud Dextrose Agar (SDA), tryptic soy broth (TSB) and yeast peptone dextrose (YPD).

Solvents of analytic grade and buffers were purchased from Merck (Madrid, Spain). Type I (18 MΩ cm) deionized water (MilliQ-Reference, Millipore, Madrid, Spain) was used throughout in this work.

2.3. Microorganisms and culture conditions

The following microorganisms from the American Type Culture Collection (ATCC) were tested: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Candida albicans* ATCC 10231. All microorganisms were acquired from Sigma–Aldrich. The stock cultures were preserved in screw-capped tubes containing TSB or YPD with 15% glycerol, for bacteria and yeast cells, respectively. Isolated colonies, selected from an 18- to 24-h agar plate, were transferred to MHB in the case of bacteria and RPMI-1640 in the case of *C. albicans* to obtain the necessary cultures for the tests.

2.4. Fast gas chromatography–mass spectrometry (FGC/MS)

Analyses of the EOs using FGC/MS were conducted using an Agilent GC7890 chromatograph coupled to an Agilent MS5975 mass spectrometry detector, with electronic impact ionization and single quadrupole analyzer. To enhance

Please cite this article in press as: Cutillas A-B, et al., Thyme essential oils from Spain: Aromatic profile ascertained by GC–MS, and their antioxidant, anti-lipoxygenase and antimicrobial activities, Journal of Food and Drug Analysis (2017), http://dx.doi.org/10.1016/j.jfda.2017.05.004