Characterization of aroma-active and phenolic profiles of wild thyme (*Thymus serpyllum*) by GC-MS-Olfactometry and LC-ESI-MS/MS

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Abstract The present study was designed to characterize the volatile, aroma-active and phenolic compounds of wild thyme. Volatile components of *T. serpyllum* were extracted by use of the purge and trap technique with dichloromethane and analyzed by gas chromatography–mass spectrometry (GC-MS). The extraction method gave highly representative aromatic extract of the studied sample based on the sensory analysis. A total of 24 compounds were identified and quantified in *Thymus serpyllum*. Terpenes were qualitatively and quantitatively the most dominant volatiles in the sample. Aroma extract dilution analysis (AEDA) was used for the first time for the determination of aroma-active compounds of *Thymus serpyllum*. In total, 12 aroma-active compounds were detected in the aromatic extract by GC-MS-Olfactometry and terpenes were the most abundant compounds. High-performance liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method was used for the phenolic compounds analysis. 18 phenolic compounds were identified and quantified in the *T. serpyllum*. Luteolin 7-*O*-glucoside, luteolin and rosmarinic acid were the most abundant phenolics in this herb.

Keywords Wild thyme · *Thymus serpyllum* · Volatile · Aroma-active · LC-MS-MS · Phenolics

Introduction

Medicinal and aromatic plants (MAPs) have been used in folk medicine for as long as human being have existed in the world. 70.000 species are estimated to have been used in folk medicine in the world. World Health Organization (WHO) has listed about 20.000 species in the world that yield drugs and over 3.000 botanical raw materials in global commerce. Turkey is very rich in MAPs and one of the biggest exporters in the world. A total of 347 native taxa and 28.000 tons of MAPs that are exported annually in Turkey (Farnsworth and Soejarto 1991; Satil et al. 2008; Cindric et al. 2013).

Species belonging to the *Lamiaceae* family are perennial herbaceous MAPs obtained from Mediterranean region. *Thymus*, a member of *Lamiaceae* family, is polymorphic comprising 60 taxa of which 39 species grow in Turkey. The genus *Thymus* has been credited with a long list of pharmacological properties, such as antiseptic antitussive, and expectorant (Schulz et al. 2005). Herbal parts of *Thymus* species are aromatic plants known as “kekik” in Turkey. Also, *Thymus serpyllum* is a well-known MAP and widely distributed in the mountainous region of Central Anatolia of Turkey.

Volatile compounds are one of the most important attributes of *Thymus* species, and can greatly influence the consumers’ acceptance of the thymus and related food products. Wild thyme (*Thymus serpyllum*) is regarded as a source of volatiles containing sesquiterpenes, alcohols, aldehydes, ketones, esters and acids (Raal et al. 2004; Lee et al. 2005). Many studies on the composition of volatiles from different *Thymus* species have been carried out (Venskutonis 1997; Condurso et al. 2013; Martins et al. 2015). The analytical

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studies performed on *Thymus* show the main volatiles are thymol, carvacrol, *p*-cymene, β -pinene, γ -terpinene, β -caryophyllene, 1-borneol, 1,8-cineole (Hudaib and Aburjai 2007; Safaei-Ghomi et al. 2009). Previously published data show remarkable differences in the volatile composition of *Thymus serpyllum* in different countries. *Thymus serpyllum* accumulates high amounts of thymol including 42.63 %, (in Pakistan), 64.6 % (in India), and 81.5 % (in Armenia) (Puri et al. 1985; Sattar et al. 1991; Loziene and Venskutonis 2002; 2006). Contrary to these countries, thymol and carvacrol were not detected in the *Thymus serpyllum* from Lithuania, Sweden, Finland and Poland (Loziene and Venskutonis 2002, 2006).

Up to now, more than 90 volatile compounds have been identified in different thyme samples (Concurso et al. 2013). But only a small fraction of this large number of volatiles in thyme actually contributes to the overall aroma. The technology of gas chromatography-olfactometry (GC-O) made it possible to divide identified volatiles into odour-active and non-odour-active compounds with regard to their existing concentration in the studied sample (Kesen et al. 2014). Additionally, the relative aroma intensity of each component can be determined by aroma extract dilution analysis (AEDA), which involves GC-O evaluation of a serial dilution series of an aromatic extract.

MAPs are known to be a rich source of phenolic compounds. Among the *Lamiaceae* species oregano, (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.), wild thyme (*Thymus serpyllum* L.) have been studied for their high content of phenolic compounds (Takacsova et al. 1995; Vichi et al. 2001). Nonetheless, LC-MS-MS analyses on these herbs were limited in the literature. Kulisic et al. (2006) reported the presence of eight phenolic compounds in the extract of wild thyme. Within the identified phenolics, rosmarinic acid was the main compound and followed by eriocitrin, luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, quercetin, luteolin, apigenin, respectively.

No work has yet been published in this field to characterize the odour-active compounds of *Thymus serpyllum*. In the present paper, the aim was to investigate volatile and aroma-active compounds of *Thymus serpyllum* produced in the Central Anatolia region of Turkey. Sensory evaluation of the aromatic extract obtained by purge and trap was done to assess its representativeness, before the GC-MS olfactometric analysis. Furthermore, there is no information available about the phenolic composition using LC-MS-MS of the studied sample. This study was also undertaken to evaluate phenolic composition of *Thymus serpyllum*.

Materials and methods

Sample and chemicals

Sample The whole-dries *Thymus serpyllum* was used as plant material and collected from the mountain region of the

Kayseri province of Central Anatolia. The sample was gathered before full flowering stage in June of 2014. The plant sample was dried at room temperature (20–25 °C) and used for analysis.

Chemicals Water used in this study was purified by a Millipore-Q system (Millipore Corp., Saint-Quentin, France). The reference volatile compounds were obtained from the following sources: α -pinene, camphene, 3-penten-2-ol, myrcene, delta-2-carene, limonene, delta-3-carene, γ -terpinene, *p*-cymene, acetic acid, linalool, terpinen-4-ol, isovaleric acid, 1-borneol, carvone, heptanoic acid, thymol, carvacrol, hexadecanoic acid, *p*-cymene-2,5-diol were purchased from Sigma-Aldrich (Steinheim, Germany). Standards of gallic acid, protocatechuic acid, caffeic acid, chlorogenic acid, naringin, rutin, rosmarinic acid and luteolin were purchased from Sigma-Aldrich (St Louis, MO, USA). Dichloromethane, sodium sulfate and 4-nonanol were obtained from Merck (Darmstadt, Germany). Dichloromethane was freshly distilled prior to use.

Representativeness test for aromatic extract

Sample preparation and presentation Wild thyme was evaluated using the descriptive and preference tests according to Poste et al. (1991). The panel was composed of ten assessors (seven females and three males between 20 and 45 years old) from Cukurova University, Food Engineering Department. The assessors were previously trained in sensory evaluation techniques. In the present study, we used a cardboard smelling strip (reference 7140 BPSI, Granger-Veyron, Lya, France) for the checking representativeness of the wild thyme aromatic extract obtained purge and trap extraction technique. Three grams of fined samples were placed in 15 mL brown coded flask as a reference for representativeness tests. Aromatic extract obtained from dichloromethane was adsorbed onto a cardboard smelling strip. After 1 min (the time necessary for solvent evaporation) the extremities of the strips were cut off, then placed in dark coded flasks (15 mL) and presented to the panel after 15 min. All the samples were assessed at room temperature (20 °C) in neutral conditions.

Similarity and intensity tests Similarity and intensity tests were performed to demonstrate the closeness between the odour of the extracts and the wild thyme. These test procedures were well documented in our previous studies (Selli et al. 2014).

Analysis of volatile compounds

Extraction of volatile compounds The purge and trap system was used for the extraction process consists in a source of nitrogen controlled by a flow-meter. The needle of the source

of N₂ and the cartridge were installed through the septum to purge and trap the aroma compounds. As adsorbent, 200 mg of Lichrolut EN resins from Merck was chosen as the most suitable material for aroma compounds retention according to the literature (Rodriguez-Bencomo et al. 2015). The temperature of the vial sample was controlled by a thermostated. 3 g sample transferred into 20 mL vial than, the sample was pre-incubated at the extraction temperature for 10 min. The purge and trap process was carried out for 90 min with a nitrogen flow of 500 mL/min. After purging process, the compounds retained in the cartridge were eluted with dichloromethane. After dehydration by anhydrous sodium sulphate, the pooled organic extract was reduced to 5 mL in a Kuderna Danish concentrator fitted with a Snyder column at 40 °C (Supelco, St Quentin, France) and then to 0.5 mL under a gentle stream of nitrogen. Extracts were then stored at −20 °C in a glass vial equipped with a Teflon-lined cap before analysis. Each sample was extracted in triplicate and the concentration of volatiles was expressed as 4-nonanol equivalent.

GC-FID, GC-MS and GC-O analysis of volatile compounds The gas chromatography (GC) system was consisted of Agilent 6890 chromatograph equipped with flame ionization detector (FID), Agilent 5973-Network-mass selective detector (MSD) (Wilmington, USA) and a Gerstel ODP-2 (Linthicum, MD, USA) sniffing port. Volatile compounds were separated on DB-Wax column (30 m length × 0.25 mm i.d. × 0.5 µm thickness, J&W Scientific Folsom, USA). 3 µL of extract was injected in pulsed splitless (40 psi; 0.5 min) mode. Injector and FID detectors were set at 270 °C and 280 °C, respectively. The flow rate of carrier gas (helium) was 1.5 mL min^{−1}. The oven temperature of the DB-Wax column was increased from 50° to 250 °C at a rate of 4 °C/min with a final hold at 250 °C for 10 min.

The same oven temperature programs were used for the mass-selective detector. The MS (electronic impact ionization) conditions were as follows: ionization energy of 70 eV, mass range m/z of 30–300 a.m.u., scan rate of 2.0 scan s^{−1}, interface temperature of 250 °C, and source temperature of 180 °C. The volatile compounds were identified by comparing their retention index and their mass spectra on the DB-Wax column with those of a commercial spectra database (Wiley 6, NBS 75 k) and of the instrument's internal library created from the previous laboratory studies. Some of the identifications were confirmed by the injection of the chemical standards into the GC-MS system. Retention indices of the compounds were calculated by using an n-alkane series.

Aroma extract dilution analysis (AEDA) The aroma extract was analyzed by GS-MS-O using two experienced sniffers. For the AEDA, the concentrated aromatic extract (200 µL) of *Thymus serpyllum* was stepwise diluted 1:1 using dichloromethane as the solvent to obtain dilutions of 1:1, 1:2, 1:4

and up to 1:1024 of the extract. The odour-active compounds perceived by the panelists were recorded when sniffing the effluent from the sniffing port. Sniffing of dilutions was continued until no odorant could be detected by GC-MS-O. Each odorant was thus assigned a flavour dilution factor (FD factor) representing the last dilution in which the odorants was still detectable. FD factor of aroma compounds increases, also the degree of odour activity increases (Selli et al. 2014).

Analysis of phenolic compounds

Extraction of phenolic compounds The extraction of phenolic compounds was carried out according to Martins et al. (2015) after some modifications. *Thymus serpyllum* sample was grinded with laboratory blender and 5 g samples were prepared in duplicate. Sample was extracted with 20 ml of 75 % methanol using magnetic stir bar for 2 h. The extract was separated and the solid was washed with 5 mL of 75 % methanol. The two extracts were combined, filtered and evaporated at vacuum at 20°. Then, extract was filtered through a 0.45-µm pore size membrane filter before injection.

LC-DAD-ESI-MS/MS analysis An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto CA-USA) operated by Windows NT based ChemStation software was used. The HPLC equipment was used with a diode array detector (DAD). System consisted of a binary pump, degasser and auto sampler. The column used was a Phenomenex reversed-phase C-18 column (4.6 mm × 250 mm, 5 µm) (Torrance, CA, USA). The mobile phase consisted of two solvents: Solvent A, water/formic acid (99:1; v/v) and Solvent B, acetonitrile/solvent A (60:40; v/v). Phenolic compounds were eluted under the following conditions: 0.7 mL min^{−1} flow rate and the temperature was set at 25 °C, isocratic conditions from 0 to 10 min with 0 % B, gradient conditions from 0 to 5 % B in 30 min, from 5 to 15 % B in 18 min, from 15 to 25 % B in 14 min, from 25 to 50 % B in 31 min, from 50 to 100 % B in 3 min, followed by washing and reconditioning the column. The DAD was set at 280, 320, and 360 nm for real-time monitoring of the peak intensity, and full spectra (190–650 nm) were continuously recorded for thyme component identification. The identification and assignation of each compounds was performed by comparing their retention times and UV spectra to authentic standards and also confirmed by an Agilent 6430 LC-MS/MS spectrometer equipped with an electrospray ionization source. The electrospray ionization mass spectrometry detection was performed in negative mode with the following optimized parameters: capillary temperature 400 °C; drying gas N₂ 12 L/min; nebulizer pressure, 45 psi (Fecka and Turek 2008; Cui et al. 2015). Data gaining was performed using multiple reactions monitoring (MRM) method that only monitors specific mass transitions during preset retention times. Dynamic MRM also has the ability to

simultaneously perform ESI in both positive and negative mode, allowing for the analysis of different phenolic classes within a single chromatographic run.

Results and discussion

Intensity and similarity evaluation

Intensity and similarity evaluation tests have great importance in evaluating the representativeness of the aromatic extracts (Selli et al. 2008). The similarity scores of the aromatic extract obtained by purge and trap was found to be 75.1 mm on a 100 mm unstructured scale. The similarity of the extract was found to be an acceptable level. When we compared it with the other studies, the similarity of the cherry tomato was found to be 70.4 by Selli et al. (2014), for olive oil extract 75.7 by Kesen et al. (2014). Regarding the intensity score of the aromatic extract, it was found to be 72.3 mm on a 100 mm

unstructured scale. This score is also high and acceptable. Briefly, the similarity and intensity results indicated that the representative aromatic extract was achieved with the purge and trap method in order to determine the wild thyme volatile and aroma-active compounds.

Volatile composition of wild thyme

Table 1 shows the volatile compounds, their retention indices and mean values ($\text{mg } 100 \text{ g}^{-1}$) with standard deviations in wild thyme. A total of 24 volatile compounds were identified in the sample (Fig. 1). Most volatiles detected in this study were consistent with those of previously published studies (Inouye et al. 2001; Paaver et al. 2008). The main chemical group of the volatile compounds in the wild thyme sample was terpenes. These compounds represented over 90 % of the total volatiles in wild thyme. Other chemical groups such as alcohols and acids were present in minor quantities. Safaei-Ghomi et al. (2009) reported that the leaf essential oil of wild thyme was

Table 1 Volatile composition of wild thyme (*Thymus serpyllum*)

No	Compound	LRI*	Concentration [#] ($\text{mg } 100 \text{ g}^{-1} \text{ DW}$)	Identification [§]
1	α -Pinene	1025	18.1 ± 0.4	LRI,MS,std
2	Camphene	1095	7.68 ± 0.1	LRI,MS,std
3	3-Penten-2-ol	1156	7.81 ± 0.1	LRI,MS,std
4	Myrcene	1159	28.4 ± 0.6	LRI,MS,std
5	Delta 2-carene	1173	25.0 ± 0.5	LRI,MS,std
6	Limonene	1195	5.99 ± 0.1	LRI,MS,std
7	Delta 3-carene	1197	8.52 ± 0.1	LRI,MS,std
8	γ -Terpinene	1199	90.4 ± 2.0	LRI,MS,std
9	p-Cymene	1205	88.8 ± 2.0	LRI,MS,std
10	4-Methyl-2 pentanol	1391	11.8 ± 0.2	LRI,MS, tent
11	Acetic acid	1404	2.22 ± 0.0	LRI,MS,std
12	Linalool	1415	4.97 ± 0.1	LRI,MS,std
13	Sabinene hydrate	1421	3.09 ± 0.0	LRI,MS, tent
14	Terpinen-4-ol	1591	7.91 ± 0.1	LRI,MS,std
15	Isovaleric acid	1665	1.85 ± 0.0	LRI,MS,std
16	Borneol	1677	7.32 ± 0.1	LRI,MS,std
17	Carvone	1751	5.54 ± 0.1	LRI,MS,std
18	Delta-cadinene	1753	2.63 ± 0.0	LRI,MS, tent
19	Heptanoic acid	1945	2.22 ± 0.0	LRI,MS,std
20	Trans ocimenone	2062	1.91 ± 0.0	LRI,MS, tent
21	Thymol	2116	1702 ± 87.3	LRI,MS,std
22	Carvacrol	2215	179 ± 4.1	LRI,MS,std
23	Hexadecanoic acid	2563	5.99 ± 0.1	LRI,MS,std
24	p-Cymene-2,5-diol	2742	12.2 ± 0.2	LRI,MS,std
Total			2231 ± 99.4	

LRI*: Linear retention index calculated on DB-WAX capillary column; Concentration[#]: Results are the means of three repetitions as $\text{mg } 100 \text{ g}^{-1}$ of dry weight (DW) with $\pm$ standard deviations; Identification[§]: Methods of identification; LRI (linear retention index), MS tent. (tentatively identified by MS), Std (chemical standard); When only MS or LRI is available for the identification of a compounds, it must be considered as an attempt of identification; $\pm$: standard deviation

Fig. 1 The GC-MS chromatogram of identified volatile compounds of *Thymus serpyllum* (Peak numbers refer to Table 1)

largely made up of oxygenated monoterpenoids, sesquiterpene and monoterpen hydrocarbons. Thymol and carvacrol are the main components of essential oil of wild thyme. Thymol and carvacrol are of special commercial interest as the preservation of food because of their high potency as antibacterials and antifungal agents (Schulz et al. 2005). Thymol was found in essential oil of wild thyme from Japan 35 %, Pakistan 42.6 %, India 64.6 %, and 81.5 % Armenia (Puri et al. 1985; Sattar et al. 1991; Loziene and Venskutonis 2002, 2006). However, the content of carvacrol is usually lower than the thymol content (Paaver et al. 2008). The obtained results revealed that the most important compounds were thymol ($1702 \text{ mg } 100 \text{ g}^{-1}$) and carvacrol ($179 \text{ mg } 100 \text{ g}^{-1}$) in the studied sample. These volatiles were distinctive for *T. serpyllum* species and found as

major compounds in other previous results, as well (Inouye et al. 2001; Saez 2001). Nonetheless, the content of the volatiles in *T. serpyllum* depends on different factors, cultivation conditions such as geographical origin, climatic conditions, soil, harvesting time etc. (Banaeva et al. 1998). Loziene and Venskutonis (2002, 2006) reported, *T. serpyllum* growing the northern climatic area do not accumulate the thymol and carvacrol in more remarkable amount.

γ -Terpinene and *p*-cymene were the other main compounds which detected in wild thyme. *p*-Cymene was previously found as precursor of the biosynthesis of thymol and carvacrol in thyme oil. The amount of these compounds was quantified $90.4 \text{ mg } 100 \text{ g}^{-1}$ and $88.8 \text{ mg } 100 \text{ g}^{-1}$ in studied sample, respectively. In the literature, high concentrations of γ -terpinene and *p*-cymene were detected in essential oils of wild thyme herbs both from Latvia and Estonia (Raal et al. 2004). The other terpenes in our sample were previously identified in *Thymus serpyllum* and various thyme species (Saez 2001; Raal et al. 2004; Schulz et al. 2005; Safaei-Ghomi et al. 2009).

Table 2 Aroma-active compounds of wild thyme (*Thymus serpyllum*)

No.	LRI ^b	Aroma compound	Odour description ^c	FD factor ^a
1	1014	Unknown	Herbal	32
2	1025	α -Pinene	Green, woody	64
3	1095	Camphene	Woody	32
4	1159	Myrcene	Green	128
5	1197	Delta 3-carene	Mint	256
6	1199	γ -Terpinene	Citrus	128
7	1421	Sabinene hydrate	Herbal	512
8	1591	Terpinene 4-ol	Vegetable	64
9	1665	Isovaleric acid	Cheesy	16
10	1702	Unknown	Flowery	256
11	1751	Carvone	Flowery	128
12	2116	Thymol	Thyme	1024

Aroma-active compounds of wild thyme

The results of the olfactometric analysis were given in Table 2. The potent odorants were determined using AEDA for the determination of flavour dilution (FD) factor. Application of the ADEA on wild thyme extract revealed 12 aroma-active compounds by using purge and trap method. The FD factors of the compounds range from 16 to 1024 including, terpenes (9), acid (1) and unknown (2) compounds. Unknown odorants were detected by GC-MS-O, but could not be identified by GC-MS. The aroma-active compounds of wild thyme were

dominated by terpenes. The compounds which have the highest FD factors were thymol (FD=1024), sabinene hydrate (512), delta-3-carene (FD=256), carvone (FD=128), γ -terpinene (FD=128), and myrcene (FD=128).

Terpenes were the major aroma-active components of wild thyme. Nine terpenes were identified in the extract as aroma active-compounds (Table 2). These terpenes are extensive, as they already have been found in other varieties of *Thymus* species (Diaz-Maroto et al. 2005; Goodner et al. 2006). Among the terpenes, thymol (FD=1024) was the most powerful aroma active compound to contribute to the aroma profile of the wild thyme providing the characteristic thyme odour, followed by sabinene hydrate (herbal) and delta-3-carene (mint). Thymol is part of a naturally occurring class of compounds known as biocides, and the threshold of it is $50 \mu\text{g L}^{-1}$ in aqueous solution (Diaz-Maroto et al. 2005). This study revealed that this compound is the main aroma active compound of *Thymus serpyllum* due to its highest concentration in the herb. Our present study supports earlier result on thyme that confirm thymol is most important contributor to thyme aroma profile (Diaz-Maroto et al. 2005; Goodner et al. 2006). The other aroma-active terpene in terms of the FD factor was sabinene hydrate. The FD factor of this compound was 512. The above mentioned compound has been detected as an important contributor to the thyme odour by Diaz-Maroto et al. (2005). Also, this compound was detected as one of the most abundant components of the *Thymus* species by many researchers (Rasooli and Mirmostafa 2002; Raal et al. 2004; Schulz et al. 2005; Safaei-Ghomi et al. 2009). Among the terpenes, α -pinene (green, woody), camphene (woody), myrcene (green), delta 3-carene (mint), γ -terpinene (citrus),

terpinene-4-ol (vegetable), carvone (flowery) were the following aroma-active compounds which have the lowest FD factors detected by GS-MS-O. However, α -pinene, camphene, myrcene, delta-3-carene, γ -terpinene and terpinene-4-ol have been previously identified as aroma-active compounds in *Thymus* cultivars in previously data on the species (Diaz-Maroto et al. 2005; Goodner et al. 2006).

Two unknown compounds may contribute to general aroma of wild thyme. Unknown 1 (LRI=1014), with herbal note and the FD factor of this compound was 32. The unknown 2 (LRI=1702) was determined in wild thyme as providing high flowery not with a FD=256.

Phenolic composition of wild thyme

The phenolic profile of *T. serpyllum*, obtained after hydro alcoholic extraction and mass spectral data for compounds identified in negative ionization mode were listed in Table 3. A total of 18 phenolic compounds were identified, eight of which were phenolic acids, ten flavan-3-ols. As for the phenolic compounds, 10 of which (gallic acid, protocatechuic acid, protocatechuic acid-hexoside, apigenin 6,8-di-*C*-glucoside, naringin, luteolin-*O*-diglucuronide, kaempferol *O*-glucuronide, rosmarinic acid-glucoside, apigenin *O*-glucuronide and methyl kaempferol *O*-rutinoside) were identified for the first time in *T. serpyllum*. LC-ESI-MS/MS chromatogram of phenolic compounds extracted from *T. serpyllum* was given in Fig. 2.

Among phenolic acids, gallic acid (peak 1), protocatechuic acid (peak2), caffeic acid (peak 5), chlorogenic acid (peak 6), and rosmarinic acid (peak 14) were identified according to

Table 3 Retention time, mass spectral data and identification of phenolic compounds of wild thyme (*Thymus serpyllum*)

Peak	UV (nm)	M – H [–] (m/z)	MS ₂ (m/z) [–]	Compound identity	Quantification (mg g ^{–1} dry herb)
1	268	169	125	Gallic acid	0.63
2	292	153	123, 109	Protocatechuic acid	4.82
3	292	315	153, 135	Protocatechuic acid-hexoside	0.46
4	336	593	503	Apigenin 6,8-di- <i>C</i> -glucoside	2.58
5	290,330	179	135, 107	Caffeic acid	1.73
6	320	353	191	Chlorogenic acid	7.88
7	284	579	459	Naringin	3.03
8	320	193	134, 117	Ferulic acid	4.54
9	350	637	461, 285	Luteolin- <i>O</i> -diglucuronide	3.40
10	360	609	301	Rutin	1.35
11	348	461	285	Luteolin 7- <i>O</i> -glucuronide	8.86
12	320	521	359,179	Rosmarinic acid-glucoside	4.36
13	346	447	285	Luteolin 7- <i>O</i> -glucoside	51.84
14	330	359	197,161	Rosmarinic acid	21.72
15	340	445	269	Apigenin <i>O</i> -glucuronide	1.93
16	336	607	299	Methyl kaempferol <i>O</i> -rutinoside	17.42
17	344	461	285	Kaempferol <i>O</i> -glucuronide	15.21
18	259, 370	285	175,199	Luteolin	48.04

Fig. 2 LC-DAD and LC-ESI-MS/MS MRM chromatograms of the identified phenolic compounds of *Thymus serpyllum*

their retention, mass and UV–vis characteristics by comparison with commercial standards and LC-ESI-MS/MS analysis. The presence of chlorogenic acid was previously reported in *T. serpyllum* by Fecka and Turek (2008) and Boros et al. (2010) also caffeic acid was identified in tea infusions and leaves in *T. serpyllum* (Kulisic et al. 2006; Fecka and Turek

2008; Boros et al. 2010; Miron et al. 2011). However, gallic acid and protocatechuic acid compounds were previously identified in *T. vulgaris* (Proestos et al. 2005; Miron et al. 2011; Roby et al. 2013; Martins et al. 2015). Peak 3 ($[M-H]^-$ at m/z 315) presented characteristic UV and MS spectra consistent with a protocatechuic acid-hexoside. This compound was

described in plants, such as those that are member of the *Prunus* genus, *Salvia officinalis* (Zimmermann et al. 2011) but never before in *T. serpyllum*. Peak 8 ($[M-H]^-$ at m/z 193, and MS^2 fragment ions at m/z 134, 117) could be assigned as ferulic acid. This compound was reported in wild thyme by Boros et al. (2010). Rosmarinic acid-glucoside (Peak 12; $[M-H]^-$ at m/z 521) was another important phenolic acid in wild thyme. This compound is probably the most abundant soluble bound phenolic acid, and is a type of non-flavonoid catecholic compound, which is present in many plants (Nagy et al. 2011; Martins et al. 2015). In general, rosmarinic acid was the most abundant phenolic acid found in the present study; this compound is well known by its potent antioxidant activity, radical-scavenging and antiviral properties. Besides, it takes part in inhibit gonadotrophin release, xanthine oxidase and adenylate cyclase activities. Fecka and Turek (2008) and Berdowska et al. (2013) studied a sample of *T. serpyllum* from Poland, after hot aqueous extraction from the examined sample. Both identified and quantified rosmarinic acid as major compound detected.

The other phenolic compounds identified in the analyzed sample belong to the groups of flavones, flavonols and flavanones. Apigenin 6,8-di-*C*-glucoside (peak 4) was identified as a $[M-H]^-$ deprotonated molecule (m/z 593) yielded ion fragments at m/z 503. Apigenin 6,8-di-*C*-glucoside was previously reported in *T. vulgaris* by Vergara-Salinas et al. (2012). Furthermore, luteolin-*O*-diglucuronide ($[M-H]^-$ at m/z 637), methyl kaempferol *O*-rutinoside ($[M-H]^-$ at m/z 607) and kaempferol-*O*-glucuronide ($[M-H]^-$ at m/z 461) were tentatively identified in *T. vulgaris* by Martins et al. (2015). Peak 11 presented a pseudo molecular ion $[M-H]^-$ at m/z 461, together with the characteristic fragment ions at m/z 285. This compound was assigned to luteolin 7-*O*-glucuronide owing to the identification of that compound in leaves from *T. vulgaris* by Dapkevicius et al. (2002) and Vergara-Salinas et al. (2012), wild thyme by Miron et al. (2011).

Luteolin 7-*O*-glucoside (peak 13) and luteolin (peak 18) were the most abundant phenolic compound detected in the sample. Luteolin 7-*O*-glucoside according to its mass spectrum, shows the pseudo molecular ion $[M-H]^-$ at m/z 447, and characteristic fragment ions at m/z 285. Luteolin 7-*O*-glucoside was among the functional compounds in thyme that suppress oxidative stress. Moreover this compound was identified in thyme infusions (Kulisic et al. 2006); leaves from *T. vulgaris* (Dapkevicius et al. 2002; Vergara-Salinas et al. 2012); and *T. serpyllum* (Miron et al. 2011). Luteolin was identified as a $[M-H]^-$ deprotonated molecule (m/z 285) yielded ion fragments at m/z 175 and 199. This compound has high radical scavenging ability. It was also largely identified in different thyme varieties and obtained by different extraction methodologies (Dapkevicius et al. 2002; Kulisic et al. 2006; Vergara-Salinas et al. 2012). Besides, Miron et al. (2011) reported that luteolin was the main identified compound in the wild thyme ethanolic extract.

In the literature, it is reported that variations in the phenolic profiles of *Thymus* species depend on cultivars, place of origin, agronomic techniques and harvesting processing. On the other hand, the use of wild thyme a dietary supplement, could support the health benefits, both in the treatment of diseases related to reactive species production and oxidative stress and against bacterial infections (Dapkevicius et al. 2002; Martins et al. 2015).

Conclusions

A total of 18 phenolic compounds were identified and quantified in the samples of which 10 compounds (peak 1, 2, 3, 4, 7, 9, 12, 15, 16 and 17) have not been reported previously in *T. serpyllum*. The phenolic profiling of *T. serpyllum* and specially the confirmation of the identity by LC-ESI-MS/MS of the three major phenolics in the herb (peak 13, 14 and 18) that could be used as chemical markers. With regard to the volatile profile, a total of 24 aroma compounds have been identified and quantified in the aromatic extract of *T. serpyllum*. Also, aroma extract dilution analysis (AEDA) was used for the first time for the determination of aroma-active compounds of *Thymus serpyllum*. In total 12 compounds were detected as aroma-active compounds. Within these thymol (FD=1024), sabinene hydrate (FD=512) and delta 3-carene (FD=256) were the most powerful contributors to the aroma of the *T. serpyllum*.

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