Industrial Crops & Products 109 (2017) 445–451

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Research Paper

Phenological changes in triterpenic and phenolic composition of *Thymus* L. species

Lina Raudone[a,*], Kristina Zymone[a], Raimondas Raudonis[a], Rimanta Vainoriene[b], Vida Motiekaityte[c], Valdimaras Janulis[a]

[a] Department of Pharmacognosy, Lithuanian University of Health Sciences, Sukileliu av. 13, LT-50162, Kaunas, Lithuania
[b] The Botanical Garden of Šiauliai University, Paitaiciu str. 4, LT-77175, Siauliai, Lithuania
[c] Department of Biomedicinal Sciences, Siauliai State College, Ausros av. 40, Siauliai LT-76241, Lithuania

ARTICLE INFO

Keywords:
Thymus
Phenolic acid
Flavone
Triterpenic acid
Phenological phase

ABSTRACT

Abundant phenolic and triterpenic compounds constitute a characteristic chemical profile of the *Thymus* species. Phenolic acids and flavone glycosides (rosmarinic acid, caffeic acid, luteolin-7-rutinoside, luteolin-7-glucoside and apigenin-7-glucoside) were identified and quantified in the samples of the *Thymus austriacus* Bernh. ex Rchb., *Thymus praecox* Opiz ssp. *arcticus* (Durand) Jalas, *Thymus serpyllum* L., *Thymus pulegioides* L., *Thymus sibthorpii* Benth, *Thymus x citriodorus* Schweig. et Korte and *Thymus x oblongifolius* Opiz, *Thymus longicaulis* C. Presl. The content of these compounds varied significantly at different phenological phases. The amounts of phenolic compounds were the greatest at the budding and massive flowering phases, declined during fruiting and were the lowest at the end of vegetation. Rosmarinic acid was determined as a predominant phenolic compound. Four triterpenic acids, oleanolic, ursolic, corosolic and betulinic were identified in the samples. Ursolic acid was found to be predominant triterpene followed by oleanolic, betulinic and corosolic acids, on the average 9594 ± 654 μg/g, 4334 ± 351 μg/g, 319 ± 38 μg/g, 84 ± 22 μg/g, respectively. Accumulation pattern of ursolic acid varied significantly depending on the *Thymus* species investigated. The phenolic-dominant and the triterpenic-dominant types of the *Thymus* species could be distinguished.

1. Introduction

The *Thymus* L. is a taxonomically and systematically complex genus comprising 220 species (Jarić et al., 2015). The genus is distributed and cultivated all over the world with the central distributional area covering the Mediterranean region (Horwath et al., 2008). The *Thymus* species are widely used as culinary herbs as well as a traditional herbal medicinal product to treat gastrointestinal disorders, respiratory infections, skin disorders (Costa et al., 2012; Jarić et al., 2015; Özgen et al., 2011; Pereira et al., 2013; (EMA/HMPC/342334/2013, 2013). Various pharmacological studies have proven the antioxidant, anti-inflammatory, spasmolytic, antitussive, expectorant, antibacterial, antifungal, antiviral, carminative, and astringent activities (Boros et al., 2010; Costa et al., 2012; Jarić et al., 2015; Kindl et al., 2015; Kontogiorgis et al., 2016; Ložienė, 2009; Nabavi et al., 2015; Zarshenas and Krenn, 2015). These health promoting properties of *Thymus* species have been attributed to the presence of phenolic compounds, triterpenic acids and especially essential oils (Öztürk, 2015). Extensive research has been performed on essential oil composition of different *Thymus* species (De Martino et al., 2009; Jamali et al., 2013; Jordán et al., 2006; Ložienė et al., 2012; Ložienė and Venskutonis, 2010, 2005; Satyal et al., 2016; Tohidi et al., 2017; Vaičiulytė et al., 2017). Several phytochemical studies have shown that *Thymus* species have characteristic phenolic and triterpenic profiles which mainly depend on genetic origin and therefore could be possibly used in taxonomical and systematical studies (Díaz-García et al., 2015; Horwath et al., 2008; Janicsák et al., 2006). Collecting raw materials from natural habitats significantly increases the risk of chemical polymorphism that is very characteristic of *Thymus* species. Cultivation ensures qualitative and chemically homogeneous raw material that can be more easily standardised and used in safety and efficacy studies (Ložienė, 2009; Vaičiulytė et al., 2017; Vaičiulytė and Ložienė, 2015). Although morphological and chemical polymorphism depends on the genetic origin, the harvesting time and environmental conditions of growing area can influence the quantitative composition of bioactive compounds (Vaičiulytė et al., 2017). Phytochemical composition of herbal material can vary significantly

* Corresponding author.
E-mail addresses: lina.raudone@lsmuni.lt, raudone.lina@gmail.com (L. Raudone), kristigai@gmail.com (K. Zymone), raimondas.raudonis@lsmuni.lt (R. Raudonis), rimanta.vainoriene@su.lt (R. Vainoriene), vmotiek@gmail.com (V. Motiekaityte), farmakog@lsmuni.lt (V. Janulis).

http://dx.doi.org/10.1016/j.indcrop.2017.08.054
Received 12 April 2017; Received in revised form 21 August 2017; Accepted 28 August 2017

during the phenological cycle. Therefore it is very important to select optimal ontogenetic stages yielding in the highest amounts of bioactive compounds and ensuring the homogeneity of the quality of raw material.

Previous reports have determined certain phenolic compounds in *T. pannonicus, T. glabrescens*, *T. pulegioides* (Boros et al., 2010), *T. serpyllum* (Boros et al., 2010; Kulisic et al., 2006), *T. praecox* (Boros et al., 2010; Sevindik et al., 2015; Turumtay et al., 2014) *T. lotocephalus* (Costa et al., 2012), *T. nummularius* (Ertas et al., 2015), *T x citriodorus* (Pereira et al., 2013; Shekarchi et al., 2012), *T. sipyleus* (Özgen et al., 2011), *T. longicaulis* (Galasso et al., 2014), *T. vulgaris* (Dapkevicius et al., 2002; Kulisic et al., 2006), *T. sibthorpii* (Kontogiorgis et al., 2016). Only a few reports regarding the composition of triterpenic acids in *T. vulgaris* (Jäger et al., 2009; Janicsák et al., 2006), *T. serpyllum*, and *T. x citriodorus* (Janicsák et al., 2006) were found.

To the best of our knowledge, no research of phenolics was performed on *T. austriacus, T. praecox* ssp. *arcticus* and *T x oblongifolius*, no studies of the triterpenic acidswere performed on *T. praecox* ssp. *arcticus, T. sibthorpii, T. austriacus, T. pulegioides, T x oblongifolius, and T. longicaulis*. The influence of plant development stages on the content of phenolic and triterpenic compounds was not determined in these species before. This is the first report on the phenological changes of phenolic and triterpenic compounds of certain cultivated *Thymus* species.

2. Materials and methods

2.1. Chemicals and solvents

Analytical and chromatographic grade reagents were used for this study: acetonitrile, methanol, caffeic acid, rosmarinic acid, apigenin-7-glucoside, luteolin-7-rutinoside, betulinic acid, corosolic acid, oleanolic acid, ursolic acid (Sigma-Aldrich GmbH, Steinheim, Germany), luteolin-7-glucoside (Extrasynthese, Genay, France), 99.8% trifluoracetic acid (Carl Roth GmbH, Karlsruhe, Germany), 96% ethanol (Vilniaus degtinė SC, Vilnius, Lithuania). The purified deionized water (18.2 mΩ/cm) was produced using the Millipore (USA) water purification system.

2.2. Plant material and growing conditions

The Thymus species were grown in the field collection of Botanical Garden of Siauliai University (55° 55′ 57″ N, 23° 16′ 59″ E. (WGS)). Native Lithuanian thyme species (*T. serpyllum L., T. pulegioides L., T. x oblongifolius* Opiz) as well as introduced species (*T. sibthorpii* Benth, *T. x citriodorus* Schweig. et Korte) were received from the Institute of Botany of the Nature Research Centre Vilnius as clones in 2008 year. Clone of *T. austriacus* Bernh. ex Rchb. was obtained from Kaunas Botanical Garden of Vytautas Magnus university in 2000 year. Two species were received as seeds: *Thymus praecox* Opiz ssp. *arcticus* (Durand) Jalas from Reykjavik Botanical Garden (natural habitat in W-Iceland: Hvalfjörbur, Flekkudalur, 64°18′ N 21° 34′ W) in 2011 and *Thymus longicaulis* C. Presl from the Garden of Medicinal Plants University of Medicine in Wroclaw in 2013 (Hortus Botanicus Reykjavikensis, 2010; Hortus Plantarum Medicinarum Universitatis Medicae Wratislaviensis, 2013).

Plant identification. Morphological criteria and their variability of the species were investigated (length and width of the leaves, leaves length and width ratio, length of inflorescences, width of calyx, length of calyx tubule, length of generative sprout, numbers of inflorescence whorls and internodes of stems, etc.). Microscope Motic ST-39 and monographs and research papers on Thymus of Lithuanian and foreign researches: (Lekavičius and Jaskonis, 1969; Kuusk et al., 1996; Cullen et al., 2000; Bojnanský and Fargašová, 2007; Jäger, 2007; Jäger et al., 2007; Jäger, 2011) were used for the identification of species. Taxa of *Thymus*, which were obtained from Institute of Botany in Vilnius are being cloned repeatedly every year in Botanic garden. This material was presented to Botanic garden by Dr. K. Loziene personally (Ložienė, 2002; Kamašina and Ložienė 2009; Ložienė and Venskutonis 2010). *Thymus* species were grown in open, non-shaded, sandy loam soil. Fertilisation and irrigation during the season have not been applied. Voucher specimens were deposited in the herbarium of the Botanical Garden of Siauliai University. The above-ground parts of plants were collected at different stages of their development between May and September 2016. The material was comprised of aboveground parts of 10 model plants in 4 phenological phases: floral budding (FB: 31/05/2016–20/06/2016), massive flowering (MF: 13/06/2016–12/07/2016), fruit maturity (FM: 10/07/2016–01/08/2016) and the end of vegetation (EV: 15/08/2016–28/09/2016). The meteorological data (temperature, °C; precipitation mm; sunshine duration, h) were obtained from the archive of Lithuanian Hydrometeorological Service under the Ministry of Environment and are presented in Fig. 1.

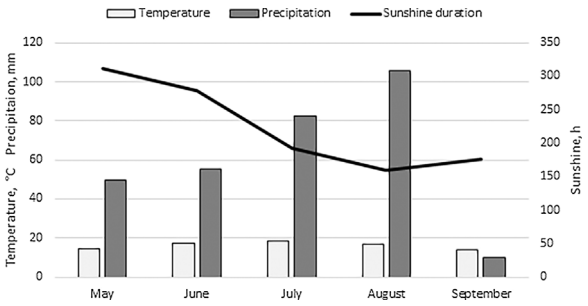

Fig. 1. Dynamics of meteorological factors: average air temperatures (°C), amount of precipitation (mm) and sunshine duration (h) during the vegetation period (May–September, 2016), Siauliai city (distance between meteorological station and Botanical garden is 3.1 km (55° 56′ 28″ N, 23° 10′ 50″ E. (WGS)).

2.3. Sample preparation

For qualitative and quantitative analysis, the thyme herb samples were crushed to particles and passed through a 355 μm sieve. The weighed raw plant material sample 0.1 g was placed into a conical flask with 10 mL of 70% ethanol for the analysis of phenolic compounds. The weighed 0.5 g sample was placed into a conical flask with 10 mL of 100% methanol for the analysis of triterpenic compounds. The extraction was performed by submerging the materials in an ultrasonic bath "Elmasonic P" (Elma Schmidbauer GmbH, Singen, Germany) for 30 min. The extracted samples were centrifuged at 8500 rpm for 4 min. Before HPLC analysis extracts were filtered through a membrane filter with a pore size of 0.22 μm (Carl Roth GmbH, Karlsruhe, Germany).

2.4. Determination of phenolic and triterpenic compounds by HPLC–UV

The qualitative and quantitative analysis was performed using a "Waters e2695 Alliance system" (Waters, Milford, MA, USA) with a photodiode array detector "Waters 2998". Separation of phenolic compounds was performed using an "ACE" (ACT, UK) column (C18, 150 mm × 4.6 mm, particle size 3 μm). The mobile phase of the optimised chromatographic method consisted of eluent A (0.05% trifluoracetic acid) and B (acetonitrile). The gradient was: 0–5 min – 12% B, 5–50 min – 12–30% B, 50–51 min – 30–90% B, 51–56 min – 90% B, 57 min – 12% B. Eluent flow rate – 0.5 mL/min, injection volume – 10 μL.

Separation of triterpenic compounds was performed using an "ACE" (ACT, UK) column (C18, 250 mm × 4.6 mm, particle size 5 μm). The mobile phase of the isocratic chromatographic method consisted of eluent 12% A (water) and 88% B (acetonitrile). Eluent flow rate – 1 mL/min, injection volume – 10 μL.

The column was maintained at 25 °C. Chromatographic peak identification was carried out according to the analyte and reference compound retention time as well as by comparing their UV absorption spectra at 200–400 nm. Calibration curves of compounds identified in