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THE VARIABILITY OF SECONDARY METABOLITES IN *MENTHA* × *PIPERITA* CV. 'PERPETA' DURING THE DEVELOPMENT OF INFLORESCENCE

KOLÍSANIE SEKUNDÁRNYCH METABOLITOV V *MENTHA* × *PIPERITA* CV. 'PERPETA' POČAS VÝVOJA KVETNÝCH ORGÁNOV

Original research article

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Abstract *Mentha* × *piperita* (L.) Huds. of the family Lamiaceae is a very important species for commercial exploitation due to the high content of essential oil. Besides the essential oils, there are also other significant secondary metabolites in peppermint, especially flavonoids and hydroxycinnamic derivatives. In this study, we evaluate the variability of essential oil constituents and of phenolic compounds in the leaves of *M.* × *piperita* during the development of inflorescence. Similar studies, dealing with the variation of the essential oils and its composition, have been performed in the past, but no attention was paid to the variation of other secondary metabolites. We examined *M.* × *piperita* cv. 'Perpeta', the domestic cultivar. The plants were cultivated in the climatic conditions of south-west Slovakia. The contents of secondary metabolites were investigated in the week periods, from the beginning of inflorescence formation to the end of flowering. The yield and the quality of essential oil have been carried out by distillation and gas chromatography mass spectrometry (GC-MS). The phenolic substances were analysed using spectrophotometric methods according to European Pharmacopoeia. The highest contents of phenolic substances have been found in leaves of plants during the flowering phenophase, the same stage when essential oil of mint also achieves the highest quality.

Slovak abstract *Mentha* × *piperita* (L.) Huds. z čeľade Lamiaceae je významný druh najmä z hľadiska komerčného využitia pre vysoký obsah silice. Okrem silice sa v mäte piepornej nachádzajú aj ďalšie významné sekundárne metabolity. Sú to najmä látky fenolového charakteru (flavonoidy a hydroxyškoricové deriváty). V tejto práci sa hodnotilo kolísanie hlavných zložiek silice a fenolových látok v listoch *Mentha* × *piperita* počas vývinu kvetných orgánov. V minulosti sa podobné práce zaoberali kolísaním obsahu silice a jej zložiek. Kolísaniu fenolových látok v tomto období sa doposiaľ nevenovala veľká pozornosť. My sme sledovali domácu odrodu *Mentha* × *piperita* cv. ‚Perpeta'. Rastliny sa pestovali v klimatických podmienkach juhozápadného Slovenska. Kolísanie obsahových látok sa sledovalo v týždenných intervaloch v období od začiatku tvorby súkvetí až po odkvitanie. Obsah a kvalita silice sa stanovili po destilácii s vodou GC-MS metódou a obsah jednotlivých fenolových látok sa stanovil spektrofotometricky s využitím metód Európskeho liekopisu. Najvyšší obsah fenolových látok v listoch sa zaznamenal v období nástupu kvitnutia rastliny, teda v tom istom období, kedy silica mäty dosahuje najvyššiu kvalitu.

Keywords *Mentha × piperita, flavonoids, flowering stage, THD, polyphenols, essential oil*

Kľúčové slová: *Mentha × piperita, flavonoidy, fáza kvitnutia, THD, polyfenoly, silica*

INTRODUCTION

Mints (*Mentha* L., Lamiaceae) are known and classified as aromatic plants with high content of essential oil (EO) and are famous medical plants in traditional and conventional medicine all over the world. *Mentha* × *piperita*, the best known mint, is used chiefly for the treatment of gastrointestinal disorders. Peppermint has also shown strong antimicrobial, antiviral, antioxidant activity and seems to have an antitumour, immunomodulating, antiallergic and chemoprotective potential (Mckay & Blumberg, 2006; Košťálová *et al.*; 2012). The pharmacological effects of mints are chiefly because of the presence of two main groups of secondary metabolites: EO and phenolic compounds. EO of mints is composed of monoterpenes and sesquiterpenes, the content of which varies from species to species. The main phenolic compounds in mints are phenolic acids (especially rosmarinic acid) and flavonoids (eriodictyol, luteolin, apigenin and their glycosides). In the

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past, some studies were carried out dealing with changes in EO composition depending on flowering stage and year/day of harvest (Lawrence, 2007; Felklová *et al.*, 1981; Neugebauerová & Kafková, 2012). The aim of this study is to determine the content of secondary metabolites in leaves of *M.* × *piperita* of different harvests according to flowering stage. This study focuses on comparing the quality of peppermint during the development of inflorescence.

2. MATERIAL AND METHODS

2.1. Plant material

The plants selected for this study were grown in the climatic conditions of the south-west Slovakia (Bratislava). Variation in the content of substances was followed at weekly intervals, in the period from the beginning of the formation of inflorescence to the phase of overblown flowers. The leaves of *M.* × *piperita* cv. 'Perpeta' were harvested in during the week period of the two summer months; during the period from 30.6.2007 to 28.8.2007 (Table 1), on sunny days, morning at 10 am.

2.2. The determination of secondary metabolites

Phenol compounds were determined in dry leaves within 1 year after the harvest using spectrophotometric methods of the European Pharmacopoeia (Ph. Eur. 6, 2007).

Total hydroxycinnamic derivatives (THD, Arnow´s assay)

The content of THD was determined using a colorimetric method with the Arnow´s reagent at 505 nm (Spectrophotometer, Thermo Electron Corporation Genesys 6, Waltham, Massachusetts, USA). The percentage content of THD were calculated with reference to the dried drug and expressed as rosmarinic acid.

Flavonoids (spectrophotometric assay)

The content of total flavonoids was determined by a spectrophotometric method using aluminium chloride (Spectrophotometer, Thermo Electron Corporation Genesys 6, Waltham, Massachusetts, USA). The percentage contents were determined at 392 nm and were calculated with reference to the dried drug and expressed as luteolin-7-*O*-glucoside (one of the most abundant flavone glycoside in mint family).

Total polyphenols (spectrophotometric assay)

The content of total polyphenols was determined using a colorimetric method with the Folin–Ciocalteau reagent at 760 nm (Spectrophotometer, Thermo Electron Corporation Genesys 6, Waltham, Massachusetts, USA). The percentage contents were calculated with reference to the dried drug and expressed as rosmarinic acid.

Steam distillation of EO

The distillation was carried out immediately after drying of the plant material. 200 ml of water as the distillation liquid was added to 20.0 g of crushed drug in a 500 ml flask. Distillation was carried out at a rate of 3-4 ml/min for 2 h. The volume of isolated EO was measured in the graduated tube.

The analysis of EO by GC-MS

The EO was analysed using Hewlett Packard GC 5890 Series 2 with MS 5971 equipped with INOWAX-MS column (60 m × 0.25 mm, film thickness 0.25 µm). The injector and detector temperatures were set at 250 and 280°C, respectively. The column temperature was programmed from 60 to 260°C at a rate of 5°C/min, with the lower and upper temperatures being held for 3 and 7 min, respectively. The flow rate of the carrier gas (helium) was 1.0 ml/min. 1.0 µl of a sample was injected. The composition was reported as a relative percentage of the total peak area.

3. RESULTS AND DISCUSSION

The examined *M.* × *piperita* cv. 'Perpeta' (domestic cultivar) was cultivated at a sunlit location in Bratislava. The content of EO and phenolic substances was observed in leaves of 1-year-old plants during the development of floral organs in the week period from 30 June 2007 (beginning of inflorescence

Table 1. Developmental stage of plant and air temperatures correspond to harvest time

Date of harvest	Average week temperature* (°C/°F)	Developmental stage of floral organ
30th June	20.3/68.54	Beginning of inflorescence formation
7th July	20.3/68.54	Inflorescence not flowering
14th July	19.2/66.56	Before flowering
22nd July	28.1/82.58	Beginning of flowering
31st July	22.6/72.68	Beginning of flowering
5th August	19.7/67.46	Full flowering
13th August	22.5/72.50	Full flowering
21st August	22.9/73.22	End of flowering
28th August	23.3/73.94	End of flowering

*average week air temperature in the period between each harvest

formation) up to the 28 August 2007 (overblown flowers). Different literatures do not agree on the most appropriate term for the harvest of plant material to be used as samples for studying EO content (Telci *et al.*, 2011; Neugebauerová & Kafková, 2012; Špaldon *et al.*, 1982; Felklová, 1975). Therefore, we decided to evaluate and compare the samples at different developmental stage. In this study, we have also examined the proportion of leaves in dry herbal drug (Table 2).

The EO was obtained by steam distillation from drug dried for 7 days at 35°C. The contents of EO in the leaves ranged from 23 ml/kg (2.3% V/m) to 36 ml/kg (3.6% V/m) (Table 2). The minimum prescribed content of EO in *M.* × *piperita* folium according to Ph. Eur. 6 is 9 ml/kg of EO for the cut drug. This same requirement is also prescribed in the currently available Ph. Eur. 8, 2014. The detected contents of EO during the whole period of development of floral organs for the domestic cultivar 'Perpeta' fulfils the Ph. Eur. requirement for the minimal content of EO in drug *M.* × *piperita* folium. The quality of the drug *M.* × *piperita* folium depends on the constitution of EO, in particular on the presence of desirable compound menthol but also on the presence of the ingredients reducing the quality of EO such as menthone, menthyl acetate and menthofuran. The relative percentage content of the menthol in EO in our samples ranged from 36% up to 52%, which also meets the requirements of Ph. Eur. for *M.* × *piperita* aetheroleum (content of menthol 30 – 55 %). The lowest value of menthol has been detected in the plants at the beginning of inflorescence formation, while the volume of EO reached the highest value (36 ml/kg). Meanwhile, the highest content of menthol was detected in the leaves of flowering plants (Fig. 1).

By comparing the values of the other constituents with respect to the quality of EO, it was found that the high quality EO could be obtained during the stage of full flowering (Fig. 2). Felklová *et al.* (1981) found the highest content of EO in *M.* × *piperita* at the beginning of flowering (Felklová *et al.*, 1981). In our study, EO from the leaves collected at the beginning of flowering had a high amount of menthol (52%) and relatively low levels of quality-reducing components menthon (8%) and menthofuran (2%). In comparison, the highest value of menthon has been recorded at the beginning of inflorescence formation. The level of menthol and menthon has changed rapidly during the investigated period (Fig. 1). The increase of menthol could be related to the growth of leaves, which corresponds at the biosynthetic level to the processes typical for older leaves and coincides with the study of several authors of (Gershenson *et al.*, 2000; Croteau & Martinkus, 1979; Brun & Voirin, 1991). It follows that in older leaves, the process of biosynthesis predominantly leads to the synthesis of menthol from its precursor menthon (McCaskill & Croteau, 1997).

The investigation of phenolic compounds in leaves harvested at different developmental stages of inflorescence of *M.* × *piperita* has been provided by spectrophotometrical methods (Ph. Eur. 6, 2007); these methods also find mention in the currently available Ph. Eur. 8, 2014. In the available literature, no relative study about the content of phenolic compounds in *M.* × *piperita* during development of inflorescence can be found. The contents of secondary metabolites contributing to therapeutic effect of mint respond for the quality of drug. Contrary to the yield of EO, which decreases during the development of inflorescence by up to 40 %, the content of phenolic compound increases until the beginning of flowering. These observations are especially evident in the content of flavonoids, the production of which conspicuously depends on UV light (Dolzhenko *et al.*, 2010). At the beginning of flowering, we detected the content of THD was 2.91%, the content of flavonoids expressed as luteolin-7-*O*-glucoside was 1.48 %

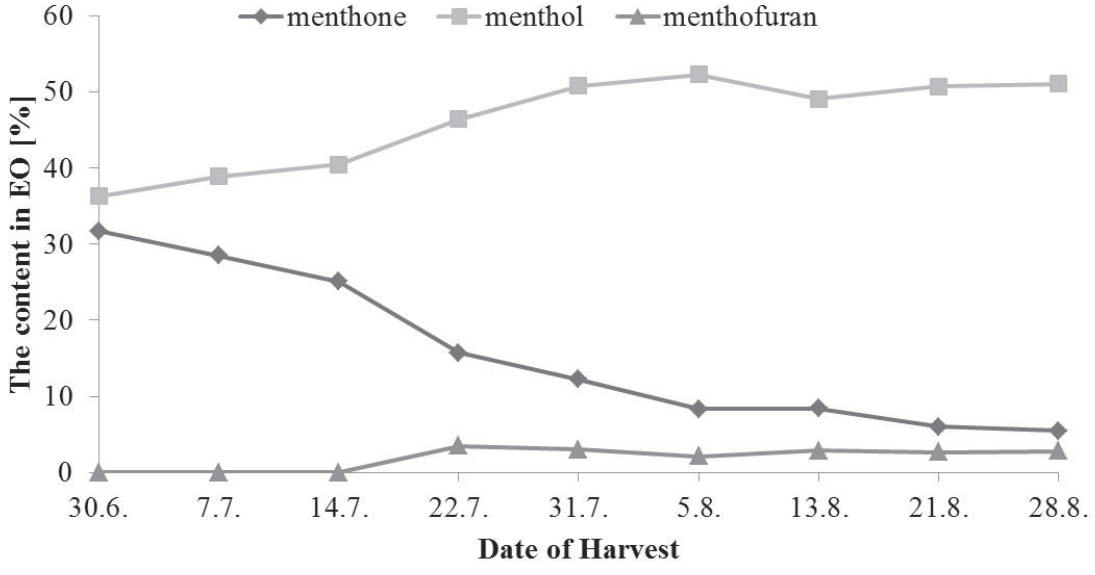

Fig. 1. The variation of major monoterpenes in EO of Mentha × piperita cv. 'Perpeta' during development of inflorescence

Table 2. The mass proportion of leaves in dry herb [%] and the content of EO [%, V/m] in dry leaves of Mentha × piperita cv. 'Perpeta' during development of inflorescence

Date of harvest	Developmental stage of inflorescence	The proportion of leaves in dry herb [%]a)	The content of EO [% V/m]b)
30th June	**Beginning of inflorescence formation**	61.0	3.6
7th July	Inflorescence not flowering	64.7	3.4
14th July	Before flowering	61.9	2.9
22nd July	Beginning of flowering	50.3	2.9
31st July	Beginning of flowering	46.6	2.7
5th August	Full flowering	36.9	2.5
13th August	Full flowering	34.0	2.3
21st August	End of flowering	33.3	2.3
28th August	End of flowering	30.7	2.5

a) n = 100, b) n = 3

and the content of total polyphenols was 10.06 %. Considering phenolic compounds, we detected the best quality drug at the beginning of flowering (Table 3, Fig. 2).

4. CONCLUSION

Our results show the best time for the harvest of *M.* × *piperita* cv. 'Perpeta' leaves considered the best quality drug depending on the contents of secondary metabolites. The optimal period for the harvest has been determined as the stage at the beginning of flowering. During this period, we can obtain from the leaves the EO with highest content of menthol. Besides it, the content of phenolic compounds in leaves such as flavonoids or THD are the highest during this period as well. Our work confirmed and complements the earlier observations on EO in peppermint.

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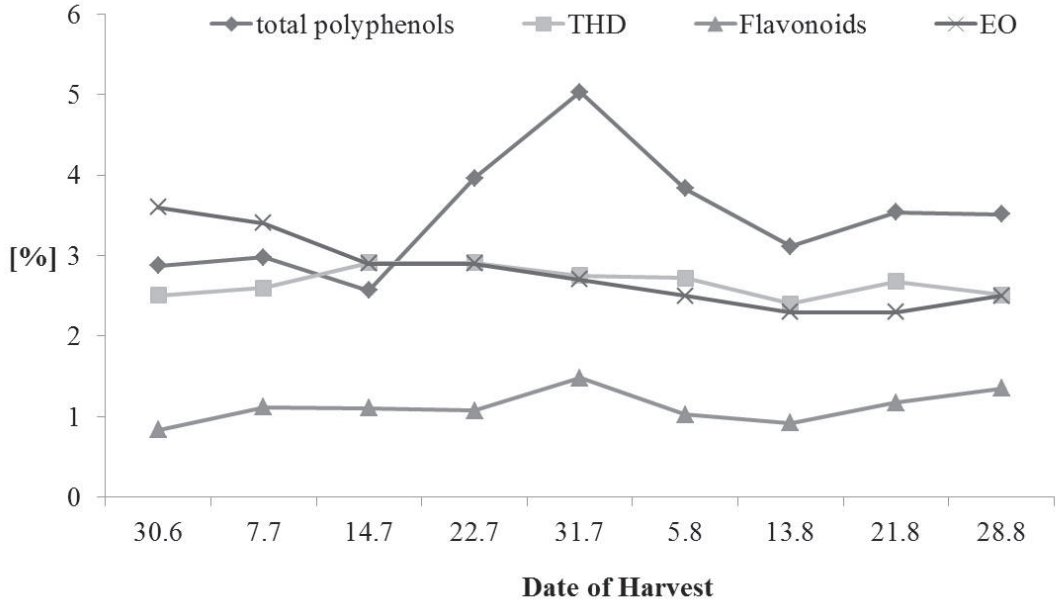

*Fig. 2. Secondary metabolites in leaves of M. × piperita cv. 'Perpeta' during development of inflorescence. The contents of total polyphenols expressed as rosmarinic acid (content [%]*0.5), total hydroxycinnamic derivatives (THD) expressed as rosmarinic acid [%], flavonoids expressed as luteolin-7-O-glucoside [%] and EO [%].*

Table 3. The content of THD expressed as rosmarinic acid [%], the content of flavonoids expressed as luteolin-7-O-glucoside [%] and the content of total polyphenols expressed as rosmarinic acid [%] in dry leaves of Mentha × piperita cv. 'Perpeta' during development of inflorescence

Date of harvest	Developmental stage of inflorescence	THD [%]*	Flavonoids [%]*	Total polyphenols [%]*
30th June	Beginning of inflorescence formation	2.51 ± 0.09	0.84 ± 0.06	5.75 ± 0.02
7th July	Inflorescence not flowering	2.60 ± 0.08	1.12 ± 0.09	5.96 ± 0.02
14th July	Before flowering	2.90 ± 0.12	1.11 ± 0.03	5.14 ± 0.05
22nd July	**Beginning of flowering**	2.91 ± 0.07	1.08 ± 0.06	7.92 ± 0.08
31st July	**Beginning of flowering**	2.75 ± 0.07	1.48 ± 0.11	10.06 ± 0.08
5th August	Full flowering	2.72 ± 0.07	1.02 ± 0.08	7.67 ± 0.37
13th August	Full flowering	2.40 ± 0.10	0.92 ± 0.03	6.23 ± 0.14
21st August	End of flowering	2.68 ± 0.05	1.18 ± 0.04	7.08 ± 0.32
28th August	End of flowering	2.51 ± 0.06	1.35 ± 0.07	7.03 ± 0.08

* Values [%] are presented as means ± standard deviation, n = 3

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