Seasonal Variation in DPPH Scavenging Activity of *Mentha × piperita*

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**ABSTRACT**

Peppermint, the sterile tetraploid hybrid of *Mentha aquatica* and *Mentha spicata*, is commonly cultivated for commercial production of the essential oil, the drug *Menthae piperitae folium*, and the fresh herb. In this study, methanol extracts of *Mentha × piperita* leaves were screened for its antioxidant properties. Samples of leaves were collected in 2010 and 2011 from field cultivated plants in different development stages. Radical scavenging activity was determined spectrophotometrically by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and expressed as SC₅₀ values. The highest antioxidant activity (SC₅₀ = 10.11 - 57.35 μg/ml) exhibited leaves collected at the stage of flowering i.e. June – August.

**Key words:** peppermint, *Mentha × piperita*, DPPH, antioxidant activity

**Introduction**

The antioxidant activity of plant extracts is an interesting object of research because of the increasing usage of natural antioxidants as additives in food and cosmetics. Peppermint, the sterile tetraploid hybrid of *Mentha aquatica* and *Mentha spicata*, is commonly cultivated for commercial production of the essential oil, the drug *Menthae piperitae folium*, and the fresh herb. This plant is widely used for its therapeutic properties and for the aromatic and culinary purposes. Secondary metabolites, such as phenolics, flavonoids, as well as the components of the peppermint essential oil, were found to possess a free radical scavenging activity.

Reactive oxygen species (ROS) are engaged in development of many different kinds of diseases. They can cause gene mutations and induce a formation of oncogenes. Prevailing state of the reactive oxygen species is called an oxidative stress and it can be created in biological systems by prooxidative enzyme systems, lipid oxidations, inflammation in the body, as well as by radiation, smoking or air pollutants [1,2]. Due to reactions of ROS with other metabolites the oxidized substances are formed. They can be often more reactive and harmful as the origin radical [3]. Damaging effects of the reactive oxygen species can be reduced by antioxidants. Many compounds of plant origin possess antioxidant properties. They are able to protect genes against mutations.

There is considerable interest in the screening of plants and other natural product extracts in modern drug discovery programs, since structurally novel chemotypes with potent and selective biological activity may be obtained [4]. Chemical constituent with antioxidant activity found in high concentrations in plants determine their important role in the prevention of various degenerative diseases. Besides the fruits and vegetables that are recommended at present as optimal sources of such components, the supplementation of human diet with herbs, containing especially high amounts of compounds capable of deactivating free radicals may have beneficial effects [5]. The evaluation of antioxidant properties of the raw material allows the determination of its suitability as high quality food beneficial for human health.

Genus *Mentha* is one of the most important genera of the family Lamiaceae. Peppermint (*Mentha × piperita*) is a sterile tetraploid hybrid of spearmint (*Mentha spicata*) and water mint (*Mentha aquatica*). This perennial herb is indigenous in Europe and has widespread in cultivation throughout all regions of the world [6]. (Vaverková et al., 2009). Peppermint tea, brewed from the plant leaves, and the essential
oil are used for the diseases prevention or treatment in traditional medicines. Experimental studies revealed considerable therapeutic properties of peppermint, such as antimicrobial and antiviral activities, antioxidant and antitumor actions, antiallergenic potential or antispasmodic, analgesic and anesthetic effects [7]. The drug of *Mentha × piperita* contains about 1.2-1.5 % essential oil, in which menthol and menthone are present in the highest amount. Other secondary metabolites found in peppermint are flavonoids, polyphenols, carotenes, tocopherols, saponin, and choline [8].

In general, accumulation of plant secondary metabolites depends on many factors including the ontogenetic and the environmental ones. The aim of the present work was to evaluate the seasonal variation in radical scavenging capacity of methanol extracts of *Mentha × piperita* leaves.

**Material and methods**

Plants of *Mentha × piperita* were cultivated in the experimental field of University of Prešov under following conditions. The degree of slope is 0. Altitude is around 240 m about sea level. Annual average temperature in the area is around 8 – 9 °C. The coldest month in this region is January with average monthly temperature about –3.5 °C, the warmest month is August with average monthly temperature about 19 °C. The soil is composed of alluvial silt from close river Torysa. Agro-chemical analysis of the soil was performed by the certificated laboratory of UKSUP Košice, Slovakia, with the following results: humus content 3.08%, Cox 1.79%, mineral nitrogen (NO₃ + NH₄) 16.7 mg/kg, P 186 mg/kg, K 95 mg/kg, Mg 249 mg/kg, Ca 5944 mg/kg, soil pH 7.4. Meteorological data of the monthly temperature average and the total monthly rainfall were obtained from the nearest hydro-meteorological station situated in Malý Šariš.

Our investigations were carried out in 2010 and 2011, during the fourth/fifth year of peppermint growth. Aerial parts of plants were collected by destructive method once in a month (May – September) from the three randomly chosen square meters. Until the extraction the fresh plant material was stored in a refrigerator at 4 °C.

Extraction procedure and DPPH assay was performed according the method described in [9]. 5 g of laves were extracted with 100 ml MeOH at room temperature for 30 days. Extracts were filtered and 200 µl of each extract was evaporated under the vacuum to dryness at temperature not exceeded 40 °C. The dry rests were weighted, redissolved in MeOH and used for DPPH assay.

Into 0.1 mM/L 2,2-diphenyl-1-picrylhydrazyl (Sigma – Aldrich, Germany) methanol p.a (Centralchem, Slovakia) solution different amount of the tested extracts was added. After 30 min, absorbance at 517 nm was measured using Shimadzu Spectrophotometer UV-1800. SC₅₀ values (i.e. concentration causing 50% decrease of absorption) were obtained from the linear part of inhibition curves.

**Results and discussion**

In the Fig. 1, the monthly temperature average and the total monthly rainfall during the vegetation period of the years 2010 and 2011 are compared to the long term average. As for the temperature, there were no principal differences between the data of the years in which peppermint plants were collected and the data of 40-years period. A slight decrease of the average monthly temperature in July 2011 corresponds to the high value of total rainfall observed in this month. In contrast to 2010, rainfall during the best part of vegetation season in 2011 not reached the level of the long-term average. Rainfall amount in 2010 was considerably higher than that of long-term average with the largest difference recorded in May.

![Fig. 1: Monthly average temperature and monthly total rainfall in 2010 and 2011 together with the long-term average (1964–2004) recorded in meteorological station Malý Šariš, Slovakia.](image-url)
Mentha × piperita is a perennial herb vegetatively propagating by underground and overground stolons. When cultivated for the herb production, peppermint plants after the autumn cropping form new shoots in the next spring. As we have observed previously, the production of aerial parts culminates at the end of summer [10]. Under climatic conditions of the experimental field in Prešov the flowering period falls especially on July and August. These months together with June are usually characterized by the high values of average temperature as well as the sufficiency of rainfall.

Evaluation of DPPH scavenging activity revealed that methanol extracts of our experimental peppermint plants had considerable antioxidant properties (Fig. 2). Lowest SC50 values were observed in leaves collected during the period of April – June 2010 (about 13.57 µg/ml) and April – June 2011 (about 14.33 µg/ml) respectively. Seasonal variation in scavenging activity manifested especially in the year 2010. Unlike 2011, there were found the large differences in the SC50 parameter between the extracts responding to individual months in the year 2010. Extreme values were recorded in August and September (between 56.29 – 57.35 µg/ml). The results suggest that plants have been adversely affected by period of drought and high temperatures. Our results indicate that the extractable constituent of peppermint leaves have the highest antioxidant activity in the end of growing season period i.e. August – September. We suppose that the relatively high antioxidant activity of extract in August and September 2011 was caused by very low rainfall (12.4 – 24.8 mm).

![Fig. 2: DPPH scavenging activity of peppermint leaf methanol extracts during the vegetation season of 2010 and 2011 expressed as SC50 values.](image)

Antioxidant effects of peppermint are attributed especially to the phenolic compounds as well as to the essential oil components [11,12]. Nickavar et al. [13] found that Mentha × piperita had the highest total phenolics content and showed the strongest antioxidant effects in DPPH assay (SC50 13.32 µg/ml) in comparison to other four Mentha species. Valentová et al. [14] found, that Mentha × piperita contains also caffeic and rosmarinic acids, which are very strong antioxidants, their SC50 values of DPPH scavenging are 1.57 respectively 3.41 µg/ml. We suppose that mainly these acids and other phenolic compounds caused relatively high antioxidative effect of peppermint plants. Yield of essential oil in plants and its composition may be influenced by environmental factors such as temperature and humidity, so these important drug parameters may vary with the time of year [15]. Also for various species of genus Mentha, changes of quantitative and qualitative properties of essential oil during the vegetation season were observed with the maximum yield reached during the flowering period [16,17].

Acknowledgments

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References