SUPPLEMENTARY MATERIAL

Screening of antioxidant activity and volatile compounds composition of *Chamerion angustifolium* (L.) Holub ecotypes grown in Lithuania

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Abstract. Since biological activity of medicinal plants is dependent on cultivation area, climatic conditions, developmental stage, genetic modifications and other factors, it is important to study flora present in different growing sites and geographical zones. This study was focused on screening of antioxidant activity of *C. angustifolium* harvested in five different locations in Lithuania. The total contents of phenolic compounds, flavonoids and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity were evaluated by spectrophotometric methods. A correlation between radical scavenging activity and total phenolic compounds content was observed (correlation coefficient 0.98). HPLC with on-line post-column DPPH radical scavenging reaction detection was used for separation of extracts. Oenothien B, rutin and one unidentified compound were predominant. Volatile compounds were analysed using solid phase microextraction coupled with gas chromatography–mass spectrometry. Based on the analysis of volatiles all samples were classified into two chemotypes: (I) with predominant α - and β -caryophyllenes and (II) with predominant anethole.

Keywords: *Chamerion angustifolium* (L.) Holub; total phenolic content; total flavonoids content; radical scavenging activity; on-line HPLC-DPPH; SPME-GC-MS.

Experimental

Plant Material

Seven samples of *Chamerion angustifolium* were collected in five different locations in Lithuania from May till July in 2012 year (Table S1). Samples were identified by prof. O. Ragažinskienė. A voucher specimens (numbers listed in Table S1) were deposited at the Herbarium of the Kaunas Botanical Garden of Vytautas Magnus University.

Table S1. List of the tested *C. angustifolium* (L.) samples.

Sample	Collection data	Harvesting place	Vegetation phase	Geographic coordinates	Voucher specimen number
1CA	2012 06 30	Kaunas district, Kazlų Rūda forest	massive blooming	54° 44' N, 23° 29' E	LT-0-KAUN-2014- O0481
2CA	2012 07 03	Trakai district, Užutrakis forest	massive blooming	54° 40' N, 24° 56' E	LT-0-KAUN-2014- O0488
3CA	2012 07 04	Kaunas (Aleksotas)	massive blooming	54°53'N, 23°53'E	LT-0-KAUN-2014- O0482
4CA	2012 05 28	Varėna district, Panara	growing	54° 6' N, 24° 7' E	LT-0-KAUN-2014- O0485
5CA	2012 07 15	Švenčionys district (village Svirkos)	massive blooming	55° 17'N, 26° 40'E	LT-0-KAUN-2014- O0487
6CA	2012 06 30	Kaunas Botanical garden	massive blooming	54°52'N, 23°54'E	LT-0-KAUN-2014- O0486
7CA	2012 06 17	Varėna district, Panara	butonization	54° 6' N, 24° 7' E	LT-0-KAUN-2014- O0489

Results

The wide UV peak with a very high antioxidant activity (DPPH line) in the beginning of HPLC-DPPH chromatogram (compound No 1, Figure S1) is attributed to oenothien B according to the published data (Tóth et al. 2009). Oenothien B is a highly polar dimeric macrocyclic ellagitannin isolated from *C. angustifolium* (L.), as well as other plant sources. Many of therapeutic activities of *C. angustifolium* are attributed to oenothien B (Kiss et al. 2006; Schepetkin et al. 2009; Ramstead et al. 2012; Kiss et al. 2011). Figure S1 shows, that this compound has the highest impact on the antioxidant activity of *C. angustifolium* herb.

Figure S1. On-line HPLC-DPPH[•] scavenging assay profiles of *C. angustifolium* (L.) extracts: the positive signal line (254 nm) of HPLC; the negative signal line (517 nm) of DPPH[•] (sample codes see Table S1)

References

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