High performance thin layer chromatography (HPTLC) assessment of Epilobii herba

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Introduction. *Epilobium parviflorum* (Onagraceae) is a perennial herb widely used in folk medicine for its anti-inflammatory, sedative and antioxidant properties. The herb contains several important bioactive compounds such as polyphenolic compounds, polysaccharides and amino acids [1, 2]. The composition of secondary metabolites undergoes significant changes in plants depending on the growth phase and the influence of environmental factors. The purpose of this work is to apply the high-performance thin layer chromatography (HPTLC) method to evaluate the *E. parviflorum* quality.

Materials and methods. *E. parviflorum* samples collected in Ukraine and Lithuania were used for the current comparative analysis. Polyphenolics were studied using HPTLC for the methanol (50%, v/v) extracts. Reference standards including chlorogenic acid, gallic acid, isoquercitrin, and hyperoside were used. The analysis was carried out using HPTLC plates Si 60 F254 (Merck) with a mobile phase of ethyl acetate: formic acid: acetic acid: water (67.5:7.5:7.5:17.5). The detection of compounds was performed at 365 nm after derivatization by 2-aminoethyldyphenylborinate 1% solution followed by 5% macrogol 400 in methanol. Chromatographic separation of polyphenols was performed using the Waters e2695 Alliance HPLC system coupled with a 2998 PDA detector according to [3].

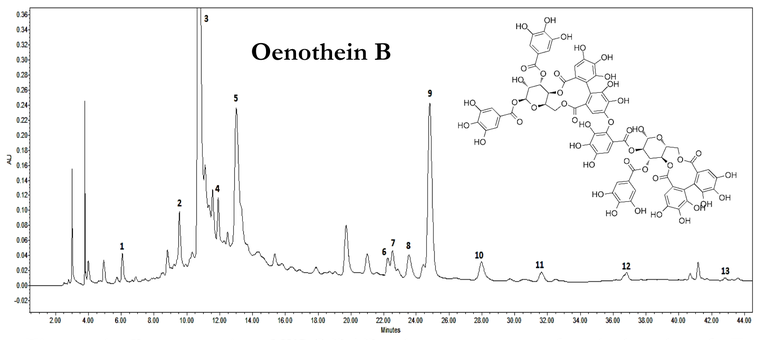

Figure 1. Representative HPLC-DAD chromatograms of polyphenols of *E. parviflorum* flowers harvested during early flowering. Peak assignments: 1—gallic acid; 2—neochlorogenic acid; 3—oenothein B; 4—chlorogenic acid; 5—oenothein A; 6—ellagic acid; 7—rutin; 8—hyperoside; 9—isoquercitrin; 10—guaijaverin (quercetin-3-O-arabinopyranoside); 11—quercitrin; 12—afzelin (kaempferol-3-O-rhamnoside); 13—quercetin.

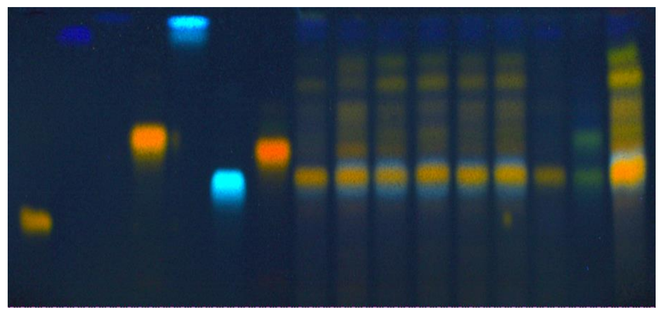

Fig. 2. HPTLC plates Si 60 F254 (Merck) in mobile phase: ethyl acetate: formic acid: acetic acid: water (67.5:7.5:7.5:17.5). Detection: at 365 nm after derivatization by 2-aminoethyldyphenylborinate 1% solution followed by 5% macrogol 400 in methanol. Reference standards: chlorogenic acid, gallic acid, isoquercitrin and hyperoside. Methanol (50%, v/v) extracts

The HTPLC showed (Fig. 2) the presence of all 4 major compounds in *Epilobium* extracts: chlorogenic acid, isoquercitrin, hyperoside and gallic acid. As a result, the optimal time for harvesting herbal raw material is the phase of plant mass flowering

Results and discussion. Using the HPLC-PDA method 13 polyphenolic compounds (Fig. 1) were identified in the plant material. It was noted that the largest content and the best polyphenol profile was in late flowering stage. The most important polyphenolic compounds in the plant material were chlorogenic acid, hyperoside, isoquercitin, and oenothein B. The HPLC data for phenolic compounds are also consistent with the results of the qualitative profile under the conditions of HPTLC analysis for samples. HTPLC showed the presence of all four compounds in *E. parviflorum* extracts. In the light blue fluorescent (Rf = 0.54), chlorogenic acid was identified as. In the light yellow fluorescent zone (Rf = 0.67), isoquercitrin was detected. In the yellow fluorescent zone (Rf = 0.60) hyperoside was suggested. In the blue fluorescent zone (Rf = 0.82), gallic acid was identified. The fluorescence was more intense in the sample from Ukraine.

Conclusions. The presented method can be further applied to assess *Epilobium* sp. chemical content

References

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