Comparison of Free Radical Scavenging Activity and Phenoloid Content of *Epilobium parviflorum* Schreb

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Abstract

***Epilobium parviflorum* Schreb. -(Onagraceae) – has been used for a long time as a medicinal plant for the treatment of various prostate symptoms, like prostate adenoma and associated disorders. *Epilobii* herba, willow herb, has not been officially recognized by any of European pharmacopoeias.**

***Epilobii* herba contains various pharmacologically-useful compounds, such as polyphenols, among which are the flavonoids: quercitrin, rutin, isoquercitrin, isomyricitrin and myricitrin. The total polyphenol and flavonoid contents were determined in crude willow herb drugs.**

A high linear correlation was observed between the total phenolic content and the reducing power of the samples. *Epilobii* herba showed marked activity as a radical scavenger, indicating that it has effective activities as hydrogen donors and primary antioxidants to react with lipid radicals.

INTRODUCTION

The genus *Epilobium* (family Onagraceae) consists of over 200 species. The commercially available plant drug *Epilobii* herba contains the dried aerial parts of several species, most notably *E. parviflorum, E. montanum* and *E. roseum*. Various members of the genus *Epilobium,* in particular *E. parviflorum* have been used in folk medicine for the treatment of adenoma and inflammation of the prostate (Hiermann et al., 1991). The aerial parts of *Epilobium* species are rich in flavonoids such as quercitrin, rutin, isoquercitrin, isomyricitrin and myricitrin, myricetinglycoside: myricitrin-3-0-β-glucoronide (Hiermann, 1985). The hydrogen–donating ability, scavenging property and the reducing activity of these active principles have not yet been investigated**.**

The aim of this study was to define the total polyphenol and flavonoid content in samples of *Epilobii* herba collected from the wild, cultivated as *E. parviflorum* in Hungary and from commercial sources.

MATERIALS AND METHODS

Epilobii herba is available commercially in Hungary and is controlled officially. For this work we used different crude drug samples (Herbaház and Schmidt and Co. 2000) as well as E. *parviflorum* collected in Budapest. Samples were identified macroscopically and microscopically in the Department of Pharmacognosy, Semmelweis University, where samples and herbarium specimens are deposited.

For biological evaluation the collected plant sample was used.

The polyphenol contents of the drugs were measured according to the modified method of the Hungarian Pharmacopoea VII using spectrophotometry (750 nm, pyrogallol as standard).

Determination of the total flavonoid content of the samples was carried out by the method of DAB 10. Flavonoids were measured as yellow Al-complexes spectrophotometrically after acidic hydrolysis and appropriate purification by solvent

extraction using ethyl acetate. Flavonoid contents were calculated as hyperoside (g/100g, A^{1cm}%=500)

The hydrogen-donating ability of methanolic extracts was examined on the basis of the method of Bois (1985), as modified by Hatano et al. (1988) using 1,1-diphenyl-2-picrylhydrasyl as the stable radical. DPPH evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants (Cao and Sofic, 1997). Absorbance of the methanolic DPPH-solution was measured at 517 nm. The degradation of DPPH was evaluated by comparison with a control sample without hydrogen-donating compounds. The amount of the samples (mg) reducing the absorbance by 50 % was determined (I_{50}).

The method of Oyaizu (1986) was applied for the analysis of reducing power. We measured the change of absorbance that accompanies the Fe^{3+}- Fe^{2+} transformation at 700 nm, in comparison with that produced by ascorbic acid. The reducing power was expressed as ASE/mg. ASE means the reducing power of 1 mg of 1 nmol ascorbic acid (AS).

The total scavenger capacities of the samples were detected by a chemiluminometric method (Blázovics and Kovács, 1999) with a Lumat LB 9051 luminometer in the H_2O_2/$^{\bullet}$OH-luminol-microperoxidase system. Unstable free radicals $^{\bullet}$OH originating from H_2O_2 via the Fenton reaction result in dissolving of luminol to amino-phthalic acid, when monochromatic light is emitted. The highest chemiluminescence intensity is given by the H_2O_2/$^{\bullet}$OH-luminol-microperoxidase system (standard light). In the presence of free radical scavenging compounds, the emitted light is reduced. The I_{90} was determined and expressed in micrograms, i.e. the amount of the sample that diminished emitted light of H_2O_2/$^{\bullet}$OH-luminol-microperoxidase by 90 %.

Results were assessed by one-way analysis of variance (ANOVA) and represent the mean ±S.E.M. of three different measurements with two parallels.

RESULTS AND DISCUSSION

The total polyphenol and flavonoid contents were determined in crude willow herb drugs. The results are summarized in Table 1, Fig. 1 and Fig. 2. The polyphenol and flavonoid contents are given in terms of pyrogallol and hyperoside, respectively. Both groups of plant phenoloids represented a higher concentration in the collected sample. The mean polyphenol and flavonoid contents in the commercial samples were 3.63 % and 0.91 % respectively.

The hydrogen-donating ability, the reducing power property and radical scavenging activity measured by the chemiluminometric method were compared with the activity of the well known antioxidant flavonolignan, silibinin, the reference bioactive agent from milk thistle (*Silybum marianum* L.), which is used clinically in Europe (Blázovics and Kovács, 1999).

Epilobii herba extracts exerted hydrogen-donating ability in the presence of DPPH stable radical. The inhibition of the samples was concentration dependent (Fig.3).

Epilobii herba extract proved to be more effective than the silibinin standard.

The reducing power property of extracts showed concentration dependence. The absorbance of the samples increased together with the reducing power. The reducing power of the extract was 1049±2.86 ASE/mg compared with 447.7±5.17 ASE/mg for silibinin (Fig.4).

The emitted light of the H_2O_2/$^{\bullet}$OH – luminol-microperoxidase system was decreased dose-dependently in the presence of different concentrations of *Epilobii* herba extract (Fig. 5). The willow herb extract proved to be much more effective in this system than silibinin.

CONCLUSION

Great variability was found both in the quantitative and qualitative composition of flavonoids in *Epilobii* herba. The polyphenol contents were more stable. A high linear correlation was observed between the total phenolic content and reducing power of

samples. *Epilobii* herba showed marked activity as a radical scavenger indicating that it has effective activities as hydrogen donors and primary antioxidants to react with lipid radicals. Willow herb is also effective in decreasing chemiluminescence intensity arising from the $H_2O_2/^{\bullet}OH$ luminol system. This result indicates that *Epilobii* herba is also a scavenger of active oxygen species in connection with the total amount of polyphenolic compounds. The overall antioxidant effect of *E. parviflorum* might be attributed to the property of scavenging free radicals and active oxygen species.

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Literature Cited

Blázovics, A., Fehér, E., Abdel Rahman M., Preehl, J., Ágoston, M. and Fehér, J. 1996. Free radicals in connection of bile and liver. Czech and Slovak Gastroenterology 3. 73-78.

Blázovics, A., Kovács, A., Lugasi, A., Hagymási, K., Bíró, L. and Fehér, J. 1999. Antioxidant defense in erythrocytes and plasma of patients with active and quescient Crohn disease and ulcerative colitis: a chemiluminescent study. Clinical Chemistry 45. 895-896.

Blázovics, A., Prónai, L., Fehér, J., Kéry, Á. and Petri, G. 1993. A natural antioxidant extract from *Sempervivum tectorum*. Phytother. Res. 7. 95-97

Bois, M.S. 1985. Antioxidant determinatiom by the use of a stable free radical. Nature 4617. 1198-1200.

Cao, G., Sofic, E. and Prior, R.L. 1997. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationship. Free Rad. Biol. and Med. 22. 749-760.

Czinner, E., Hagymási, K., Blázovics, A., Kéry, Á., Szőke, É. and Lemberkovics, É. 2000. In vitro antioxidant properties of *Helichrrysum arenarium* (L.) Moench. Journal of Ethnopharmacology 73. 437-443

Fejes, Sz., Blázovics, A., Lemberkovics, É., Petri, G., Szőke, É. and Kéry, Á. 2000. Free radical scavenging and membrane protective effects of metanol extracted fractions of parsley. Acta Alimentaria 29. (1). 81-87.

Hagymási, K., Blázovics, A., Lugosi, A., Kristó, T. SZ., Fehér, J. and Kéry, Á. 2000. In vitro antioxidant evaluation of dandelion (*Taraxacum officinale* Web.) water extract. Acta Alimentaria 29. (1): 1-7.

Hatano, T., Kagawa, H., Yasuhara, T. and Okuda, T. 1988. Two new flavonoids and other constituents in licore root: their relative astringency and radical scavenging effescts. Chemical and Pharmaceutical Bull. 36. 2091-2097.

Hiermann, A. 1985. Die Untersuchung potentieller Wirkstoffe in *Epilobium-Arten*. Scientia Pharmaceutica 53. 39-44.

Hiermann, A., Reidlinger, M., Juan, H., Sametz, W. 1991. Plant Med 57. 357-360.

Hollman, P.C. and Katan, M.B. 1998. Bioavilibilty and health effects of dietary flavonols in man. Arch. Toxicol. Suppl., 20: 237-248.

Oyaizu, M. 1986. Studies on products of browning reaction: Antioxidaive activities of products of browning reaction prepared trom glucoseamin. Japanese Jour. Nutr. 44. 307-315.

Roth, H.J. and Schmid, W. 1976. Hagers Handbuch der Pharmazeutischen Praxis. Springer-Verlag, Berlin, 34-37

Tables

Table 1. Total polyphenol and flavonoid contents of commercial and collected *Epilobii* herba samples

Samples		Flavonoid (g/100g)	Total polyphenol (g/100g)
Commercial	1.	0.81±0.01	3.96±0.02
Commercial	2.	0.60±0.15	3.44±0.12
Commercial	3.	0.65±0.02	3.70±0.01
Commercial	4.	0.55±0.01	3.34±0.11
Commercial	5.	0.60±0.15	3.02±0.11
Commercial	6.	0.62±0.02	3.02±0.02
Collected	7.	0.92±0.17	3.98±0.01

Figures

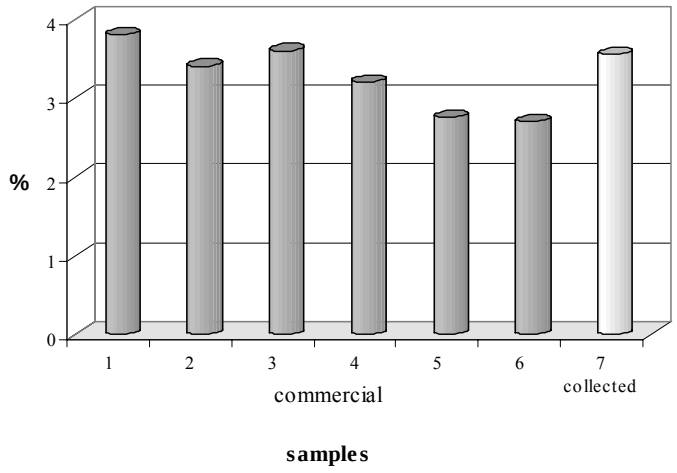

Fig. 1. Total polyphenol content of commercial and collected samples

(marking see Table 1.)

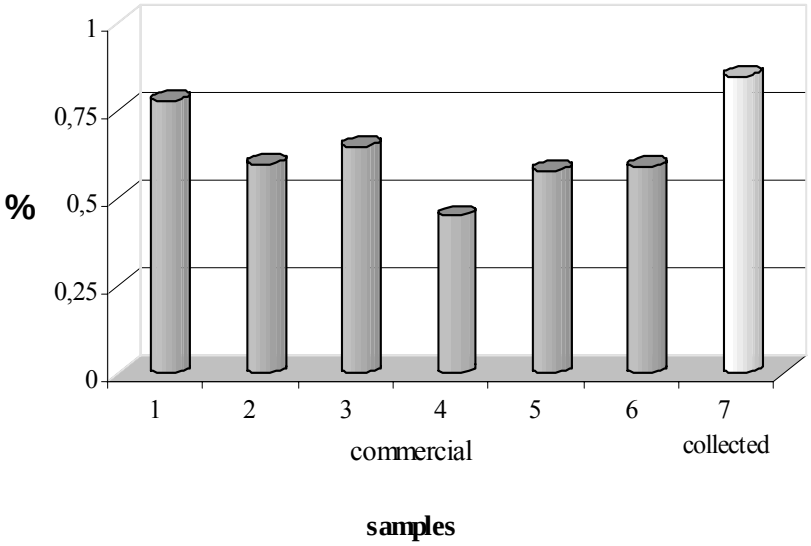

Fig. 2. Total flavonoid content of commercial and collected samples (marking see Table 1.)

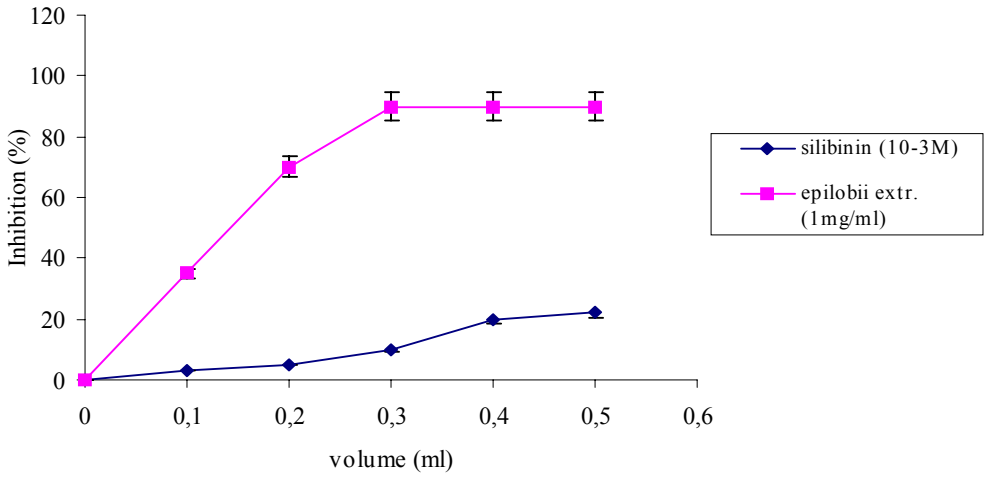

Fig. 3. Concentration-dependent hydrogen-donating ability (DPPH 517 nm)

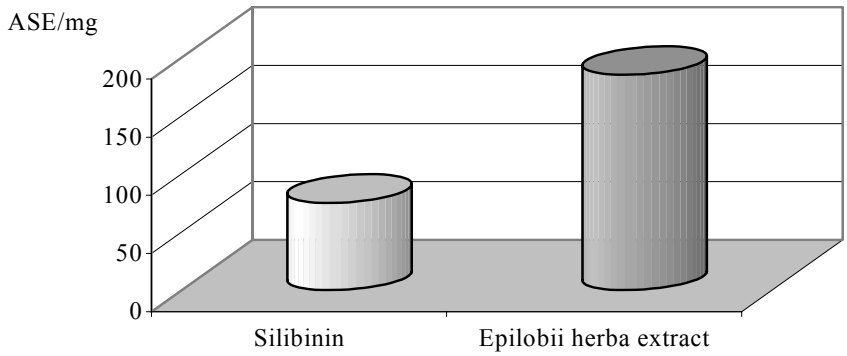

Fig. 4. Reducing power of *Epilobii* herba extract (700 nm)

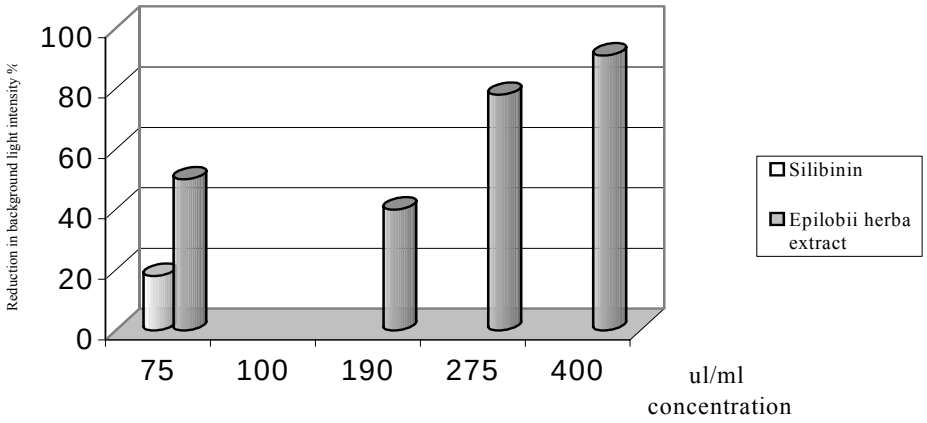

Fig. 5. Total scavenger capacity of *Epilobii* herba extract