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A QUALITATIVE AND QUANTITATIVE ANALYSIS OF POLYPHENOLIC COMPOUNDS IN FIVE *EPILOBIUM* SPP. WITH A POSSIBLE POTENTIAL TO ALLEVIATE BENIGN PROSTATIC HYPERPLASIA

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*Benign prostatic hyperplasia (BPH) is a widespread male disease, affecting more than 50 % of men over the age of 60 years. Inhibition of the enzyme 5 α -reductase is a common treatment strategy for this condition. Such potential can be found in willow flowers (*Epilobium* spp.), which are known in folk medicine for treating of prostate ailments, mainly benign prostatitis, hypertrophy and prostatitis. Smallflower hairy willowherb (*E. parviflorum*), which is rare, is the most recommended for treating BPH.*

The aim. The aim of the study was to investigate the qualitative and quantitative content of polyphenols in five *Epilobium* species (*E. adenocaulon* Hausskn., *E. hirsutum* L., *E. montanum* L., *E. parviflorum* Schreb. and *E. palustre* L.) growing in Estonia, to find the most promising species in terms of chemical composition to alleviate BPH.

Materials and Methods. The qualitative and quantitative analyses of polyphenols in herbs of *Epilobium* spp. were performed using HPLC/MS. All five species were collected from the pond's shore in Pilkuse village (Otepää municipality, Valga county, Estonia) in July 2008.

Research results. It was found that 20 % ethanol was optimal for extracting polyphenolic compounds from the herb *Epilobium* spp. with subsequent UV chromatogram analysis at 350 nm. In the analyzed *Epilobium* species, 12 polyphenolic compounds were identified. Oenothetin B, myricetin rhamnoside and myricetin glucoside were the principal polyphenolic compounds among other identified constituents in the *Epilobium* spp. herbs. *E. montanum* had the highest content of oenothetin B. The highest was the total content of myricetin glycosides for all five compared species, the total content of quercetin glycosides was slightly lower, and the total content of kaempferol glycosides was the lowest.

Conclusions. The content of polyphenols is highest in *E. adenocaulon* and the lowest in *E. parviflorum*. Thus, *E. parviflorum* does not offer the best therapeutic potential for the relief of BPH in terms of the quantitative content of polyphenolic compounds

Keywords: willowherb, oenothetin B, myricetin rhamnoside, myricetin glycoside, prostatic hyperplasia, Estonia

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1. Introduction

Benign prostatic hyperplasia (BPH) usually begins in men after the age of 40 and increases as the average population ages increase [1]. BPH occurs in more than 50 % of men over the age of 60 [2]. Despite its heterogeneous aetiology, pathology and pathophysiology, BPH is primarily an androgen-dependent process. Dihydroxytestosterone, metabolized from testosterone by the enzyme 5 α -reductase, accumulates on the androgen receptor of the prostate and thus promotes prostate enlargement [3, 4].

In Europe, drug therapy of BPH [5, 6] is preferred, for example, with synthetic α -receptor blockers [6, 7] or 5 α -reductase inhibitors [8, 9]. Since most synthetic drugs cause serious side effects [10], it would be better to use herbal remedies that do not have side effects similar to synthetic ones to treat prostate ailments [11]. In addition to industrial phytopreparations, raw herbal material can be used in the supportive therapy of BPH [12], which can be collected by anyone used into tea [13, 14].

Inhibition of 5 α -reductase helps willow herb flower (*Epilobium* L., syn. *Chamaenerion* Ség.) species, which are known in folk medicine for the treatment of prostate ailments, mainly benign prostatitis, hypertrophy and prostatitis [14, 15]. Tea made from dried above-ground parts of plants has been very common in central Europe [15, 16], due to the widespread prevalence of benign prostate enlargement in the population [17, 18].

In 2016, the European Medicines Agency (EMA) released a public document “Herbal medicine: summary for the public”, which concluded that the use of willow herb in patients with benign prostatic hyperplasia is beneficial in the relief of lower urinary tract symptoms, such as frequent need to urinate or difficulty starting urination [19].

Until now, the hoary willowherb or smallflower hairy willowherb (*E. parviflorum* Schreb.) is the most well-known of the *Epilobium* spp. However, several species in the willow-flower family still need attention in this context [20].

The genus *Epilobium*, belonging to the family *Onagraceae* Juss., includes more than 200 species [21]. Of these, 27 grow in Europe [22]. In Estonia, 9 species belonging to the genus of willowherbs (*Epilobium* L.) occur naturally: *Epilobium angustifolium* L., *E. hirsutum* L., *E. parviflorum* Schreb., *E. palustre* L., *E. montanum* L., *E. collinum* C.C.Gmel., *E. tetragonum* L., *E. adenocaulon* Hausskn and *E. roseum* (Schreb.) Schreb [23, 24]. Hybrids have also been found among almost all the *Epilobium* spp. found in Estonia. Willowherbs growing in Estonia are annual herbaceous plants with a height of 10-80 cm (hairy willowherb (*E. hirsutum*) up to 150 cm) [25]. *Epilobium* spp. mostly grow near water bodies, on ditch banks, along forest paths, as well as in gardens as weeds [26, 27].

The main biologically active constituents of *Epilobium* spp. are: macrocyclic ellagitannins oenothein A and oenothein B (Fig. 1) [28, 29], flavonoids quercetin [30, 31], isoquercetin, myricetin [32], isomyricetin, kaempferol and their glycosides [33, 34], sterols β -sitosterol [35, 36] and its esters [37–39], etc. The ellagitannins [40, 41], oenothein A and B [42, 43] inhibit the enzyme 5 α -reductase [44, 45]. The polyphenols quercetin, myricetin, and kaempferol [44], with antioxidant properties contained in *Epilobium* spp. and their glycosides [30, 46], also play an important role in the

prevention and supportive treatment of prostate disorders [47, 48].

In our previous work, the total content of polyphenols, tannins and flavonoids in five species of *Epilobium* spp. was determined, as well as the amount of some individual polyphenols in different parts of the plants [49]. The flowers and leaves were the richest in active substances in all willowherb species, while the roots and stems were the most active. Ellagic acid was most commonly found in the flowers of *E. palustre*, quercetin in the flowers of *E. parviflorum*, and gallic acid in the flowers of *E. hirsutum* and *E. parviflorum*. It was concluded that, given the total content of these active substances, one species of *Epilobium* spp. could not be preferred to others. Besides these five species of *Epilobium* spp., we also showed that fireweed *Chamaenerion angustifolium* (L.) Scop., syn. *E. angustifolium* L., with a wider natural distribution, could potentially be of interest [50].

This research aimed to reveal the qualitative and quantitative content of polyphenols in five species of willowherbs (*Epilobium* spp.), to identify the most suitable species for alleviating BPH. To the best of our knowledge, we have estimated the qualitative and quantitative content of individual polyphenolic compounds in *Epilobium* spp. for the first time. species growing in Estonia.

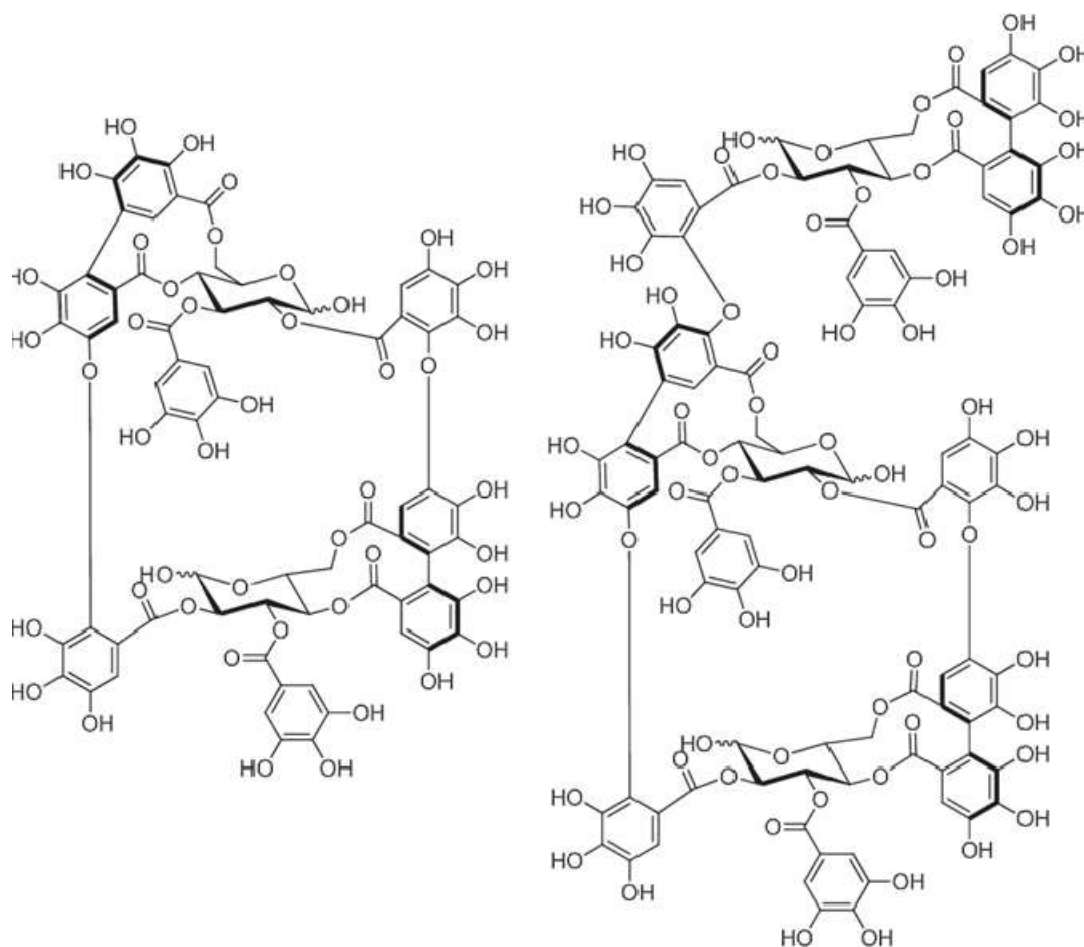


Fig. 1. Ellagitannins dimeric oenothein B (MF: $C_{68}H_{48}O_{44}$, MW: 1569.1 g/mol) and trimeric oenothein A (MF: $C_{102}H_{72}O_{66}$, MW: 2353.6 g/mol) (structures from Pubchem)

2. Planning (methodology) of research

The following flow chart presents the study protocol describing the different stages of the present research work (Fig. 2).

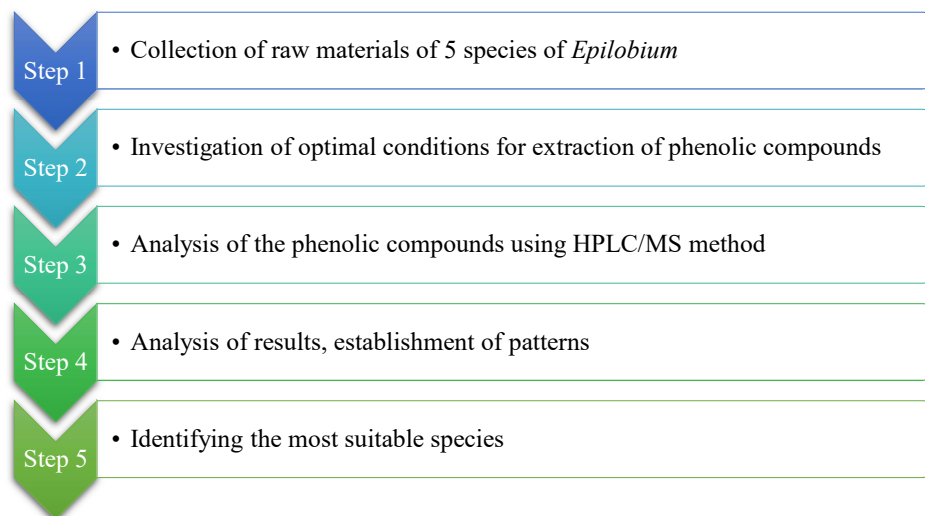


Fig. 2. Study protocol

3. Materials and methods

Plant materials.

All five species of *Epilobium* spp., such as *E. parviflorum* Shreb. (small flower hairy willowherb), *E. adenocaulon* (iron fireweed), *E. montanum* (mountain willow herb), *E. palustre* (marsh willow herb), and *E. hirsutum* (hairy willow herb) were collected in July 2008 from the village of Pilkuse (Otepää municipality, Valga County) on the shore of the pond (geographical coordinates N58.049385, E26.546264). *Epilobium* spp. plant species were identified by Dr. A. Raal from the Institute of Pharmacy of the University of Tartu and based on generally recognized plant atlases [23, 26]. A herbarium sheet for all five species of *Epilobium* spp. is available from the Institute of Pharmacy of the University of Tartu (No Ona/Epil-5, Supplemental material: the herbarium sheets and photos of *Epilobium* spp. studied are also available (Supplementary material).

The flowering tops of the studied species with a length of 30 cm were collected (five plants of each species). The plant material was dried at room temperature for 10 days. Woody stems were removed and stored in paper bags in a dark and dry place. The raw materials were analyzed within a year of collecting. The dry plant material was ground and sifted through a sieve with apertures of 3 mm immediately before making the extracts.

Extraction of polyphenolic compounds.

During preliminary tests, water extracts with different extraction times (15, 30, 60 min and 6 h) were made. An exact weight (~1 g) of the chopped herb of *E. hirsutum* was heated to boiling in a beaker with 20 ml of distilled water and allowed to stand for 15, 30 or 60 minutes, then strained through cotton and gauze and made up to 20 ml with water. The fourth extract was poured into a thermos immediately after boiling,

strained after six hours and topped up with distilled water to 20 ml.

Preparation of ethanolic extracts: an exact weight (~1 g) of the mentioned drug was placed in a plastic test tube with a cap. 20 ml of 20 %, 40 % or 80 % ethanol was poured on top and allowed to stand for 24 hours. It was then strained through cotton and gauze and topped up to 20 ml with ethanol of the appropriate concentration. Before the HPLC analysis, the images were centrifuged at 20 °C for 15 minutes at a speed of 4000 rpm.

HPLC/MS analysis of polyphenolic compounds in *Epilobium* spp.

Agilent 1100 Series LC/MSD Trap-XCT with ESI ionization unit was used. Blocks: autosampler, solvent degasser, binary pump, column in thermostat and UV-Vis diode array detector. Column: Zorbax 300SB-C18 column (2.1×150 mm), particle diameter 5 µm. HPLC 2D ChemStation software was used in combination with the ChemStation Spectral SW module to control the process. 5 µl of the test solution was injected into the column, the elution time was 50 minutes, the UV-Vis diode detector operated in the wavelength range of 190–530 nm, the temperature of the column was kept at 35 °C. The analytes were separated using a C18 reversed-phase column and an ascending gradient of an aqueous 0.1 % formic acid solution (solution A) and acetonitrile (solution B). Polyphenols were identified by an ion trap with an MS/MS detector using negative ionization mode (Table 1, Fig. 2). The particle mass-to-charge ratio range (m/z) under study was 50–1700, with a target mass of 1000. The flow rate was 0.3 ml/min and the maximum pressure was 400 bar.

To determine the quantitative content of polyphenols, solutions of a certain concentration of 96 % ethanol were prepared from the standard substances and chromatographed under the same conditions, with the difference that the target mass of the characteristic substances was 700 m/z . With the help of a computer program, the base areas of the characteristic peaks were determined, and a calibration graph was prepared for each characteristic substance. Ingredients used: quercetin glucoside (*Sigma-Aldrich*), ≥90 % HPLC purity, quercetin galactoside (*Sigma-Aldrich*), ≥97 % HPLC purity, myricetin (*Sigma-Aldrich*), ≥96 % HPLC purity, kaempferol (*Sigma-Aldrich*), ≥90 % HPLC purity, quercitrin (*Alpha-Aesar*), caffeic acid (*Sigma-Aldrich*). A similar methodology has been used in our previous studies.

By comparing the basal areas of the characteristic peaks with those of *Epilobium* spp., the content of substances in 1 g of herbal drug was calculated. Since some of the standard polyphenols were in the form of aglycones (for

example, myricetin and kaempferol), but in the plant material they were present as glycosides, a coefficient was calculated for the aglycone, with the help of which the concentration of glycoside was obtained. The coefficient (x) was calculated according to the following formula:

$$x = \frac{\text{glycoside molecular weight}}{\text{aglycone molecular weight}}$$

The content of a particular substance in the dried herbal drug was calculated according to the straight formula of the calibration graph of the characteristic substance:

$$x = \frac{(y \times b)}{m} \times z \times 20,$$

where x – substance content in dried herbal drug ($\mu\text{g/g}$); y – area under the peak of the test substance (PÜ); b – straight intersection with the y-axis; m – straight ascent; z – coefficient

4. Results

Extraction and qualitative content of polyphenolic compounds in Epilobium spp. samples.

The substances detected on the chromatograms of *Epilobium* spp. were identified by molecu-

lar weight and basic collision fragments based on previous research and literature from the Department of Food Hygiene, Estonian University of Life Sciences, Tartu, Estonia. Based on the literature of previous *Epilobium* spp. studies [48, 51], qualitative analysis was carried out using the HPLC method to compare the peak areas found on chromatograms (Table 1, Fig. 3).

Table 1

Retention times and molecular weights of substances found in *Epilobium* spp. extracts with collision fragments

t_R (min)	[M-H] ⁻	Collision fragments	Substance
4.0-4.3	311	149, 179, 135	Caffeoyl tartaric acid
7.6	1567	765, 935, 451, 1209	Oenothien B
16.4	631	479, 317, 299	Myricetin galloyl glycoside
17.9	479	316, 317, 271, 179, 151	Myricetin glucoside
20.2–20.9	615	463, 301, 271	Quercetin galloyl-glucosides
21.1	463	316, 317, 271	Myricetin rhamnoside
21.9	463	301, 316, 179	Quercetin galactoside or glucoside
24.7	433	301, 300, 179	Quercetin pentoside
25.0–25.6	447	284, 285, 255, 151	Kaempferol glucoside
26.6	447	301, 300, 179, 151	Quercetin rhamnoside (Quercitrin)
27.9	417	284, 285, 255, 327	Kaempferol pentoside
31.0	431	285, 284, 255, 327	Kaempferol rhamnoside

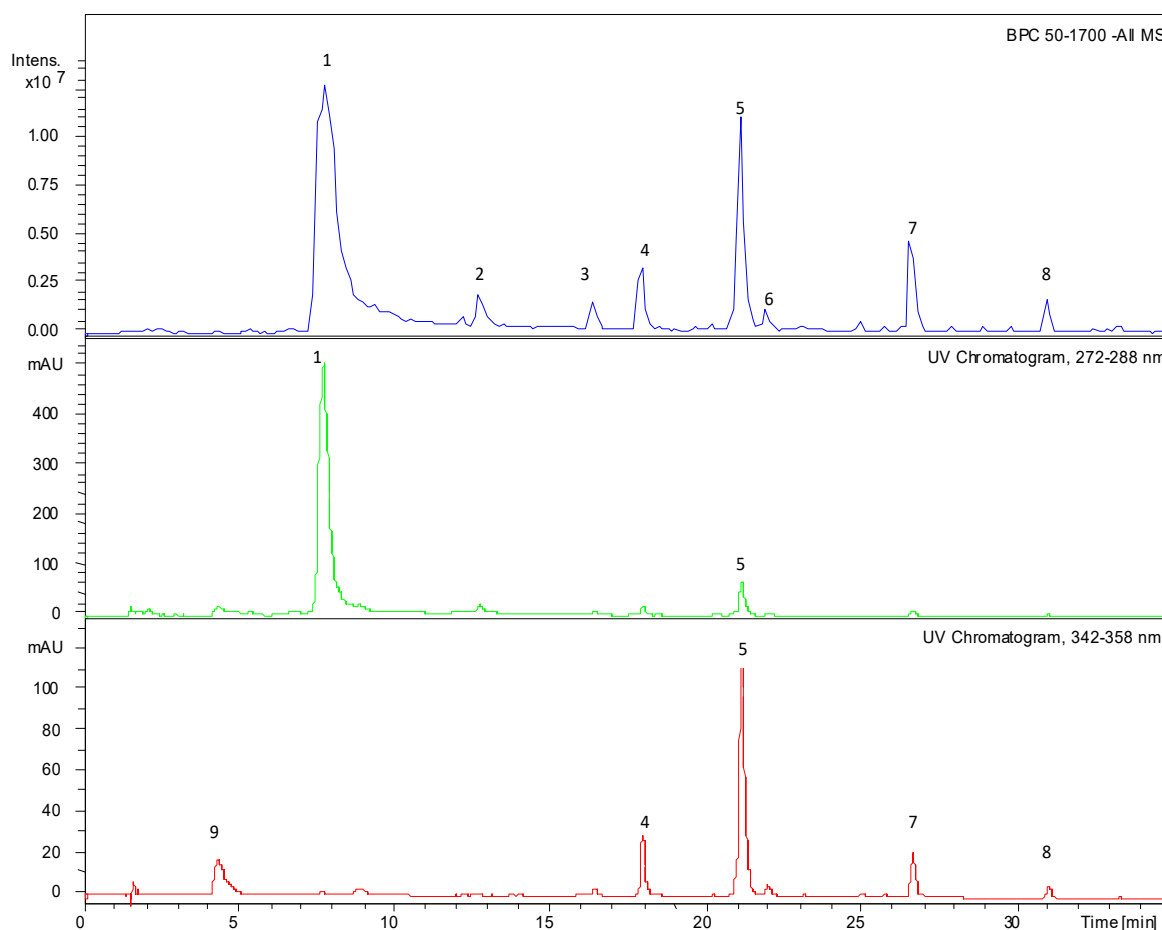


Fig. 3. Chromatograms of quantified compounds in *E. parviflorum* herbs. 1 – oenothien B, 2 – unknown substance, 3 – myricetin galloyl glucoside, 4 – myricetin glucoside, 5 – myricetin rhamnoside, 6 – quercetin galactoside or glucoside, 7 – kaempferol pentoside, 8 – kaempferol rhamnoside, 9 – caffeoyl tartaric acid

Preliminary experiments for extracting polyphenols were carried out using *E. hirsutum* herb. Although the results of the water extracts were noteworthy, water additionally extracts polysaccharides, which do not have a very good effect on the chromatographic column. Considering all this, it was still decided to study the five *Epilobium* spp. with 20 % ethanol, as their chromatograms were clearer and the peaks of the different substances were better separated (Table 2). A longer extraction time and stronger alcohol caused the decrease of peaks areas. However, oenothien B was the most abundant found in *Epilobium* spp., concentrations of polyphenols, especially of myricetin rhamnoside and myricetin glucoside were also high.

Based on the preliminary test results, it was decided to use 20 % ethanol/water as an extractant during further experiments, with which alcoholic extracts of the five studied willowherb species were prepared. Three parallel experiments were performed on each *Epilobium* spp., and the mean standard deviation and percentage of the mean were calculated. The mean standard deviation between the three parallel tests was ± 9.1 %.

The quantitative content of polyphenolic compounds in Epilobium spp. samples.

Based on the literature of previous *Epilobium* spp. studies [48, 51], qualitative analysis was carried out using the HPLC method to compare the peak areas found in the chromatograms (Table 3). The largest sum of peak areas of all identified compounds is observed in *E. montanum* (28981), the smallest – in *E. palustre* (17435).

The analysis of the absolute content of polyphenols ($\mu\text{g/g}$) also showed that the content of different polyphenolic compounds in *Epilobium* spp. is relatively high and varies within fairly large limits (Table 4).

There is a strong positive correlation between the content of quercetin and kaempferol derivatives with a high Pearson coefficient ($r=0.78$) and strong negative correlations between the content of total flavonoids and caffeoyl tartaric acid ($r=-0.65$), as well as between the content of myricetin and caffeoyl tartaric acid ($r=-0.78$, Fig. 4).

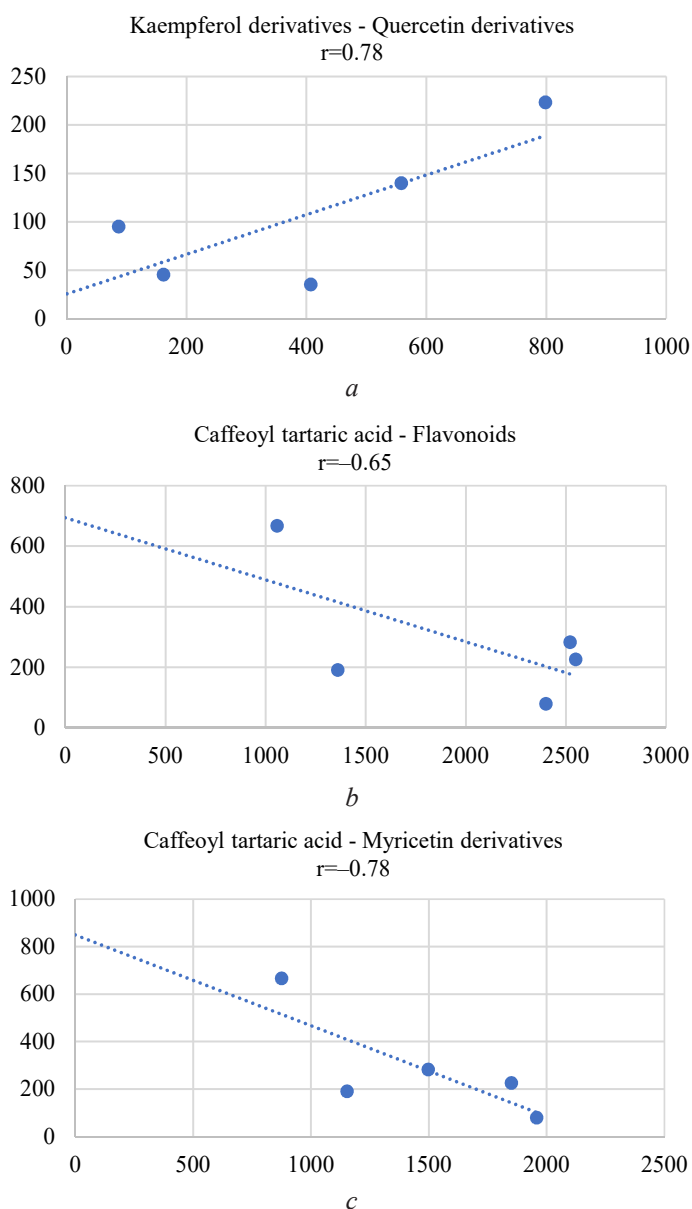


Fig. 4. Correlations between several polyphenolic compounds in *Epilobium* spp. studied: a – kaempferol derivatives-quercetin derivatives; b – caffeoyl tartaric acid-flavonoids; c – caffeoyl tartaric acid-myricetin derivatives

Table 2

Results of preliminary tests (areas under the peaks) of *Epilobium hirsutum* herb

Polyphenol	Images analysed (extraction time, ethanol concentration)						
	Water strokes				Ethanol extracts		
	15 min	30 min	60 min	6 h	20 %	40 %	80 %
Caffeoyl tartaric acid	441	549	311	352	434	495	72
Oenothien B	17132	22454	14452	14505	19006	13032	3310
Myricetin glucoside	2647	3515	2102	1884	2461	3953	2688
Quercetin galloyl glucoside	439	595	416	317	421	703	–
Myricetin rhamnoside	1034	1414	780	813	1081	1575	1527
Quercetin glucoside/galactoside	457	616	379	336	442	690	476
Quercetin pentoside	154	210	111	115	152	227	158
Quercitrin	195	273	153	158	216	373	214
Kaempferol rhamnoside	15	22	12	45	15	22	15
Myricetin galloyl glucoside	18	24	14	11	16	29	–
Myricetin rhamnoside hexoside	57	74	44	–	57	116	73
Quercetin hexoside-rhamnoside	–	–	–	–	–	19	12

Table 3
Peak areas (PA) of various substances *Epilobium* spp. chromatograms (350 nm)

Polyphenol	<i>E. parviflorum</i>	<i>E. adenocaulon</i>	<i>E. montanum</i>	<i>E. palustre</i>	<i>E. hirsutum</i>
Caffeoyl tartaric acid	822	1216	971	2855	347
Oenothain B	16654	12243	23579	12854	17990
Myricetin glucoside	312	940	376	942	2251
Quercetin galloyl glycoside	–	175	–	–	386
Myricetin rhamnoside	1597	1544	2734	468	978
Quercetin galactoside	70	558	231	135	416
Quercetin pentoside	–	223	67	13	143
Quercitrin	239	719	763	17	201
Kaempferol pentoside	–	153	41	71	22
Kaempferol rhamnoside	56	132	134	11	14
Myricetin galloyl glycoside	50	200	–	–	13
Kaempferol glycoside	14	166	71	30	–

Table 4
The content of different polyphenolic substances (µg/g) in different *Epilobium* spp.

Compound	<i>E. parviflorum</i>	<i>E. adenocaulon</i>	<i>E. montanum</i>	<i>E. palustre</i>	<i>E. hirsutum</i>
Myricetin glucoside	207	584	246	586	1372
Myricetin rhamnoside	946	915	1605	290	587
Quercetin pentoside	0	138	41	8	89
Kaempferol glucoside	14	85	40	21	0
Kaempferol pentoside	0	73	24	37	16
Kaempferol rhamnoside	32	66	67	12	19
Caffeoyl tartaric acid	190	283	225	667	79
Quercitrin	123	373	397	7	104
Quercetin galactoside	39	288	121	72	215
Kaempferol glucoside	0	0	9	25	0
Total	1551	2805	2775	1725	2481

5. Discussion

The results of the study (Table 2) revealed that water and 20–40 % ethanol solutions provide better extraction of phenolic compounds compared to concentrated ethanol solutions. Most often, *Epilobium* spp. are used as teas, so it was interesting to see how the situation changes over time and 30 minutes of infusion is enough for it.

As can be seen from Table 3, according to the area at the base of the peaks, the species *E. montanum* had the highest content of oenothain B, whose area under the peak was almost twice as large as *E. adenocaulon* with the smallest content of oenothain B. Other designated polyphenols are also contained quite a lot in *E. montanum*. The base areas of the peaks of different constituents varied within relatively large limits between different *Epilobium* spp., being even tens of times larger in one species than in another.

The dimeric hydrolysable tannin, oenothain B, dose-dependently induces several phagocyte functions *in vitro* and *in vivo*. Only the whole molecule of oenothain B is biologically active, its substructures (pyrocatecholic, gallic acid, pyrogallol, 3,4-dihydroxybenzoic acid) are not active [41]. According to a study by Miyamoto and co-authors [46], the antitumor effect of oenothain B is expressed by potentiating the activation of immune system macrophages. On the other hand, Lin et al. assessed the anti-inflammatory activity of an ethanol fraction of *E. angustifolium* from which oenothain B was removed and

the activity was much stronger than that of oenothain B [52]. This suggests a synergistic mechanism of action of the plant extract, which should possess a phytocomplex rather than a higher concentration of a certain bioactive compound.

The action of *Epilobium* spp. is based on the antitumor effect of oenothain B. The main promoter of BPH is hormone, which is indirectly regulated by the enzymes 5 α -reductase and aromatase. Oenothain B inhibits these enzymes [13, 53]. Willowherb extracts inhibit the proliferation of human PZ-HPV7 cells. These cells that originate from normal prostate cells are virilically transformed and are the basis for the development of BPH [54, 55]. The proliferation inhibitor is presumably oenothain B with an antitumor effect, as well as other components contained in the extract, such as polyphenols kaempferol, quercetin, myricetin and their glycosides [55]. Phenolic components are also important as antioxidants [13]. Quercetin

and kaempferol inhibit the proliferation of androgen-dependent and non-dependent cancer cells by affecting cell cycle development [55]. They have also been found to inhibit 5 α -reductase isoenzymes [56]. Thus, the effect of *Epilobium* spp. on BPH could be explained by the presence of various active substances.

The largest area of myricetin glycosides peaks has *E. hirsutum* and *E. montanum*, and the smallest – *E. palustre*. Myricetin is a bioflavonoid found to have both antioxidant and prooxidant properties, as well as anticarcinogenic and antimutagenic properties [57, 58].

The largest quercetin glycoside peak is observed in the case of *E. adenocaulon*, and the smallest in the case of *E. palustre*. Only galactoside and rhamnoside (quercitrin) are found among the quercetin glycosides of all species. Quercetin galloyl glycoside was present only in *E. adenocaulon* and *E. hirsutum*, and quercetin glycoside was present only in *E. montanum* and *E. palustre*. *In vitro* studies have shown that quercetin has anti-proliferative effects on various cells [59, 60].

The sum of the peak areas of kaempferol glycosides is the largest in the case of *E. adenocaulon* and the smallest in the case of *E. hirsutum*. Caffeoyl tartaric acid was found in all studied species. At the same time, the largest peak area was observed in case of *E. palustre*, the smallest – in case of *E. hirsutum*. Due to free hydroxyl groups, caffeoyl tartaric acid behaves *in vitro* as an antioxidant [61, 62].

In general, the content of myricetin glycosides (Table 4) is the highest for all species, with a slightly lower content of quercetin glycosides and the lowest content of kaempferol glycosides.

Myricetin rhamnoside was the most abundant polyphenol in *Epilobium* spp., with concentrations of 1605 µg/g in and 946 µg/g in *E. montanum* and *E. parviflorum*, respectively. This was followed by myricetin glucoside, most commonly found in *E. hirsutum* at 1372 µg/g. The sum of contents of myricetin glycosides is higher than that of quercetin and kaempferol glycosides. It corresponds to the following pattern of accumulation: *E. hirsutum*>*E. montanum*>*E. adenocaulon*>*E. parviflorum*>*E. palustre*.

The following variation in the content of quercetin glycosides has been established: *E. adenocaulon*>*E. montanum*>*E. hirsutum*>*E. parviflorum*>*E. palustre*.

The lowest content in *Epilobium* herbs had kaempferol pentoside and glycoside. Kaempferol glycosides are characterized by the following accumulation depending on the species: *E. adenocaulon*>*E. montanum*>*E. palustre*>*E. parviflorum*>*E. hirsutum*.

The content of caffeoyl tartaric acid decreases in the following order: *E. palustre* > *E. adenocaulon*>*E. montanum*>*E. parviflorum*>*E. hirsutum*.

The total polyphenol content was highest in the *E. adenocaulon* and lowest in the *E. parviflorum* at 2803 µg/g and 1551 µg/g, respectively. Although the difference is almost twofold, the content of polyphenols is still so low that one species cannot be preferred over the others. Based on the results obtained, it can be said that the content of polyphenols in the *E. parviflorum* herb is lower than that of other species. Unfortunately, we could not determine the absolute content of oenothetin B, because no standard substance was available. Thus, there is no scientific basis for recommending *E. parviflorum* specifically for treating BPH.

The established correlation between the content of quercetin and kaempferol derivatives, the content of total flavonoids and caffeoyl tartaric acid, and the content of myricetin and caffeoyl tartaric acid can be used to purposefully predict *Epilobium* remedies' chemical composition and correspond to their predictable pharmacological activity. It can also be used as species and genus markers.

The high content of phenolic compounds in *E. adenocaulon* can be explained by their adaptive functions in the life of this introduced plant. This is one of the adventitious (occasional) species that has seen the most dramatic increase in range and abundance in Great Britain during the 20th century [63]. During evolution, plants have synthesized various phenolic compounds to cope with constantly changing environmental conditions. Plants accumulate phenolic compounds in their tissues as an adaptive response to unfavourable environmental conditions. They play a key role in regulating various environmental stresses such as high light, low temperatures, pathogen infection, herbivory and other factors [64, 65]. *E. adenocaulon* aggressively invades new habitats [66] and can reduce the biodiversity of natural communities and displace native species [67, 68].

When entering a new habitat, the plant optimizes physiological and biochemical mechanisms that increase the organism's resistance to the destructive effects of biotic and abiotic stress factors. As a result, the accumulation level of both primary and secondary metabolites increases or decreases [69, 70].

Study limitations. Samples of raw materials of 5 types of *Epilobium* spp. from Estonia were used. It would be appropriate to study raw material samples of Ukraine and other species growing in Estonia and Ukraine. Also, the availability of standard samples of all the identified substances would improve the results of the analyses.

The prospects for further research. Prospects for further research include expanding the geography of the origin of raw materials and identifying other promising species of the genus. The research results create conditions for a targeted search for sources of biologically active substances among the species of the genus *Epilobium*.

6. Conclusions

The qualitative and quantitative content of polyphenols in five *Epilobium* species (*E. adenocaulon* Hausskn., *E. hirsutum* L., *E. montanum* L., *E. parviflorum* Schreb. and *E. palustre* L.) growing in Estonia, were determined. In the analyzed *Epilobium* spp., 12 polyphenolic compounds were identified. The quantitatively main phenolic ingredients of willowherbs were oenothetin B, myricetin rhamnoside and myricetin glycoside. The content of polyphenols was highest in *E. adenocaulon*. In contrast, *E. parviflorum* had the lowest polyphenol content compared. The established correlation between phenolics can be used for purposeful prediction of *Epilobium* remedy's chemical composition and corresponding to their predictable pharmacological activity. A comparative study of polyphenols in the raw materials of 5 species makes it possible to choose the most promising species for targeted study of pharmacological activity depending on the qualitative composition and quantitative content of substances.

Conflicts of interest

The authors declare that they have no conflict of interest concerning this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

The datasets used and/or analyzed during the current study are available from the author and/or corresponding author upon reasonable request.

Use of artificial intelligence

The authors confirm they did not use artificial intelligence technologies when creating the current work.

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