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TOWARDS THE CHEMICAL STANDARDISATION OF *EPILOBIUM HIRSUTUM* LEAVES

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Epilobium hirsutum L. is widely used in traditional European medicine to treat urological and inflammatory conditions. Despite its widespread use, no pharmacopoeial monographs on its plant material have been published to date. The ***aim*** *of this study was to establish physicochemical parameters and identify marker phenolic compounds for the standardisation of plant leaves.*

Method*. Seven E. hirsutum leaf samples collected in Ukraine, Lithuania, and Poland for analysis. The following parameters were analysed: loss on drying, total ash, and acid-insoluble ash content according to the State Pharmacopoeia of Ukraine. The qualitative composition was analysed using high-performance thin-layer chromatography (HPTLC), and the quantitative analysis of total phenolic compounds, flavonoids, and specific marker compounds was performed using UV spectrophotometry and HPLC-DAD.*

Results*. As a result, it was established that the content of foreign impurities did not exceed 2% in the analysed samples, the drying loss was 6.4–8.1%, and the total ash was 4.0–5.7%. The HPTLC method was used to determine gallic acid, isoquercitrin, avicularin, guijaverin and hyperoside as key compounds for the plant quality control. Total phenolics ranged between 0.78 and 1.52 mg GAE/g dw, while total flavonoid content was 1.9–5.5 mg HE/g dw. The HPLC method showed that the dominant polyphenolics is oenothein B (39.9–65.7 mg/g), followed by oenothein A, gallic, chlorogenic, and ellagic acids, hyperoside, isoquercitrin, and quercetin. The present components can be proposed for the development of raw material standardisation parameters.*

Conclusion*. The obtained data confirm that E. hirsutum leaves meet requirements of the State Pharmacopeia of Ukraine and European Pharmacopoeia and can serve as the basis for developing a monograph. Oenothein B, hyperoside, and gallic acid can be proposed as identification markers; in addition, the total phenolic content can be assessed*

Keywords*: Epilobium hirsutum, standardisation, phenolic compounds, oenothein B, flavonoids, HPTLC, HPLC-DAD*

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1. Introduction

Standardisation of herbal raw materials is a critical step in ensuring the safety, reproducibility, and pharmacological efficacy of herbal medicinal products [1]. Differences in the biological and geographic origin of samples, as well as differences in collection times, methods, and extraction procedures, can lead to significant variability in the qualitative and quantitative composition of bioactive compounds. Without establishing strict criteria for the quality of raw materials, comparability of pharmacological results and regulatory assessment become virtually impossible [2]. Pharmacopoeia standards for plants, including physicochemical parameters and the selection of marker compounds for identification, provide the basis for the control of raw materials and herbal extracts, thereby minimising the risks of toxicity and improving clinical predictability [3–5].

Epilobium hirsutum L. (family Onagraceae) is widespread in Ukraine, Poland, Germany, Lithuania, and other European countries. The plant is traditionally used to treat urological, inflammatory, and dermatological diseases [6]. The plant raw material is widely available on the pharmaceutical market as teas and dietary supplements [7] and *Epilobium* samples are represented in herbal monographs of the European Medicines Agency [8] and ESCOP (mainly concerning *E. angustifolium* and *E. parviflorum*) [9]. At the same time a separate pharmacopoeia monograph with analysis of quality composition for *E. hirsutum* plant material is currently absent in any pharmacopoeia of the world. This creates methodological and regulatory challenges in standardising this species, particularly with regard to the development of herbal preparations and the design of clinical trials.

The pharmacological potential of *Epilobium* species is due to polyphenolic compounds such as flavonoids, phenolic acids, and, in particular, hydrolysable tannins such as oenothein B [10–14]. Modern pharmacological studies confirm the anti-inflammatory, antiproliferative, urogenital, and antiviral activities of *E. hirsutum* extracts, supporting their potential development as phytopharmaceuticals [15–17].

However, the chemical profile of *E. hirsutum* exhibits its significant intraspecific variability. The composition and concentration of marker polyphenols depend on the geographical origin, phenological stage, and post-harvest processing methods [18]. Therefore, the **aim of this study** was to establish parameters for the standardisation of *E. hirsutum* leaves by determining key physicochemical characteristics and quantifying marker phenolic compounds. The obtained results provide a foundation for ensuring the quality and safety of *E. hirsutum*-based herbal preparations and for developing future regulatory documentation for this plant species.

from the steams and used for this study. All samples were dried, ground, and authenticated at the National University of Pharmacy (Ukraine).

Table 1

Location and elevation of Epilobium sampling sites in Lithuania and Ukraine

Code	Country	Location	Altitude, m	Geographical coordinators	Voucher specimens
EH_1	Ukraine	Zhuravlivskyi Hydropark, Kharkiv	120	50.01787; 36.30147	OM2022-1506
EH_2	Ukraine	Pokotilovka village, Kharkiv region	103	49.91098; 36.18950	OM2022-2006
EH_3	Ukraine	Kvasy village, Carpathian mountains	560	48.21077; 24.30930	OM2022-1006
EH_4	Ukraine	“Valley of Narcissuses”, Carpathian Biosphere Reserve	200	48.18390; 23.35667	OM2022-1006
EH_5	Ukraine	Kuziy-Tribushansky massif, Carpathian Biosphere Reserve	410	48.02590; 24.16705	OM2022-1006
EH_6	Lithuania	Rasava village, Kaišiadorys district	78	54.84746; 24.59177	OM2022-2307
EH_7	Poland	Surazh village, Podlaskie Voivodeship	138	52.91405; 22.99083	OM2022-2406

2. Research planning

The stages of the seven samples of *E. hirsutum* leaves research included:

Stage 1. Collect plant leaves from different regions of Ukraine and two neighbouring countries for research.

Stage 2. Determine the quantitative parameters of samples according to the requirements of the State Pharmacopoeia of Ukraine and European Pharmacopoeia. Samples must meet the requirements for plant raw materials: foreign impurities ≤ 2%, loss on drying ≤ 8%, total ash ≤ 5%, acid-insoluble ash ≤ 0.5%.

Stage 3. Conduct qualitative analysis using HPTLC of samples.

Stage 4. Determine the total polyphenolics and total flavonoids content of samples using UV-spectrophotometry method.

Stage 5. Analyse the component composition and quantitative content of polyphenolics, including flavonoids, hydroxycinnamic acids, and ellagitannins, in samples using HPLC.

Stage 6. Based on the obtained results, the average values of all parameters were proposed as reference criteria for the standardisation of *E. hirsutum* leaves.

3. Materials and methods

3. 1. Plant material and reagents

The following chemicals were obtained from Sigma (St. Louis, USA): gallic acid, ellagic acid, chlorogenic acid, oenothein B, hyperoside, isoquercitrin, avicularin, afzelin, guaijaverin, rutin, quercitrin, quercetin, myricetin, and Folin-Ciocalteu reagent. Water used in this study was purified using an Ultrapure water system (Millipore, Germany). Acetonitrile, methanol, and glacial acetic acid were of HPLC grade (Merck KGaA; Fisher Scientific Ltd).

Seven series of *Epilobium* samples were collected from natural habitats (Table 1). Leaves were separated

3. 2. Physicochemical Parameters

Seven batches of *E. hirsutum* leaves were analysed according to the State Pharmacopoeia of Ukraine (SPhU) and European Pharmacopoeia. Since the SPhU is a branch of the European Pharmacopoeia, the monographs and quality control methods for herbal raw materials are the same. The following parameters were determined using standard gravimetric methods: foreign matter (2.8.2), loss on drying (2.3.32), total ash (2.4.16), and acid-insoluble ash (2.8.1). All measurements were expressed as percentage of dry weight (% m/m) [19].

3. 3. HPTLC fingerprint analysis

Each leaf sample (50 mg) was extracted with 5.0 mL of 50% (v/v) methanol using ultrasound-assisted extraction (20 min, 40°C), then filtered through a 0.45 μm membrane filter. HPTLC analysis was performed on silica gel 60 F_{254} plates (20 × 10 cm, Merck, Darmstadt, Germany) using a CAMAG system (Linomat 5, ADC2, TLC Visualizer, visionCATS 3.1 software). The mobile phase consisted of ethyl acetate–formic acid–water (68:8:8, v/v/v). After development to 70 mm distance, plates were dried, derivatised with Natural Product A reagent followed by PEG 400, heated at 100°C for 5 min, and visualised under white light, UV 254 nm, and UV 366 nm. Chromatographic profiles were recorded and processed using visionCATS software.

3. 4. Determination of total phenolics compounds

Determination of the total phenolics content using Folin-Ciocalteu reagent [20]. The 50% (*v/v*) methanolic extract of *Epilobium* leaves samples was mixed with the Folin-Ciocalteu phenol reagent, 9 mL of 7% sodium carbonate was added, and the mixture was kept in a dark place for 90 min. Absorbance at 750 nm was read after 2 h on the Halo DB-20 UV-Vis spectrophotometer (Techcomp Europe, UK). The obtained data were evaluated according

to the linear regression equation of the end acid calibration graph: $y = 0.9068x + 0.0617$; $R^2 = 0.9960$; y – absorption intensity; x – total phenolic compounds expressed as gallic acid equivalent per gram dry weight (mg GAE/g dw).

3. 5. Determination of total flavonoid content

Total flavonoid content was determined according to the Eur. Ph. [21] with minor modifications: 0.4 g of crushed plant material was extracted twice with 60% (v/v) ethanol under heating (60°C, 10 min each time) and filtered. The combined extracts were made up to 100 ml with 60% ethanol to obtain a stock solution. A 5 ml aliquot of the stock solution was evaporated to dryness, redissolved in a mixture of methanol and glacial acetic acid, and reacted with boric and oxalic acids in a formic acid medium. After 30 min, absorbance was measured at 410 nm against a compensating solution prepared in a similar manner but without reagents. Total flavonoid content, expressed as hyperoside equivalents (%), was calculated using a specific absorbance value of 405. For better comparability with other quantitative parameters, total flavonoid content expressed in% was additionally recalculated to mg/g dry weight (1% = 10 mg/g) and given as mg HE/g dw.

3. 6. Quantitative determination by the HPLC

For HPLC analysis, 0.20 g of powdered *E. hirsutum* leaves was extracted with 50% (v/v) methanol (1:50) using ultrasound-assisted extraction (20 min, 45 ± 2°C) and filtered through a 0.45 µm syringe filter. Separation of phenolic compounds was performed on an ACE Super C18 column (250 × 4.6 mm, 3 µm) using a Waters e2695 Alliance HPLC system with a PDA detector. The mobile phase consisted of 0.1% trifluoroacetic acid in water (A) and acetonitrile (B) under a gradient elution at 1.0 mL/min. The detection wavelength range was 200–400 nm. Identification and quantification were carried out by comparing retention times and UV spectra with authentic standards. The concentration of oenothein A was calculated relative to oenothein B and isomyricetrin was calculated relative to myricetin. Method validation and quantification followed previously described protocols [22].

3. 7. Statistical analysis

The data was processed using the Microsoft Office Excel 2010 (Microsoft, JAV) software package. All data processing was carried out using the LabSolutions Analysis Data System. The results of descriptive statistics are presented as mean and standard deviation (mean ± SD) from three replicates ($n = 3$) for each sample. The value of $p < 0.05$ was taken as the significance level.

4. Research results

The results of foreign matter determination, as well as the parameters “loss on drying,” “total ash,” and “acid-insoluble ash,” together with the quantitative determination of total polyphenolics and flavonoids, are presented in Table 2.

Table 2

Results of the analysis of *Epilobium hirsutum* leaves samples (mean ± SD, $n = 3$)

Sample	Foreign matter (% m/m)	Loss on drying (% m/m)	Total ash (% m/m)	Acid-insoluble ash (% m/m)	Total polyphenolics, mg GAE/g dw	Total flavonoids,%	Total flavonoids mg HE/g dw
EH_1	1.6 ± 0.20	8.08 ± 0.05	5.3 ± 0.25	0.22 ± 0.04	1.02 ± 0.10	0.26± 0.03	2.6± 0.03
EH_2	1.3 ± 0.18	7.04 ± 0.06	5.7 ± 0.18	0.18 ± 0.05	1.52± 0.11	0.27±0.02	2.7±0.02
EH_3	1.8 ± 0.30	7.56 ± 0.04	5.1 ± 0.30	0.25 ± 0.06	1.19± 0.08	0.19±0.02	1.9±0.02
EH_4	1.4 ± 0.22	6.81 ± 0.05	4.0 ± 0.22	0.20 ± 0.05	0.87± 0.09	0.24±0.02	2.4±0.02
EH_5	1.9 ± 0.35	6.42 ± 0.07	4.5 ± 0.28	0.30 ± 0.07	1.05± 0.07	0.27±0.03	2.7±0.03
EH_6	1.2 ± 0.15	7.00 ± 0.06	5.4 ± 0.20	0.17 ± 0.04	0.78± 0.08	0.55±0.05	5.5±0.05
EH_7	1.5 ± 0.21	7.53 ± 0.05	5.6 ± 0.24	0.23 ± 0.05	0.79± 0.07	0.41±0.04	4.1±0.04
Average content	1.5 ± 0.24	7.21 ± 0.51	5.0 ± 0.27	0.22 ± 0.04	1.03 ± 0.24	0.31 ± 0.12	3.1 ± 0.12

The content of foreign impurities in the studied samples ranged from 1.2 to 1.9%, with an average value of 1.53 ± 0.24% across all batches, which corresponds to the pharmacopoeial standards (≤ 2%). Loss on drying ranged from approximately 6.42 to 8.08%, with an average value of 7.21 ± 0.51%. The average total ash content was 5.09 ± 0.27%, while acid-insoluble ash values were low in all batches (0.17–0.30%).

A HPTLC method was proposed for identifying the components. The sequence of zones observed in the chromatograms of the test solutions and standard solutions such as gallic acid, isoquercitrin, avicularin, guaijaverin, and hyperoside, is presented on Fig. 1. Additional fluorescent zones were also detected in the chromatograms of the test solutions.

Based on R_f values and the characteristic yellow fluorescence, the presence of the following flavonoids was established in the tested samples: isoquercitrin (R_f 0.45–0.50), guaijaverin (R_f 0.60–0.65), avicularin (R_f 0.65–0.70), and hyperoside (R_f 0.70–0.75). In EH 1–3 samples the intensity of these zones was higher, indicating the higher content of flavonoids [23]. The zone corresponding to gallic acid (R_f 0.85–0.90, blue fluorescence) was detected in most samples, indicating the presence of phenolic acids. A blue zone in the samples, located below the yellow hyperoside zone, corresponds to chlorogenic acid (R_f 0.33–0.36) [24]. Thus, all seven *E. hirsutum* samples exhibited a similar chromatographic profile of phenolic compounds, differing only in the intensity of certain zones, which may be related to the growing conditions and the plants’ vegetative stage.

The total polyphenolics and flavonoids content in *E. hirsutum* leaves varied among the samples. Total

polyphenol content ranged from 0.78 to 1.52 mg GAE/g dry weight, while total flavonoid content ranged from 1.9 to 5.5 mg HE/g dry weight (or 0.19 to 0.55%). The highest phenolic content was found in sample EH_2 (1.52 ± 0.11 mg GAE/g), whereas the lowest was observed in sample EH_6 (0.78 ± ± 0.08 mg GAE/g). Sample EH_6 exhibited the highest total flavonoid content (5.5 ± 0.05 mg HE/g or 0.55 ± 0.05%). These results indicate differences in the distribution of phenolic compounds across classes depending on growing conditions and the vegetative stage. The low variability (SD ≤ 10%) demonstrates good reproducibility of the analytical method and stability of the chemical composition across batches.

The qualitative and quantitative analysis of components of *E. hirsutum* was done by HPLC (Fig. 2). Fourteen phenolic components were identified in the samples, including gallic acid, chlorogenic acid, ellagic acid, as well as flavonol glycosides (rutin, hyperoside, isoquercitrin, quercetin, isomyricertin). Among the tannins, ellagitannins oenothein A and oenothein B were predominant (Table 3).

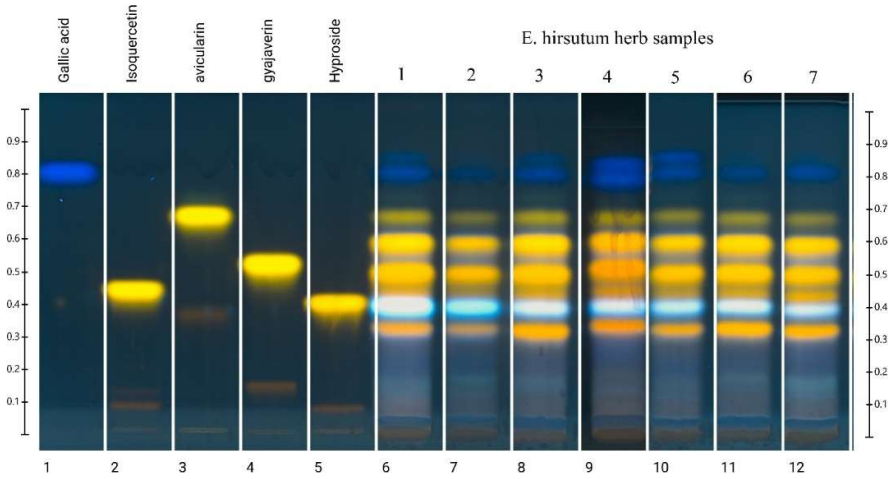

Fig. 1. HPTLC profile of *Epilobium* samples under UV 366 nm after derivatisation

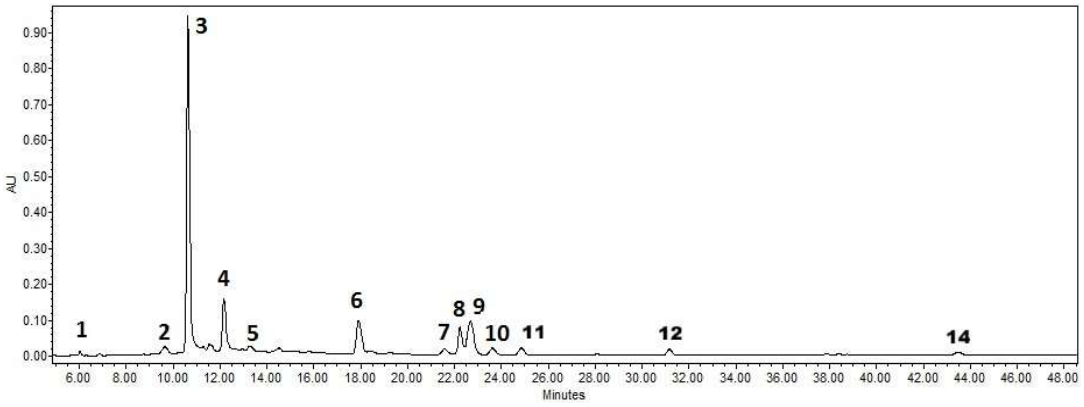

Fig. 2. The HPLC-DAD chromatograms of *E. hirsutum* (EH_2 sample) leaves at 219 nm: 1 – gallic acid; 2 – neochlorogenic acid; 3 – oenothein B; 4 – chlorogenic acid; 5 – oenothein A; 6 – isomyricitrin; 7 – ellagic acid; 8 – rutin; 9 – hyperoside; 10 – isoquercitrin; 11 – guaijaverin; 12 – quercitrin; 13 – quercetin; 14 – afzelin

Table 3

Compounds content in *E. hirsutum* samples by HPLC method (mg/g).

N*	Compound	RT	EH_1	EH_2	EH_3	EH_4	EH_5	EH_6	EH_7
1	Gallic acid	6.06	0.49 ± 0.03	0.34 ± 0.02	0.57 ± 0.04	0.72 ± 0.05	0.37 ± 0.03	0.23 ± 0.02	0.26 ± 0.02
2	Neochlorogenic acid	9.53	0.04 ± 0.01	0.07 ±0.02	0.06 ±0.02	0.08 ±0.03	0.06 ±0.03	0.04 ±0.02	0.03 ±0.01
3	Oenothein B	10.68	55.47 ± 3.88	39.92 ± 2.79	50.76 ± 3.55	65.69 ± 4.6	62.22 ± 4.36	41.13 ± 2.74	52.34 ± 2.88
4	Chlorogenic acid	11.68	0.91 ± 0.06	0.44 ± 0.03	–	–	1.23 ± 0.09	53.72 ± 3.76	75.08 ± 5.26
5	Oenothein A	13.04	7.04 ± 0.49	3.69 ± 0.26	5.90 ± 0.41	5.83 ± 0.41	2.69 ± 0.19	1.50 ± 0.11	2.19 ± 0.15
6	Isomyricitrin	18.51	0.76 ± 0.05	0.83 ± 0.04	0.66 ±0.03	0.73 ±0.03	0.81 ±0.04	0.63 ±0.03	0.58 ±0.03
7	Ellagic acid	22.72	0.45 ± 0.03	0.69 ± 0.05	0.50 ± 0.04	0.64 ± 0.04	0.74 ± 0.05	0.77 ± 0.05	0.93 ± 0.07
8	Rutin	22.80	0.80 ± 0.06	0.62 ± 0.04	0.64 ± 0.04	2.17 ± 0.15	0.27 ± 0.02	0.35 ± 0.02	0.40 ± 0.03
9	Hyperoside	23.65	0.71 ± 0.05	0.55 ± 0.04	0.55 ± 0.04	1.93 ± 0.14	0.23 ± 0.02	1.12 ± 0.08	1.96 ± 0.14
10	Isoquercitrin	24.90	0.04 ± 0.0	0.04 ± 0.0	0.05 ± 0.0	0.08 ± 0.01	0.02 ± 0.0	0.99 ± 0.07	1.74 ± 0.12
11	Guaijaverin	27.82	0.86 ± 0.06	0.69 ± 0.05	0.92 ± 0.06	1.78 ± 0.12	0.13 ± 0.01	0.16 ± 0.01	0.34 ± 0.02
12	Quercitrin	31.11	0.99 ± 0.07	0.21 ± 0.01	0.98 ± 0.07	0.79 ± 0.06	0.51 ± 0.04	0.05 ± 0.0	0.07 ± 0.0
13	Quercetin	31.13	0.01 ± 0.0	0.09 ± 0.01	0.02 ± 0.0	0.12 ± 0.01	0.01 ± 0.0	0.90 ± 0.06	1.84 ± 0.13
14	Afzelin	37.74	0.17 ± 0.01	–	0.11 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	–	0.02 ± 0.0

*Note: * – the compound number in the Table 3 corresponds to the peak number of the compound in Fig. 2.*

Oenothein B content ranged from 39.9 to 65.7 mg/g, with an average of 55.3 mg/g, highlighting its role as the dominant phenolic metabolite in *E. hirsutum*. Oenothein A was present at concentrations of 1.5–7.0 mg/g. Flavonol content varied from 0.5 to 2.0 mg/g, with the highest levels of hyperoside observed in samples EH_4 and EH_7 (1.9 mg/g and 1.96 mg/g, respectively). Gallic and chlorogenic acids were detected at concentrations of 0.3–1.2 mg/g, while ellagic acid content was slightly lower, ranging from 0.45 to 0.93 mg/g.

5. Discussion of research results

Epilobium hirsutum was chosen for the development of standardisation parameters due to its widespread use in traditional medicine and its high content of bioactive phenolic compounds. The morphological and anatomical characteristics of the raw material were previously studied and described [25], so this study focuses on its chemical composition.

The obtained values for loss on drying (~ 7.2%), total ash (~ 8.5%), HCl-insoluble ash (~ 0.22%), and foreign matter content (< 2%) indicate that the tested *E. hirsutum* raw material complies with the requirements of the SPhU for plant material and can serve as a basis for developing standardisation criteria. These results are also consistent with available literature data for *E. hirsutum* and related species [14]. Low total ash and HCl-insoluble ash values suggest minimal mineral contaminatio (e.g., sand, silica) and reflect proper collection of the raw material. Based on these findings, the following testing parameters for the raw material are recommended: foreign matter not exceeding 1.5%; loss on drying not exceeding 7.5% (1,000 g of powdered material dried at 105°C for 2 h); total ash not exceeding 5%; and HCl-insoluble ash not exceeding 0.5%.

Qualitative analysis and the HPTLC profiles of all seven *E. hirsutum* samples showed a typical pattern of zones and colouration characteristic for flavonoid glycosides (quercetin derivatives) and phenolic acids. The main zones ($R_f \approx 0.67, \approx 0.50, \approx 0.44, \approx 0.38$) were present in all samples, indicating the consistent presence of these compounds in the plant material. The intensity of the zones varied among the samples, such as reflecting differences in metabolite amount, growing location, or the plant's vegetative stage. The presence of identified marker compounds, such as avicularin, isoquercitrin, hyperoside, and gallic acid, is consistent with our previous studies on *E. hirsutum* [18].

The polyphenolic profile of *E. hirsutum* is known for its high content of tannins and flavonoid compounds, particularly ellagitannins (oenothein A and B) and flavonols (hyperoside, isoquercitrin, quercetin) [22]. The quantitative data obtained for total phenolic content (0.78–1.52 mg GAE/g) are in agreement with previously published results for *E. hirsutum* leaves [12, 22], as well as for related species such as *E. parviflorum* and *E. angustifolium*, where content ranged from 0.9 to 1.8 mg GAE/g [26, 27]. The highest flavonoid content observed in sample EH_6 (5.5 mg HE/g or 0.55%) probable because of the plant's response to intense sunlight, as this sample was collected in July [28]. Thus, the obtained results may be further implemented in the development of a monograph for *E. hirsutum* raw material.

In some samples, the calculated total flavonoid content, expressed in mg/g, exceeded the total phenolic content determined by the Folin-Ciocalteu method. This difference in content is due to methodological differences between the analyses and the use of different reagents. The Folin-Ciocalteu reagent is known to not react equally with all phenolic compounds [29]. For example, in the case of large-structure compounds (ellagitannins, particularly oenothein B), they may react weakly, partially precipitate, or exhibit low reactivity and exhibit reduced quantitative values of high-molecular-weight polyphenols, which predominate in *E. hirsutum*. Although the Folin-Ciocalteu method is more sensitive to flavonol glycosides, it was specifically used in the study as a widely used screening method for assessing total phenolic content [29]. This also allows for comparison of our own data with those of other authors [6, 10, 16, 22, 27, 28]. This method provides a value for the total reducing power of phenolic compounds and complements the results of the HPLC method.

The high total polyphenolics content observed in the samples prompted a further quantitative analysis of individual marker compounds in *E. hirsutum* leaves using HPLC. Comparison of the obtained data with published results shows that the concentrations of phenolic metabolites fall within the ranges reported by other authors [6, 27]. Similarly, studies [12, 16] confirm the dominance of oenothein B among the phenolic compounds and report comparable levels (40–60 mg/g) under similar chromatographic conditions.

The observed differences between samples EH_1–EH_7 (SD within 0.2–0.5% for key parameters) are most likely due to environmental factors (soil type, humidity, light exposure), plant growth stage, drying time and method, and possible differences in sample preparation (grinding, sieving). This is consistent with data [13], which highlight the significant influence of environmental and technological factors on the flavonoid content of *Epilobium* species. In this study, samples from Poland and Lithuania were included as neighboring countries to demonstrate differences in compound content depending on the country of origin.

The results further confirm that oenothein B can serve as a marker compound for the standardisation of *E. hirsutum* raw materials, while hyperoside and gallic acid can be used as additional identification markers. This combination of parameters is consistent with approaches used in pharmacopoeial monographs for other plant species.

Practical relevance. The obtained data can be used in the future to develop a monograph on the quality of plant raw materials of *E. hirsutum* leaves for inclusion in the State Pharmacopoeia of Ukraine.

Study limitations. This study focused on the chemical standardisation of *E. hirsutum* leaves. However, morphological and anatomical characteristics were not assessed, total tannin content was not estimated, and the number of batches analysed was limited ($n = 7$). These data could complement the standardisation parameters of raw materials. Future research should include a larger number of samples from different habitats and collection periods to fully assess the variability of chemical composition.

Prospects for further research. Prospects for further research include expanding the geographical and

phenological sampling across the territory of Ukraine to determine the optimal conditions and terms of harvesting of *E. hirsutum* leaves; resource studies to ensure the supply process of raw materials; additional quantitative determination of total tannins; validation of analytical methods for the preparation of a pharmacopoeial monograph.

6. Conclusion

The results of chemical standardisation of *Epilobium hirsutum* leaves are presented. The numerical parameters correspond to the standards for plant raw materials. HPTLC profiling revealed zones corresponding to gallic acid, chlorogenic acid, hyperoside, isoquercitrin, avicularin, and guaiaverin, confirming the characteristic phenolic profile of this species. Spectrophotometric analysis revealed variable total polyphenolics (0.78 to 1.52 mg GAE/g) and total flavonoid (1.9 to 5.5 mg HE/g) contents. Quantitative analysis revealed oenothein B as the dominant compound (39.9–65.7 mg/g), followed by oenothein A, hyperoside, isoquercitrin, and gallic acid. Similar results obtained for samples from different geographical regions confirm the reproducibility of the analytical methods. The obtained data can be further used to develop a monograph on *E. hirsutum* leaves for inclusion in the both Pharmacopoeia.

Conflict of interests

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

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Data availability

The manuscript has no associated data.

Use of artificial intelligence technologies

The authors confirm that they did not use artificial intelligence technologies in creating the submitted work.

Authors' contributions

Kateryna Uminska: Formal analysis; Methodology; Writing – Original Draft; **Liudas Ivanauskas:** Data curation; Resources, Validation; **Laurynas Jarukas:** Formal analysis; Visualization**; Victoriya Georgiyants:** Resource; Project Administration; Writing – Review & Editing**; Olha Mykhailenko:** Conceptualization; Data curation; Writing – Review & Editing; Supervision.

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