



Qualitative and quantitative standardisation of *Epilobium angustifolium* L. leaves according to Pharmacopoeial requirements

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ABSTRACT

Epilobium angustifolium L. is a medicinal plant widely used for its anti-inflammatory, antioxidant, antimicrobial, and antiproliferative properties, attributed to the presence of ellagitannins, flavonoids, and phenolic acids. However, the lack of pharmacopoeial standards for its raw materials limits consistent quality control and broader pharmaceutical use. The aim of this study was to establish approaches to the qualitative and quantitative standardisation of *Epilobium angustifolium* leaves collected from different geographic regions. Physicochemical parameters, including impurity content, loss on drying, total ash, and acid-insoluble ash, were determined according to the European and Ukrainian Pharmacopoeias. Chromatographic profiling was performed using HPTLC and HPLC-DAD, and the total phenolic compound and flavonoid contents were measured spectrophotometrically. The results showed low levels of foreign matter (1.2%–1.5%) and moisture (6.8%–8.1%), with total ash ranging from 4.2% to 6.0% and acid-insoluble ash from 0.15% to 0.38%. Total phenolic content ranged from 0.77 to 1.44 mg GAE/g dry weight, while total flavonoid content ranged from 0.19% to 0.55%. HPLC analysis identified and quantified 13 major polyphenols, including oenotherin B (24.8–60.6 mg/g), oenotherin A (0.6–5.8 mg/g), isomyricitrin (17.8–32.1 mg/g), and quercitrin (4.8–10.5 mg/g) as the dominant components. The resulting chromatographic fingerprints and quantitative data demonstrate chemical consistency between samples, with regional variability due to environmental factors. The proposed parameters can serve as a basis for developing quality control standards for raw materials and a pharmacopoeial monograph for *Epilobium angustifolium* leaves, ensuring reproducible quality and efficacy of herbal preparations.

1. Introduction

Epilobium angustifolium L. [syn. *Chamaenerion angustifolium* (L.) Scop.], commonly known as fireweed, belongs to the family Onagraceae. The plant is widespread in temperate regions of Europe, Asia, and North America [1]. This perennial herb is traditionally used in local and traditional medicine for the treatment of inflammatory diseases of the urogenital tract, gastrointestinal disorders, skin conditions, and as a tea for fatigue, colds, insomnia, anemia, or migraines [2–4]. Extracts of *Epilobium angustifolium* exhibit pronounced anti-inflammatory, antioxidant, antimicrobial, and antiproliferative activity, primarily due to

ellagitannins (e.g., oenotherin A and B), flavonoids (e.g., quercetin derivatives), and phenolic acids (e.g., gallic acid, ellagic acid) [5–8].

The plant raw material is widely available on the pharmaceutical market as teas and dietary supplements [9] and *Epilobium* samples are represented in herbal monographs of the European Medicines Agency [4] and ESCOP (mainly concerning *Epilobium angustifolium* and *Epilobium parviflorum*) [10].

With the growing popularity of *Epilobium angustifolium* as a component of herbal preparations and dietary supplements, particularly in the treatment of prostate and urinary tract diseases, ensuring the quality, safety, and efficacy of *Epilobium angustifolium* raw materials is becoming increasingly important [2]. The trend of using *Epilobium angustifolium*

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Abbreviation list:

HPTLC - High-Performance Thin-Layer Chromatography
 HPLC–DAD - High-Performance Liquid Chromatography–Diode Array Detection
 UV-Vis - Ultraviolet-visible spectroscopy

and *Epilobium parviflorum* as medicinal teas began in the late 1970s and early 1980s in several European countries [11,12]. Products containing *Epilobium* herba are still available on the market in Austria, the Czech Republic, Hungary, Germany, Finland, Ukraine, Estonia, and Lithuania [12].

Despite extensive pharmacological data supporting the drug's therapeutic potential, the lack of harmonised pharmacopoeial standards for plant raw materials complicates their quality control and limits their wider pharmaceutical use [13,14]. The chemical composition of *Epilobium angustifolium* can vary significantly depending on environmental factors, geographical origin, and processing conditions [15], highlighting the need to develop standardisation parameters.

Standardisation of medicinal plant materials involves macroscopic, microscopic, and physicochemical evaluation, chromatographic profiling for quality control and the quantitative determination of bioactive compounds. While we have previously assessed and described in detail the anatomical features of *Epilobium angustifolium* leaves [16], data on the development of quality control parameters for raw materials remains limited.

Therefore, the aim of this study was to develop approaches to the qualitative and quantitative standardisation of *Epilobium angustifolium* leaves based on physicochemical and chromatographic (HPTLC, UV, HPLC) analysis, using general principles and analytical methods adopted in the European and Ukrainian Pharmacopoeias. To date, no harmonised pharmacopoeial standard has been proposed for *Epilobium angustifolium* leaves, despite their widespread use in herbal preparations. Existing research has primarily focused on phytochemical profiling or pharmacological activity, rather than developing quality control approaches for this herbal raw material. This study, for the first time, provides a framework for standardising *Epilobium angustifolium* raw materials based on pharmacopoeial principles and validated analytical markers, which can subsequently be implemented to develop a monograph for this herbal raw material.

2. Materials and methods

2.1. Plant material and reagents

Seven batches of *Epilobium angustifolium* (L.) Scop. samples were collected during the flowering stage from natural habitats across different regions of Ukraine, as well as from Romania, Lithuania, and Switzerland (Table 1). The plant material was authenticated at the Department of Pharmaceutical Chemistry, National University of Pharmacy (Kharkiv, Ukraine), where voucher specimens were deposited. From each population, leaves were harvested from 5 to 8 individual plants. The leaves were manually separated from the stems, air-dried under shade at room temperature, thoroughly mixed, and used for further analyses.

For physicochemical and chromatographic analyses, 10 g of the dried leaves were taken and ground using a household electric grinder (Tube Mill 100 control, IKA®) to obtain a particle size of approximately 2–3 mm. Whole dried leaves were stored in paper bags, while the powdered material was used immediately for analytical purposes.

Reference standards including gallic acid, ellagic acid, chlorogenic acid, caffeic acid, oenothien B, hyperoside, isoquercitrin, avicularin, guaijaverin, rutin, quercitrin, and quercetin were obtained from Sigma-

Table 1Location and elevation of *Epilobium angustifolium* sampling sites.

Code	Country	Location	Altitude, m	Geographical coordinates	Voucher specimens
EA_1	Ukraine	Czarnohora massif, Rakhiv Forestry, Chornogirsk, Lypiv area	800	48.06568 N; 24.53255 E	OM2023-1501
EA_2	Ukraine	Carpathian mountains, Kvasy village	1180	48.15655 °N 24.33730 °E	OM2023-1502
EA_3	Ukraine	Shpilchina village, Lviv region	376	49.66174 N; 24.27277 E	OM2023-1503
EA_4	Ukraine	Krasnokutsk village, Kharkiv region	140	50.00831 °N 35.21047 °E	OM2023-1504
EA_5	Romania	Cavnic, Maramureş massif, mountain pass to Sighet	610	47.66518 N; 23.88702 E	OM2023-1505
EA_6	Lithuania	Kaišiadorys district, 1 km south of Gegužinė	78	54.99942 N; 24.50541 E	OM2023-1506
EA_7	Switzerland	Kriens, Lucerne region	490	47.03252 N; 8.28071 E	OM2023-1507

Aldrich (St. Louis, MO, USA). The Folin–Ciocalteu reagent was also supplied by Sigma-Aldrich. Ultrapure water was generated using a Milli-Q purification system (Millipore, Germany). HPLC-grade organic solvents (acetonitrile, methanol, glacial acetic acid) were purchased from Merck KGaA (Darmstadt, Germany) and Fisher Scientific (Loughborough, UK). HPTLC plates precoated with silica gel 60 F₂₅₄ were obtained from Merck KGaA. All other reagents were of analytical grade and used without further purification.

2.2. Physicochemical parameters

Seven batches of *Epilobium angustifolium* leaves were evaluated following the procedures described in the State Pharmacopoeia of Ukraine [17], applying general pharmacopoeial approaches for the assessment of herbal drugs. The physicochemical assessment included the determination of foreign matter (2.8.2), loss on drying (2.3.32), total ash (2.4.16), and acid-insoluble ash (2.8.1) using standard gravimetric techniques. All results were expressed as percentage values relative to the dry weight of the samples (% m/m).

Prior to analysis, the collected plant material, consisting primarily of the upper two-thirds of the aerial parts, was manually cleaned and visually inspected. Impurities were determined by manual separation according to pharmacopoeial definitions; coarse stems, flower fragments, and extraneous plant material were considered impurities for analytical purposes.

2.3. Preparation of plant extracts and HPTLC analysis

For analysis, 50 mg of powdered *Epilobium angustifolium* leaves were extracted with 5.0 mL of 50% (v/v) methanol using ultrasound-assisted extraction for 20 min at 40 °C [15]. The obtained extracts were filtered through a 0.45 µm membrane filter prior to chromatographic analysis.

HPTLC was performed using a CAMAG system comprising a Linomat 5 semi-automatic applicator, Automatic Developing Chamber 2 (ADC2), TLC Plate Heater III, and TLC Visualizer, all operated via visionCATS 3.1 software. Chromatographic separation was carried out on 20 × 10 cm HPTLC plates pre-coated with silica gel 60 F₂₅₄ (layer thickness 0.20

mm; Merck KGaA, Darmstadt, Germany). Sample solutions were applied as 8 mm bands using a 100 μ L CAMAG syringe under a gentle nitrogen flow, with the following parameters: 15 mm from the left edge, 10 mm from the lower edge, and 10 mm track spacing.

Plates were developed in a chamber pre-saturated for 20 min at room temperature with a mobile phase consisting of ethyl acetate–formic acid–water (68:8:8, v/v/v) to a migration distance of 70 mm. After development, the plates were air-dried and sequentially derivatised by immersion in Natural Product A reagent (2 g 2-aminoethyl diphenylborinate in 200 mL methanol) followed by PEG 400 reagent (10 g polyethylene glycol 400 in 200 mL dichloromethane) using a CAMAG immersion device. The treated plates were heated at 100 °C for 5 min and visualized under white light, UV 254 nm, and UV 366 nm using the CAMAG Visualizer. Chromatograms were documented and processed with visionCATS software.

2.4. Determination of total phenolics and flavonoids in *Epilobium angustifolium* leaves

The total phenolic and flavonoid contents of *Epilobium angustifolium* leaf extracts were assessed using spectrophotometric methods adapted from established protocols [18,19].

Total phenolics: 1 mL aliquot of the 50% (v/v) methanolic extracts of leaves were combined with 1 mL of Folin–Ciocalteu reagent and 9 mL of distilled water. After 5 min, 10 mL of a 7% Na₂CO₃ solution was added, and the volume was adjusted to 25 mL with distilled water. The reaction mixture was kept in the dark at room temperature for 90 min. The absorbance of the solution was measured at 750 nm using a UV–Vis spectrophotometer (Halo DB-20, Techcomp Europe, UK). The total phenolic compounds content was calculated based on a gallic acid calibration curve ($y = 0.9068x + 0.0617$, $R^2 = 0.9960$) and expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

Total flavonoids: Flavonoid content was determined following the European Pharmacopoeia procedure for *Crataegus* leaves and flowers [20], with slight modifications. About 0.4 g of powdered leaf material was extracted twice with 60% ethanol under heating (60 °C, 10 min each). The combined extracts were filtered and brought to 100 mL with 60% ethanol to prepare a stock solution. A 5 mL portion of this solution was evaporated to dryness, reconstituted in a methanol–glacial acetic acid mixture, and reacted with boric and oxalic acids in a formic acid medium. After 30 min, absorbance was recorded at 410 nm against a blank prepared in the same way without reagents. Total flavonoid content was expressed as hyperoside equivalents, calculated using a specific absorbance value of 405.

2.5. HPLC analysis of polyphenolic compounds

For HPLC analysis, 0.20 g of powdered *Epilobium angustifolium* leaf material was extracted with 50% (v/v) methanol at a 1:50 (w/v) ratio using an ultrasonic bath at 45 ± 2 °C for 20 min. After extraction, the mixture was allowed to stand, and the supernatant was collected. The extracts were then filtered through a 0.22 μ m syringe filter (Millex R, Merck, USA); the first portion of the filtrate was discarded, and the remaining filtrate was used for HPLC injection. Extraction procedures were conducted in triplicate ($n = 3$) for each sample.

Chromatographic separation was carried out using a Waters 2695 Alliance HPLC system equipped with a Waters 2998 photodiode array detector (Waters, Milford, MA, USA). Polyphenols were separated on an ACE Super C18 column (250 $\times$ 4.6 mm, 3 μ m; ACT, Aberdeen, UK) at 25 °C. A gradient elution program was applied with 0.1% trifluoroacetic acid in water (solvent A) and acetonitrile (solvent B): 0 min, 5% B; 8–30 min, 20% B; 30–48 min, 40% B; 48–58 min, 50% B; 58–65 min, 50% B; 65–66 min, 95% B; 66–70 min, 95% B; 70–81 min, 5% B. The flow rate was 1.0 mL/min, and injection volume was 10 μ L.

Peak identification was achieved by comparing retention times and UV–Vis spectra with reference standards. Each sample was analysed in

triplicate. The content of oenothien A was quantified using recalculation based on oenothien B standards. Method validation and quantification of polyphenolic compounds were performed using the external standard approach according to previously published protocols [19]. The results were adjusted to reflect values for absolutely dry raw material (DW).

All experimental data were processed using Microsoft Excel 2010 (Microsoft, USA) and Waters® Empower 3 software. Results are expressed as mean values $\pm$ standard deviation (SD) based on three independent measurements ($n = 3$) for each sample. Statistical significance was determined at the level of $p < 0.05$.

3. Results

3.1. Physicochemical and quantitative analysis

The results of the physicochemical and quantitative analysis of *Epilobium angustifolium* leaf samples from seven geographic zones are presented in Table 2. The content of foreign impurities in all samples complied with pharmacopoeial requirements and ranged from 1.20% $\pm$ 0.08% to 1.50% $\pm$ 0.11%, indicating high purity of the raw material (the harvest includes 2/3 of the plant top). Loss on drying values ranged from 6.81% $\pm$ 0.48% to 8.08% $\pm$ 0.57%, which are within the ranges generally considered acceptable for dried herbal materials. Total ash content ranged from 4.21% $\pm$ 0.29% to 6.03% $\pm$ 0.42%, while acid-insoluble ash content ranged from 0.15% $\pm$ 0.01% to 0.38% $\pm$ 0.03%, confirming low levels of mineral impurities and the absence of soil contamination.

Total phenolic content, expressed as gallic acid equivalents (GAE), varied moderately between the samples, ranging from 0.77 ± 0.05 to 1.44 ± 0.10 mg GAE/g dry weight, with the highest values observed in the Carpathian region (EA₁) and the lowest in the Lithuanian sample (EA₆). Similarly, the total flavonoid content, expressed as hyperoside equivalents, ranged from 0.19% $\pm$ 0.01% to 0.55% $\pm$ 0.04%. The Lithuanian sample (EA₆) demonstrated the highest flavonoid concentration, while the Carpathian sample (EA₂) had the lowest.

Overall, the physicochemical parameters of all batches were stable and fell within ranges considered acceptable according to general pharmacopoeial criteria, confirming the satisfactory quality of the raw materials. Variability in the total phenolic compound and flavonoid content across collection sites likely reflects the influence of environmental and climatic factors on the accumulation of secondary metabolites. These results establish baseline values for the standardisation of *Epilobium angustifolium* leaves and can serve as reference criteria for the development of a pharmacopoeial monograph.

3.2. HPTLC analysis

Qualitative HPTLC analysis of methanol extracts of *Epilobium angustifolium* leaves revealed distinct chromatographic profiles under various imaging modes (Fig. 1). Before derivatisation, chromatograms observed under UV 254 nm light showed several dark bands corresponding to phenolic acids and ellagitannins, while weakly fluorescent bands were observed under UV 366 nm light, indicating the presence of flavonoid glycosides.

After derivatisation using Natural Product A reagent and PEG 400, chromatograms under UV 366 nm light showed multiple bright yellow, orange, and green, fluorescent bands characteristic of flavonol derivatives such as hyperoside, isoquercitrin, quercitrin, and quercetin. Under white light, additional brownish and violet zones belonging to tannins and phenolic acids became visible.

The HPTLC profiles of all studied samples (EA₁–EA₇) demonstrated similar general patterns, confirming the constancy of the qualitative composition of phenolic compounds regardless of the geographical origin of the plant material. However, slight differences in the intensity and number of zones were noted, particularly in the regions corresponding to both oenothien and flavonoid glycosides, reflecting the

Table 2

Physicochemical parameters and total compounds content (mg/g DW; mean $\pm$ SD, standard deviation) in the raw material of the studied *Epilobium angustifolium* samples.

Sample	Foreign matter (% m/m)	Loss on drying (% m/m)	Total ash (% m/m)	Acid-insoluble ash (% m/m)	Total phenolic content, mg GAE/g DW	Total flavonoids (%)	Total flavonoids (mg HE/g DW)
EA_1	1.30 $\pm$ 0.09	6.81 $\pm$ 0.48	4.21 $\pm$ 0.29	0.31 $\pm$ 0.02	1.44 $\pm$ 0.10	0.24 $\pm$ 0.02	2.4 $\pm$ 0.02
EA_2	1.50 $\pm$ 0.11	7.56 $\pm$ 0.53	5.04 $\pm$ 0.35	0.38 $\pm$ 0.03	1.14 $\pm$ 0.08	0.19 $\pm$ 0.01	1.9 $\pm$ 0.01
EA_3	1.40 $\pm$ 0.10	7.34 $\pm$ 0.51	5.62 $\pm$ 0.39	0.25 $\pm$ 0.02	0.85 $\pm$ 0.06	0.25 $\pm$ 0.02	2.5 $\pm$ 0.02
EA_4	1.38 $\pm$ 0.10	7.58 $\pm$ 0.50	5.15 $\pm$ 0.30	0.33 $\pm$ 0.03	1.08 $\pm$ 0.08	0.21 $\pm$ 0.01	2.1 $\pm$ 0.01
EA_5	1.20 $\pm$ 0.08	7.04 $\pm$ 0.49	4.74 $\pm$ 0.33	0.15 $\pm$ 0.01	1.01 $\pm$ 0.07	0.27 $\pm$ 0.02	2.7 $\pm$ 0.02
EA_6	1.40 $\pm$ 0.10	7.00 $\pm$ 0.49	4.82 $\pm$ 0.34	0.27 $\pm$ 0.02	0.77 $\pm$ 0.05	0.55 $\pm$ 0.04	5.5 $\pm$ 0.04
EA_7	1.30 $\pm$ 0.09	8.08 $\pm$ 0.57	6.03 $\pm$ 0.42	0.32 $\pm$ 0.02	0.98 $\pm$ 0.07	0.26 $\pm$ 0.02	2.6 $\pm$ 0.02
Average value	1.35 $\pm$ 0.09	7.30 $\pm$ 0.51	5.08 $\pm$ 0.36	0.28 $\pm$ 0.02	1.03 $\pm$ 0.07	0.29 $\pm$ 0.02	2.9 $\pm$ 0.02

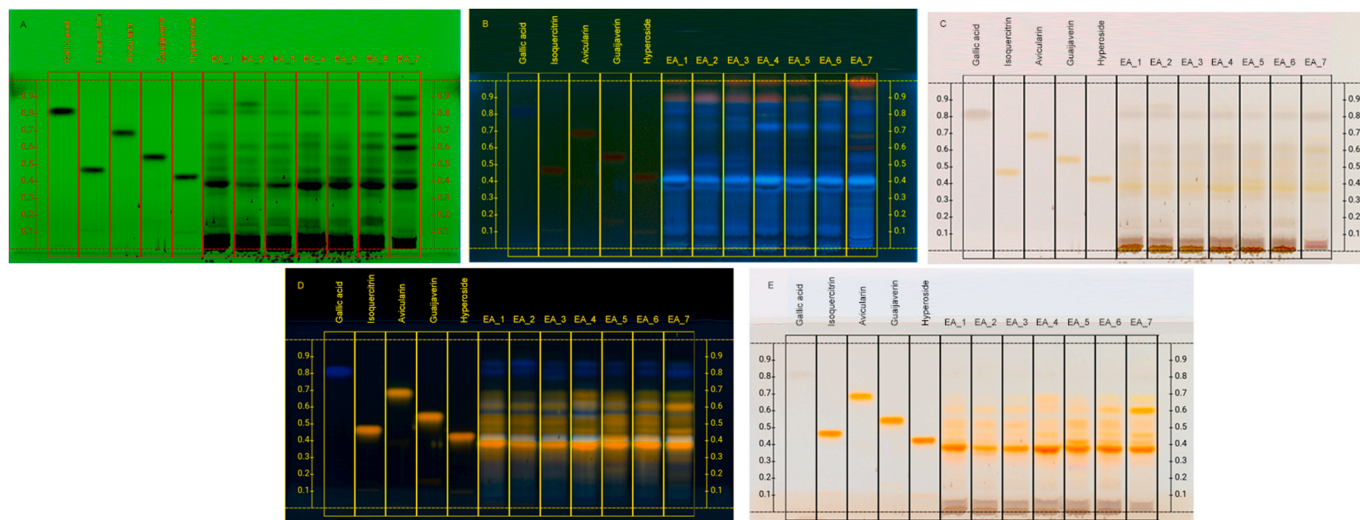


Fig. 1. HPTLC profile of *Epilobium angustifolium* extracts under UV 254 nm (A), UV 366 nm (B) and white light (C) prior to derivatisation, and under UV 366 nm after derivatisation (D), and white light after derivatisation (E).

variability of the quantitative content determined by HPLC.

The developed chromatographic system, such as ethyl acetate: formic acid: water (68:8:8, v/v/v) was effective for the simultaneous separation of the main phenolic components of *Epilobium angustifolium*. Thus, the obtained HPTLC fingerprints can serve as specific raw material identification profiles, complementing the quantitative assessment by HPLC and providing a fast and reliable tool for quality control and standardisation in accordance with pharmacopoeial requirements.

3.3. HPLC analysis

The HPLC-DAD analysis enabled the separation and identification of thirteen major phenolic components in methanolic extracts of fireweed leaves by comparison of retention times and UV-Vis spectra with those of reference standards (Fig. 2). Compounds were identified based on retention times and UV spectra compared to standards, including gallic acid, neochlorogenic acid, chlorogenic acid, ellagic acid, oenotherins A and B, and several flavonoid glycosides such as isomyricitrin, hyperoside, isoquercitrin, guajaverin, quercitrin, and quercetin.

Of the detected compounds, oenotherin B was the most abundant in all samples (Table 3), with concentrations ranging from 24.85 $\pm$ 1.74 mg/g (EA₂) to 60.61 $\pm$ 4.24 mg/g (EA₆), confirming its role as a key chemical marker for the species. Oenotherin A content varied significantly from 0.61 $\pm$ 0.04 to 5.83 $\pm$ 0.41 mg/g, with the highest values in the Romanian and Swiss samples (EA₅, EA₇). These results are consistent with literature data indicating the influence of environmental conditions and altitude on ellagitannin biosynthesis.

Flavonoid glycosides were also well represented, with isomyricitrin

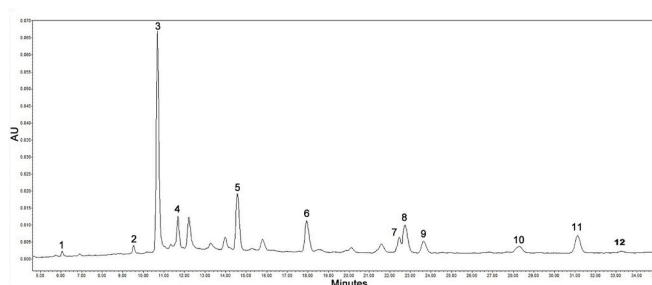


Fig. 2. The HPLC-DAD chromatograms of *Epilobium angustifolium* (EA₁ sample) leaves at 219 nm: gallic acid (1); neochlorogenic acid (2); oenotherin B (3); oenotherin A (4); chlorogenic acid (5); isomyricitrin (6); ellagic acid (7); rutin (8); hyperoside (9); isoquercitrin (10); guajaverin (11); quercitrin (12); quercetin (13).

(17.82–32.14 mg/g) and quercitrin (4.77–10.49 mg/g) being the most abundant. Other flavonoids, such as hyperoside, isoquercitrin, and guajaverin, were present at lower concentrations (0.46–3.40 mg/g). The major phenolic acids detected were chlorogenic acid (0.47–2.20 mg/g) and gallic acid (0.19–0.56 mg/g). Neochlorogenic acid was present at very low levels (0.02–0.08 mg/g), while rutin occurred at lower concentrations compared to the major phenolic acids, ranging from 0.20 to 0.28 mg/g.

HPLC analysis revealed a characteristic and reproducible profile across all *Epilobium* batches, dominated by ellagitannins and flavonol glycosides. Despite quantitative differences between populations, the

Table 3Content of phenolic compounds (mg/g DW; mean $\pm$ SD, standard deviation) in *Epilobium angustifolium* samples by HPLC method.

N ^a	Compound	RT	EA_1	EA_2	EA_3	EA_4	EA_5	EA_6	EA_7
1	Gallic acid	6.06	0.40 $\pm$ 0.03	0.49 $\pm$ 0.03	0.30 $\pm$ 0.02	0.56 $\pm$ 0.04	0.37 $\pm$ 0.03	0.23 $\pm$ 0.02	0.19 $\pm$ 0.01
2	Neochlorogenic acid	9.53	0.04 $\pm$ 0.00	0.08 $\pm$ 0.01	0.04 $\pm$ 0.00	0.03 $\pm$ 0.00	0.03 $\pm$ 0.00	0.04 $\pm$ 0.00	0.02 $\pm$ 0.00
3	Oenothien B	10.66	44.79 $\pm$ 3.14	24.85 $\pm$ 1.74	41.28 $\pm$ 2.89	39.71 $\pm$ 2.54	38.33 $\pm$ 2.68	60.61 $\pm$ 4.24	46.09 $\pm$ 3.23
4	Oenothien A	13.04	2.10 $\pm$ 0.15	0.61 $\pm$ 0.04	1.81 $\pm$ 0.13	1.02 $\pm$ 0.10	5.83 $\pm$ 0.41	2.69 $\pm$ 0.19	5.55 $\pm$ 0.39
5	Chlorogenic acid	14.35	1.46 $\pm$ 0.10	0.47 $\pm$ 0.03	2.20 $\pm$ 0.15	1.30 $\pm$ 0.12	1.25 $\pm$ 0.09	1.88 $\pm$ 0.13	1.59 $\pm$ 0.11
6	Isomyricitrin	18.51	21.15 $\pm$ 1.48	18.43 $\pm$ 1.29	23.12 $\pm$ 1.62	20.12 $\pm$ 1.23	17.82 $\pm$ 1.25	32.14 $\pm$ 2.25	22.21 $\pm$ 1.55
7	Ellagic acid	22.72	1.53 $\pm$ 0.11	0.36 $\pm$ 0.03	2.24 $\pm$ 0.16	1.92 $\pm$ 0.15	1.57 $\pm$ 0.11	1.99 $\pm$ 0.14	1.69 $\pm$ 0.12
8	Rutin	22.80	0.28 $\pm$ 0.02	0.27 $\pm$ 0.02	0.23 $\pm$ 0.02	0.22 $\pm$ 0.02	0.25 $\pm$ 0.02	0.23 $\pm$ 0.02	0.20 $\pm$ 0.01
9	Hyperoside	23.65	2.55 $\pm$ 0.18	0.52 $\pm$ 0.04	1.93 $\pm$ 0.14	0.79 $\pm$ 0.05	0.76 $\pm$ 0.05	1.56 $\pm$ 0.11	1.32 $\pm$ 0.09
10	Isoquercitrin	24.90	2.27 $\pm$ 0.16	0.46 $\pm$ 0.03	1.71 $\pm$ 0.12	0.70 $\pm$ 0.10	0.68 $\pm$ 0.05	1.39 $\pm$ 0.10	1.17 $\pm$ 0.08
11	Guaijaverin	27.82	0.17 $\pm$ 0.01	0.74 $\pm$ 0.05	3.40 $\pm$ 0.24	1.49 $\pm$ 0.15	0.93 $\pm$ 0.07	1.36 $\pm$ 0.10	1.35 $\pm$ 0.09
12	Quercitrin	31.11	6.45 $\pm$ 0.45	4.77 $\pm$ 0.33	10.49 $\pm$ 0.73	7.80 $\pm$ 0.50	7.41 $\pm$ 0.52	7.88 $\pm$ 0.55	4.85 $\pm$ 0.34
13	Quercetin	33.13	0.01 $\pm$ 0.00	0.96 $\pm$ 0.07	2.41 $\pm$ 0.17	1.33 $\pm$ 0.10	0.88 $\pm$ 0.06	1.27 $\pm$ 0.09	1.16 $\pm$ 0.08

^a The compound number in Table 3 corresponds to the peak number of the compound in Fig. 2.

qualitative composition remained stable, confirming the chemical integrity of *Epilobium angustifolium* leaves collected from various habitats. The predominance of oenothiens and quercetin derivatives confirms their suitability as diagnostic markers for standardisation and pharmacopoeial identification of the species.

4. Discussion

Epilobium angustifolium 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 [16], so this study focuses on its chemical composition.

The results of this study provide data on the physicochemical and phytochemical characteristics of *Epilobium angustifolium* leaves collected in different geographic regions. The physicochemical parameters of all samples were consistent with general requirements of the European and Ukrainian Pharmacopoeias for plant material and can serve as a basis for developing standardisation criteria. Low levels of foreign impurities (<1.5%) and acid-insoluble ash (~0.38%) indicate appropriate collection and processing methods, while the homogeneity of total ash values (~6%) suggests a consistent mineral composition across the samples. These results are also consistent with available literature data for *Epilobium angustifolium* and related species compound content [21–23]. The proposed physicochemical limits are comparable to those established for other tannin-rich herbal drugs in European pharmacopoeial practice (e. g., *Tormentillae* rhizoma 01/2024:1478; *Agrimoniae* herba 01/2011:1587; *Hamamelidis* folium 01/2016:2532, Ph. Eur. 11th Ed), supporting the feasibility of incorporating *Epilobium angustifolium* into a harmonised regulatory framework. 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 8% (1000 g of powdered material dried at 105 °C for 2 h); total ash not exceeding 6%; and HCl-insoluble ash not exceeding 0.5%.

Qualitative analysis and the HPTLC profiles of all seven *Epilobium angustifolium* samples showed a typical pattern of fluorescent showed a typical pattern of fluorescent band colouration characteristic of flavonoid glycosides (quercetin derivatives) and phenolic acids at UV 366 nm after derivatisation provides an effective means of visual identification. The main zones ($R_f \approx 0.65$, ≈ 0.50 , ≈ 0.45 , ≈ 0.40) were present in all samples, indicating the consistent presence of these compounds in the plant material. Similar HPTLC profiles were described by Pirvu et al. [24] and Granica et al. [25], demonstrating the suitability of this chromatographic approach for the authentication of *Epilobium angustifolium* leaves. The intensity of the zones varied among the samples, such 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 *Epilobium angustifolium* [19]. The reproducibility of the HPTLC fingerprint across batches indicates its suitability as a routine identification method in quality control laboratories, particularly where rapid authentication of herbal raw materials is required [26].

The polyphenolic profile of *Epilobium angustifolium* is known for its high content of tannins and flavonoid compounds, particularly ellagitannins (oenothien A and B) and flavonols (e.g., hyperoside, isoquercitrin, quercetin). Quantitative analysis revealed moderate variability in the content of total phenolic compounds (0.77–1.44 mg GAE/g DW) and flavonoids (0.19–0.55%), which may be related to environmental and geographic factors influencing the biosynthesis of secondary metabolites. The quantitative data obtained for total phenolic content in tested *Epilobium angustifolium* samples are in agreement with previously published results for related species such as *Epilobium hirsutum* leaves [27,28], *Epilobium parviflorum* [3,29] with phenolic compound levels ranging from 0.9 to 1.8 mg GAE/g. Similar interpopulation differences were previously described by Monschein et al. [30], who found that altitude and sunlight exposure significantly influenced the accumulation of ellagitannins and flavonoids in *Epilobium angustifolium*. Baert et al. [31] and Maruška et al. [32] also noted strong environmental modulation of phenolic profiles in wild *Epilobium angustifolium* populations, confirming our findings of regional variation.

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 [33]. For example, in the case of large-structure compounds (ellagitannins, particularly oenothien B), they may react weakly, partially precipitate, or exhibit low reactivity and reduced quantitative values of high-molecular-weight polyphenols, which predominate in *Epilobium angustifolium*. 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 [34]. This also allows for comparison of our own data with those of other authors [22,29,33,35]. This method provides a value for the total reducing power of phenolic compounds and complements the results of the HPLC method.

HPLC profiling confirmed that the dominant components of *Epilobium angustifolium* leaves are ellagitannins, primarily oenothien B (24.8–60.6 mg/g), and flavonol glycosides such as isomyricitrin, quercitrin, and hyperoside. These results are in good agreement with the data published by Granica et al. [25], who developed and validated an HPLC-DAD-MS method for the quantitative determination of oenothien B, and with the data of Agnieszka et al. [36], who described similar phenolic profiles in *Epilobium angustifolium* at different harvest times. The oenothien B to oenothien A ratio observed in our samples (approximately 8:1) is consistent with that described by Ref. [30],

indicating that oenothien B is the most reliable marker for standardisation. Its predominance and quantitative stability across samples further support its suitability as a primary marker for standardisation purposes [37]. The observed differences between samples EA₁–EA₇ 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 [37], which highlight the significant influence of environmental and technological factors on the flavonoid content of *Epilobium* species. In this study, samples from Romania, Lithuania, and Switzerland were included as neighboring countries to demonstrate differences in compound content depending on the country of origin.

The results further confirm that oenothien B can serve as a marker compound for the standardisation of *Epilobium angustifolium* raw materials, while hyperoside, isomyricitrin, 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. Unlike previous studies, which primarily focused on phytochemical variability, this study combines marker-based quantitative assessment with pharmacopoeial physicochemical criteria. The combination of physicochemical data, spectrophotometric quantification, and chromatographic fingerprinting provides a robust basis for developing a pharmacopoeial monograph on *Epilobium angustifolium* and ensuring the reproducibility of herbal preparations based on this species. This presented approach to quality control of herbal raw materials can be adapted to the harmonised standardisation of *Epilobium angustifolium* as a medicinal herbal raw material.

5. Limitations

The aim of this study was to evaluate the feasibility of chemical standardisation methods for *Epilobium angustifolium* leaves, the results of which could be used to develop a monograph for this plant. However, several limitations should be noted.

First, although representative lots from different countries were included in the study, the number of populations analysed ($n = 7$) does not allow for a full assessment of intra- and interannual variability. Seasonal dynamics and phenological stage were not systematically examined in this study. However, the authors have conducted such studies previously [15,19].

Secondly, although the major ellagitannins and flavonoid glycosides were individually quantified using HPLC, the total hydrolysable tannin content was not defined as a separate pharmacopoeial parameter, which may be important for the regulatory standardisation of tannin-rich herbal medicines. This should be explored in the future.

Third, advanced metabolomic approaches (e.g., LC-MS-based profiling) and multivariate chemometric analysis, which could provide a deeper understanding of potential geographic differences and the robustness of marker selection, were not applied.

Therefore, future studies should expand the sample to cover multiple harvest seasons and years, include chemometric evaluation, and evaluate additional pharmacopoeial parameters to further strengthen the development of a consensus monograph on *Epilobium angustifolium* leaves.

6. Conclusion

This study established reproducible and harmonised quality standards for *Epilobium angustifolium* (fireweed) leaves from different geographical regions, integrating physicochemical, spectrophotometric, and chromatographic data. All samples complied with physicochemical criteria adapted from the European and Ukrainian Pharmacopoeias, confirming the high quality and purity of the raw materials. Key phenolic compounds, including ellagitannins (oenothien A and B) and flavonol glycosides (e.g., isomyricitrin, hyperoside, and quercitrin), were identified consistently, while moderate variability in total phenolic

and flavonoid contents reflected environmental influences. The resulting chromatographic fingerprints provide reliable tools for species authentication and raw material standardisation, supporting the development of a pharmacopoeial monograph. These findings highlight the pharmaceutical potential of *Epilobium angustifolium* and contribute to the consistent production of safe and effective herbal preparations.

CRedit authorship contribution statement

Kateryna Uminska: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Victoriya Georgiyants:** Writing – review & editing, Resources. **Iryna Drapak:** Investigation. **Liudas Ivanauskas:** Resources, Investigation. **Olha Mykhailenko:** Writing – review & editing, Supervision, Resources, Investigation, Conceptualization.

Ethics approval

All plant materials used were collected in accordance with relevant institutional, national, and international guidelines and regulations. No endangered or protected species were involved. The authors declare that ethical standards were upheld throughout the research process.

Declaration of generative AI in scientific writing

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

Funding information

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The manuscript has no associated data.

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