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Chemotypic Plasticity of *Potentilla erecta* (L.) Raeusch. Across Elevational Gradients in the Ukrainian Carpathians

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Abstract

Potentilla erecta (L.) Raeusch. is a widely used medicinal species valued for its astringent, anti-inflammatory, and antimicrobial effects. This study examined the variation in hydrolysable tannin and flavonoid content in rhizomes of wild-growing populations collected along an elevational gradient in the Ukrainian Carpathians (180–2020 m above sea level). Rhizomes from fifteen populations were analyzed using pharmacopoeial methods, including thin-layer chromatography for tannins and spectrophotometry for flavonoids. Tannin levels ranged from 15.57% to 31.82%, while flavonoid contents varied between 0.23% and 0.40%, expressed as a percentage of dry weight. Both metabolites showed a strong positive correlation with altitude (r = 0.88 for tannins; r = 0.84 for flavonoids), indicating a clear influence of elevation on their accumulation. The highest concentrations were consistently found in high-mountain populations. These results suggest that environmental factors associated with increasing elevation, such as reduced temperature and enhanced ultraviolet radiation, play a significant role in shaping the phytochemical profile of *P. erecta*. The study contributes to the understanding of altitudinal effects on secondary metabolite accumulation in mountain plants and provides a basis for further ecological and pharmacological-oriented research related to this species.

Keywords: Carpathian Mountains; mountain ecosystems; altitudinal gradient; medicinal plants; phytochemical variation; chemotype selection

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1. Introduction

Medicinal plants adapted to mountainous environments often exhibit pronounced biochemical diversity as a response to the harsh and variable ecological conditions typical

of high altitudes, including low temperatures, increased ultraviolet radiation, and limited nutrient availability. These environmental pressures influence the biosynthesis and accumulation of secondary metabolites, particularly phenolic compounds such as flavonoids and tannins, which are widely recognized for their antioxidant, antimicrobial, and anti-inflammatory properties [1,2]. Consequently, mountain habitats are considered hotspots for the discovery of chemically rich plant populations with potential pharmacological value [3,4].

Potentilla erecta (L.) Raeusch. is a perennial herbaceous species in the Rosaceae family, widely distributed across Europe and western Asia. It typically grows in acidic soils of meadows, moorlands, and subalpine ecosystems [5,6]. Traditionally, it has been used for its astringent, antimicrobial, and anti-inflammatory properties, and its rhizomes are officially recognized in the national pharmacopeias of several countries, including Ukraine and the European Union [7,8]. Numerous pharmacological studies have confirmed these effects, linking them to the presence of hydrolysable tannins and flavonoids, particularly catechins and hyperoside [9–14]. Additionally, phenolic acids found in the rhizomes of *P. erecta* highlight its pharmacological complexity [15].

While several studies have reported that altitude can significantly influence the accumulation of phenolic compounds in other medicinal plants [16–18], the altitudinal effects on wild populations of *P. erecta* have not yet been systematically assessed. With its widespread presence across various elevations in the Carpathian Mountains and the growing interest in its pharmacological applications, examining this species' biochemical variation along an altitudinal gradient is both timely and relevant.

This study aimed to investigate the impact of altitude on the accumulation of tannins and flavonoids in the rhizomes of wild *P. erecta* populations across the Ukrainian Carpathians. Understanding the variation in phytochemicals along environmental gradients is crucial, as these compounds are directly linked to the medicinal efficacy of plants. Moreover, identifying high-yielding chemotypes could support the establishment of cultivation systems that reduce pressure on wild populations [19]. Such insights are becoming increasingly relevant as climate variability and overharvesting continue to impact alpine medicinal flora worldwide. In particular, understanding how secondary metabolite profiles vary along ecological gradients contributes to the chemotaxonomic classification and ecological specialization of species.

2. Materials and Methods

2.1. Study Area and Plant Collection

Fifteen natural populations of *P. erecta* were sampled in the Ukrainian Carpathians in July 2023, spanning a broad elevational gradient from 180 to 2020 m above sea level. The study sites encompassed a variety of habitats, including colline foothills, montane meadows, upper montane forests, and exposed subalpine–alpine summits, each differing in edaphic and climatic characteristics. The geographical data of the sites (altitude, latitude, longitude) are summarized in Table 1.

From each population, five mature individuals were randomly selected, ensuring a minimum distance of 5 m between them to prevent clonal repetitions. Entire rhizomes were collected manually using stainless-steel tools, washed with distilled water, and initially air-dried in a shaded, ventilated environment at ambient temperature. Further dehydration was performed at 50 °C using a laboratory-grade dehydrator. We ground the dried rhizomes into a fine powder and stored them in sealed, light-proof containers for analysis. Voucher specimens were placed at the Herbarium of Uzhhorod National University.

Table 1. *Potentilla erecta* (L.) Raeusch. sampling sites in the Ukrainian Carpathians, within the Tysa River basin.

No.	Sampling Site (Locality)	Altitude (m)	Latitude (°)	Longitude (°)
1.	Ostra wildlife sanctuary, Kamianytsia, Uzhhorod Rayon	250	49.1479	22.6582
2.	Offshoot slopes of Synatoriya Range between Perechyn and Simer	180	49.2210	22.8547
3.	Mt Antalovetska Poliana (Synatoriya Range)	968	49.1256	22.8235
4.	Mt Lutianska Holytsia (western part of Polonyna Range)	1375	49.4219	23.2694
5.	Mt Polonyna Runa (western part of Polonyna Range)	1479	49.3410	23.3519
6.	Environs of Uzhok Village, Uzhhorod Rayon	600	49.6392	23.4367
7.	Mt Pikui (West Beskydy Range)	1408	49.3834	23.0024
8.	Mt Stoi (Borzhava Polonynas Range)	1681	49.0358	23.3192
9.	Mt Darvaika (Pishkonia Range)	1502	48.8074	24.2876
10.	Mt Topas (Krasna Range)	1548	48.6383	24.2481
11.	Mt Syhliansky (Krasna Range)	1563	48.6085	24.3273
12.	Mt Tempa (western part of Svydovets Range)	1634	48.4581	24.0776
13.	Mt Blyznytsi (eastern part of Svydovets Range)	1883	48.3733	24.385
14.	Mt Sheshul (western part of Chornohora Range)	1726	48.2593	24.6174
15.	Mt Petros (Chornohora Range)	2020	48.2883	24.7043

Note: Altitudes may vary by ±10 m due to summit terrain disturbance at tourist sites.

2.2. Determination of Tannin Content

The total tannin content was assessed using the procedure described in the State Pharmacopoeia of Ukraine (2014). A 0.5 g powdered rhizome sample was extracted with 50% ethanol using ultrasonic-assisted extraction (30 min, 40 °C). Catechin served as the pharmacopoeial reference standard. Test and standard solutions were applied to silica gel TLC plates and developed in a solvent system comprising ethyl acetate, formic acid, acetic acid, and water (100:11:11:26 v/v). Tannins were visualized using a 1% ferric chloride solution, and quantitative evaluation was performed densitometrically at 550 nm using a Camag TLC Scanner 3. The results were calculated as a percentage of dry weight (%DW). The presence of catechin was confirmed by TLC, showing intense reddish-brown zones at matching Rf values in both reference and sample solutions.

2.3. Determination of Flavonoid Content

Flavonoid content was determined spectrophotometrically using the aluminum chloride colorimetric assay, as per the methodology adopted from the State Pharmacopoeia of Ukraine (2014). A 10.0 mL aliquot of the stock extract was diluted to 25.0 mL with 5% (v/v) glacial acetic acid in methanol (compensation solution). Absorbance was measured

after 30 min at 425 nm against the blank. The concentration of flavonoids, expressed as a percentage of dry weight (%DW), was calculated using the following formula:

$$C = \frac{A \cdot 1.25}{m \cdot 500}$$

where *C*—flavonoid content (%DW); *A*—absorbance of the test solution at 425 nm; *m*—weight of the test sample (g); 1.25—correction factor for dilution; 500—specific absorption coefficient of hyperoside ($E^1\%_1$ cm).

2.4. Statistical Analysis

All statistical analyses were conducted using R software (version 4.2.2; R Core Team, 2023). The relationship between elevation and the levels of tannins and flavonoids was assessed using one-way analysis of variance (ANOVA) and correlation analysis. Results were considered statistically significant at $p < 0.05$. Values are presented as mean ± standard deviation (SD). For each population, the coefficient of variation (CV = SD/mean × 100) was also calculated to characterize relative variability.

3. Results

3.1. Tanin Content

The total tannin content in the rhizomes of *P. erecta* varied notably among the studied populations (Table 2). Quantified as a percentage of dry weight (% DW) using catechin as the pharmacopoeial reference standard, the values ranged from 15.57% in population No. 1 (Ostra, 250 m a.s.l.) to 31.82% in population No. 13 (Mt Blyznytsi, 1883 m a.s.l.). The coefficient of variation (CV) ranged from 3.55% to 10.50%, consistent with the expected diversity in a species that reproduces apomictically and vegetatively via rhizomes.

Table 2. Total tannin content (catechin equivalents, %) in rhizomes of *Potentilla erecta* (L.) Raeusch. from 15 populations in the Ukrainian Carpathians.

Population *	Mean ± SD (%)	CV (%)
1	15.57 ± 1.16	7.45
2	17.05 ± 1.79	10.50
3	20.71 ± 1.54	7.44
4	18.05 ± 1.22	6.76
5	22.70 ± 1.75	7.71
6	19.98 ± 0.87	4.35
7	25.16 ± 1.18	4.69
8	28.00 ± 1.07	3.82
9	24.82 ± 1.51	6.08
10	24.31 ± 1.62	6.66
11	25.86 ± 1.19	4.60
12	29.55 ± 1.26	4.26
13	31.82 ± 1.13	3.55
14	29.48 ± 1.79	6.07
15	30.79 ± 1.77	5.75

* Locality numbers correspond to those in Table 1.

3.2. Total Flavonoid Content

The total flavonoid content, measured using hyperoside as a reference and expressed as a percentage of dry weight (%DW), is shown in Table 3. The recorded values ranged from 0.23% in population No. 1 (Ostra, 250 m a.s.l) to 0.40% in population No. 15 (Mt. Petros, 2020 m a.s.l.). The coefficient of variation (CV) ranged between 4.10% and 9.20%, closely reflecting the pattern observed for tannins. This variation likely reflects limited gene flow and local ecological adaptation. Further experimental studies are needed to distinguish the environmental and genetic components of this variation.

Table 3. Total flavonoid content (hyperoside equivalents, %) in rhizomes of *Potentilla erecta* (L.) Raeusch. from 15 populations in the Ukrainian Carpathians.

Population *	Mean ± SD (%)	CV (%)
1	0.23 ± 0.016	6.96
2	0.25 ± 0.023	9.20
3	0.34 ± 0.024	7.06
4	0.27 ± 0.016	5.93
5	0.33 ± 0.021	6.36
6	0.31 ± 0.016	5.16
7	0.32 ± 0.021	6.56
8	0.35 ± 0.016	4.57
9	0.34 ± 0.016	4.71
10	0.33 ± 0.021	6.36
11	0.32 ± 0.016	5.00
12	0.33 ± 0.024	7.27
13	0.39 ± 0.016	4.10
14	0.39 ± 0.024	6.15
15	0.40 ± 0.021	5.25

* Locality numbers correspond to those in Table 1.

3.3. Relationship with Altitude

Tables 1 and 2 suggest a positive trend between elevation and the accumulation of both tannins and flavonoids. To evaluate this relationship, correlation and regression analyses were performed. The findings are illustrated in Figures 1–3.

3.4. Statistical Interpretation

A strong and positive correlation was found between altitude and tannin content, with a Pearson correlation coefficient of r = 0.88, as illustrated in Figure 1. Similarly, the relationship between altitude and total flavonoid content yielded a coefficient of r = 0.84 (Figure 2). These values confirm a clear tendency for higher elevations to be associated with increased levels of secondary metabolites in the rhizomes of *P. erecta*.

A three-dimensional scatter plot (Figure 3) displaying tannin content, flavonoid content, and altitude jointly further reinforces this pattern. The highest phytochemical concentrations were consistently recorded in the high-elevation populations (Nos. 13–15), suggesting a cumulative effect of elevation-related stress factors on metabolite accumulation.

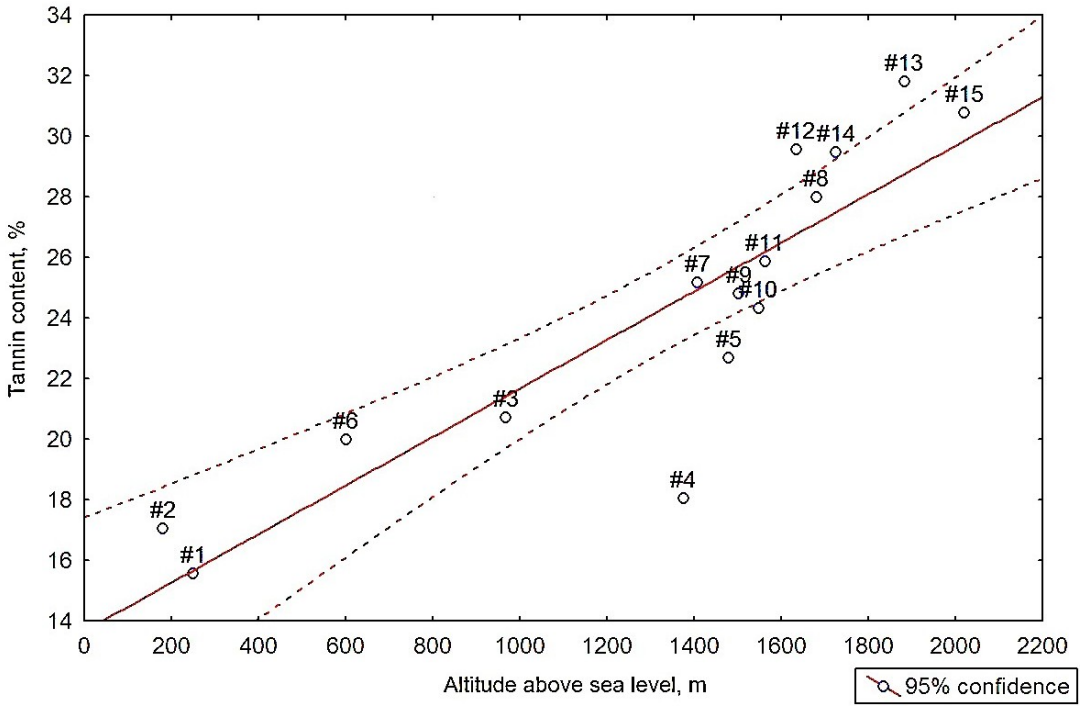


Figure 1. Correlation between altitude and total tannin content in rhizomes of *Potentilla erecta* (L.) Raeusch. Linear regression equation: y = 0.01x + 12.3, R^2 = 0.88. The population numbers correspond to sampling sites listed in Table 1.

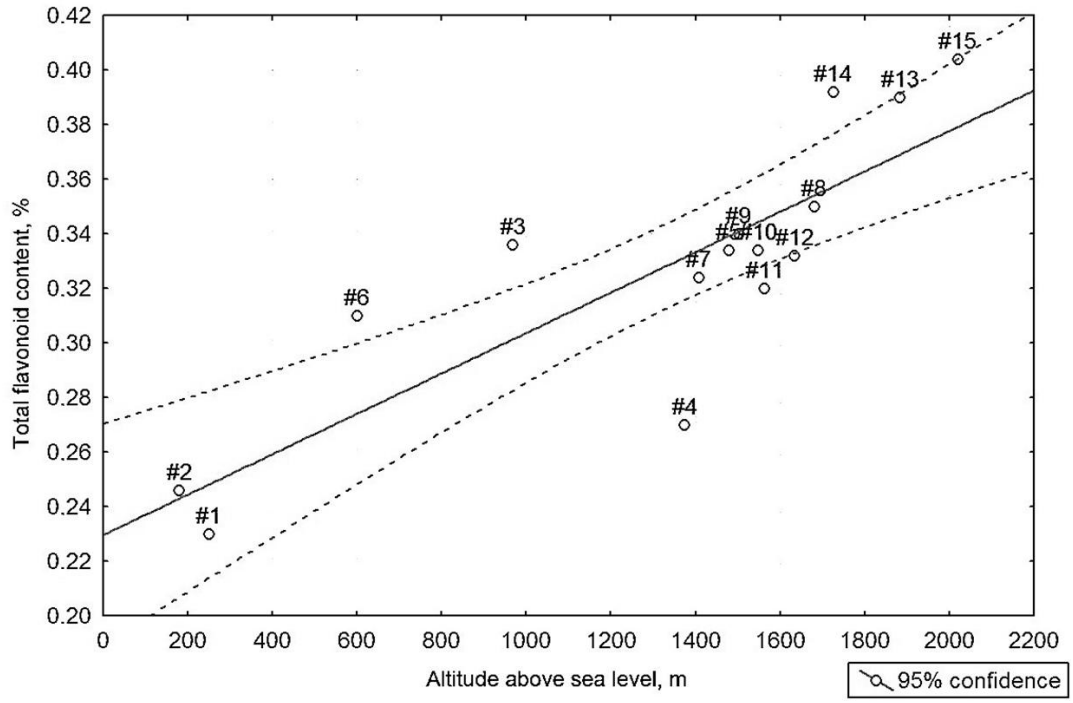


Figure 2. Correlation between altitude and total flavonoid content in rhizomes of *Potentilla erecta* (L.) Raeusch. Linear regression equation: y = 0.0001x + 0.2043, R^2 = 0.84. The numbers on the plot correspond to the population IDs listed in Table 1.

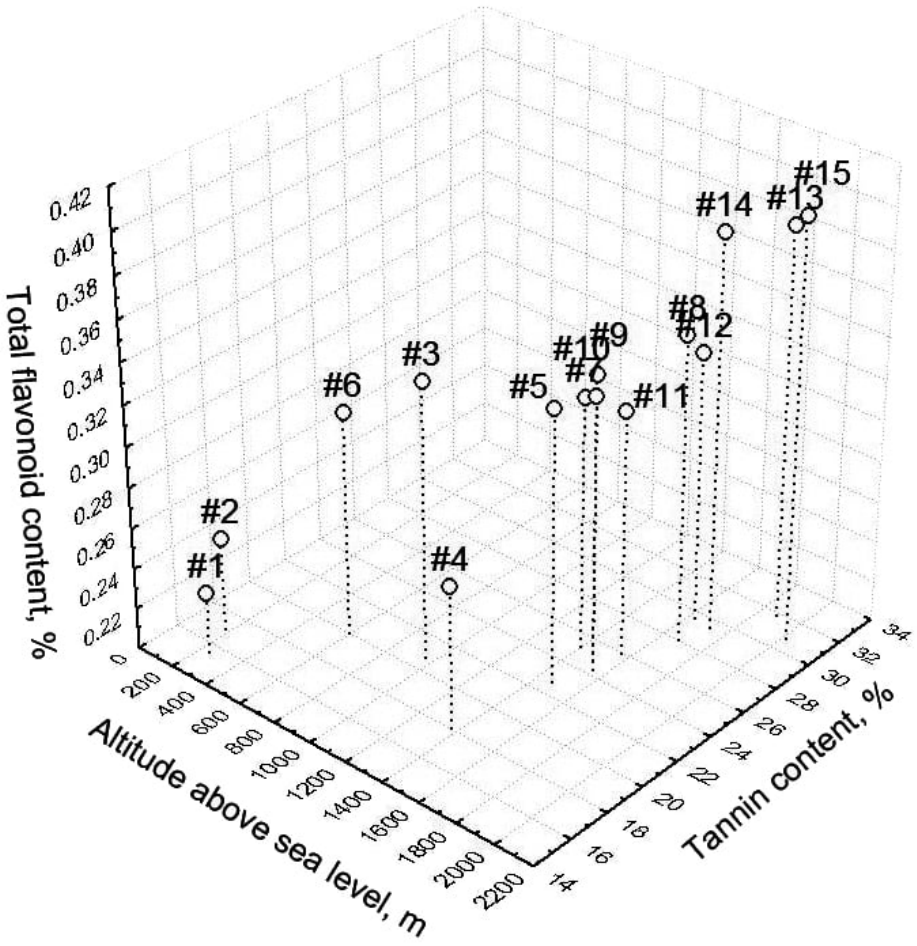


Figure 3. Three-dimensional scatter plot showing the relationship between altitude, total tannin content, and total flavonoid content in rhizomes of *Potentilla erecta* (L.) Raeusch. The point numbers correspond to the sample locations.

4. Discussion

Our results indicate that altitude affects the levels of flavonoids and tannins in *P. erecta* rhizomes. At higher sites, plants tended to accumulate more of these compounds. This observation agrees with earlier reports that stronger UV radiation, colder temperatures, and oxidative stress can stimulate phenolic biosynthesis [20]. In *P. erecta*, hyperoside and catechins are among the main flavonoids [21–24], and their changes along altitudinal gradients support the view that elevation is an important factor in biochemical differentiation. Similar effects have been described in other medicinal plants, for example, in *Hypericum* [16,17,25], *Epilobium angustifolium* [18], *Spiraea* spp. [26], and *Erythroxylum suberosum* [27].

Our results can be compared with those from Synowiec et al. (2014), who reported tannin contents of 17–22% in rhizomes collected at lowland sites in Poland (122 m a.s.l.) [28]. These values align with the lower end of our range, while high-mountain populations in our study (above 1600 m) reached up to 31.82%. This indicates that the unusually high tannin levels we observed are likely related to elevational stress conditions. In addition to tannins and flavonoids, *P. erecta* rhizomes are also rich in gallic and p-hydroxybenzoic acids, as well as chlorogenic and protocatechuic acids, which boost their antioxidant properties [15]. These compounds support our findings by confirming that *P. erecta* contains a wide array of pharmacologically relevant phenolics.

The high metabolite levels in rhizomes from elevated sites indicate that these populations could represent superior chemotypes with notable pharmacological potential. The

systematic identification of wild chemotypes is gaining importance in medicinal plant research and cultivation [29–32]. The broad ecological range of *P. erecta* and its presence in varied plant communities point to ecological plasticity and suggest potential for domestication. This variability, repeatedly documented across environments [33], may reflect underlying genetic differentiation, consistent with evolutionary studies in the genus *Potentilla* [34]. Mountain ecosystems, characterized by steep gradients and population isolation, are widely recognized as centers of genetic diversity and unique biochemical traits [35].

Taken together, our results indicate that wild populations of *P. erecta*, particularly those growing at higher altitudes, may provide valuable resources for developing phytomedicinal products. Protecting these populations and investigating their potential for cultivation should be a priority to secure both pharmacological benefits and the sustainable use of mountain medicinal plants. The observed increase in tannin and flavonoid contents with altitude likely reflects adaptive responses to stressors such as UV exposure and temperature variation.

5. Conclusions

Our results indicate that altitude affects the secondary metabolite levels in *P. erecta* rhizomes. Tannin and flavonoid levels increase with elevation, and at the highest sites, tannin concentration reached 31.82%. This pattern demonstrates the ecological adaptability of *P. erecta* and suggests that mountain environments significantly influence its phytochemical profile.

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Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of Variance
DW	Dry Weight
HSD	Honestly Significant Difference (Tukey's test)
Rf	Retention Factor (in thin-layer chromatography)
SD	Standard Deviation
TLC	Thin-Layer Chromatography
UV	Ultraviolet

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