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Coumarins of *Ferulopsis hystrix*: LC–MS Profiling and Gastroprotective and Antioxidant Activities of Skimmin and Peucenidin

Yanina G. Razuvaeva [1], Anyuta A. Toropova [1], Saizana M. Salchak [1] and Daniil N. Olennikov [2,*]

[1] Biologically Active Substances Safety Laboratory, Institute of General and Experimental Biology, Siberian Division, Russian Academy of Science, 6 Sakhyanovoy Street, 670047 Ulan-Ude, Russia; tatur75@mail.ru (Y.G.R.); anyuta-tor@mail.ru (A.A.T.); saizana_salchak@mail.ru (S.M.S.)

[2] Laboratory of Biomedical Research, Institute of General and Experimental Biology, Siberian Division, Russian Academy of Science, 6 Sakhyanovoy Street, 670047 Ulan-Ude, Russia

* Correspondence: olennikovdn@mail.ru; Tel.: +7-902-160-06-27

Abstract: *Ferulopsis hystrix* is a perennial plant of the Apiaceae family. In Buryat and Mongolian medicine, it is used as a substitute for *Costus speciosus* roots (ru rta), and in Tibetan medicine, it is used to cure digestive system diseases and used as a wound-healing agent. However, its metabolites and their bioactivities are still poorly understood. High-performance liquid chromatography with photodiode array detection and electrospray ionization triple quadrupole mass-spectrometric detection (HPLC–PDA–ESI–tQ–MS/MS) were used to identify approximately 42 phenolic compounds in *F. hystrix*, and 30 coumarins were characterized and quantified. The major compounds in *F. hystrix* roots were skimmin (umbelliferone 7-*O*-glucoside) and peucenidin (vaginidiol 3′-*O*-acetyl-4′-*O*-senecioyl ester), of which, gastroprotective and antioxidant effects were found for the first time. The abovementioned compounds exhibit a gastroprotective effect against indomethacin and steroid gastropathy by reducing the amount of damage (point, large, and strip-like erosions) in the gastric mucosa and lowering the corresponding Paul's index. The most pronounced gastroprotective effect was exhibited by skimmin at a dose of 1 mg/kg and by peucenidin at doses of 16 and 48 mg/kg; these compounds help to limit the development of pronounced erosive–necrotic processes in the gastric mucosa. In pathological conditions, these compounds reduce malondialdehyde, increase the activity of catalase, and increase the content of reduced glutathione in the blood. Thus, this study demonstrates that *F. hystrix* roots are a good source of bioactive coumarins with gastroprotective potential, which supports earlier ethnopharmacological studies.

Keywords: *Ferulopsis hystrix*; coumarins; skimmin; peucenidin; LC–MS; gastroptotective activity; antioxidants

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1. Introduction

Digestive system diseases have one of the highest incidence rates in the population. The most common illnesses are chronic gastritis, peptic ulcers of the stomach, and duodenum [1,2]. The pathogenesis of these diseases is complex, and it includes three factors that are currently considered to be important in their development and relapse, specifically, genetic pre-disposition, imbalance between the "aggression" and "defense" factors, and the presence of *Helicobacter pylori* [3–6]. Drinking alcohol, taking nonsteroidal anti-inflammatory drugs (NSAIDs), smoking, and stress contribute to ulcerative defect formation in the mucous of the gastrointestinal tract [1,2,7,8].

Currently, complex treatments are used for peptic ulcer diseases; these treatments target the disease's pathogenesis, and they take concomitant diseases into account [9]. Despite the effectiveness and regular improvement of methods for treating peptic ulcers, and despite the reduction in chronic gastritis and stomach ulcer incidences, frequent

disease relapses, complicated disease forms, and drug resistance remain, leading to more disabilities and greater mortality in the population [10–12].

The use of plant remedies is of particular interest for treating stomach diseases because such remedies can simultaneously affect several pathogenetic links and provide a total therapeutic effect. Phytopreparations can prolong the therapeutic effects of drugs, which is especially important for long-term use in chronic diseases, including gastric ulcers [13–17]. However, plant remedies (in terms of the strength and speed of their effect) cannot fully replace synthetic drugs; therefore, they can be used as an auxiliary or additional therapy in the complex treatment of gastrointestinal tract diseases [18–20].

The Apiaceae family perennial plant, *Ferulopsis hystrix* (Bunge) Pimenov (syn.: *Peucedanum hystrix* Bunge; *Phlojodicarpus turczaninovii* Sipliv.; Figure 1) is of interest for the prevention and complex treatment of stomach diseases. In folk medicine, *F. hystrix* roots are widely used as an antitumor, coronary dilator, anticoagulant, choleretic, antispasmodic, and bacteriostatic agent [21]. In Mongolian medicine, it is found in recipes for the treatment of lung, stomach, and esophageal cancers [22]. In Mongolian and Buryat medicine, *F. hystrix* is a substitute for *Costus speciosus* (ru rta), and it is used in Tibetan medicine to treat digestive and respiratory system diseases and as a wound healing agent [23–25]. In Tuvan folk medicine, this plant (known as "*chuksugbai*") has been widely used, and it continues to play a leading role as an anti-inflammatory wound healing agent, and as a treatment for oncological and infectious diseases, including tuberculosis [26].

(**a**) (**b**)

Figure 1. *Ferulopsis hystrix* in its natural habitat (Khorinsky District, Buryatia Republic, Russia (**a**); CC BY-NC 4.0) and *Ferulopsis hystrix* roots purchased at a local herbal market (**b**).

In animal experiments, *F. hystrix* root extract (in a dose of 100–300 mg/kg) has been found to have a gastroprotective effect against ethanol, steroid, neurogenic, and indomethacin gastropathy, and it can prevent damage to the gastric mucosa (according to Shay); this effect was achieved by increasing the resistance of the gastric mucosa to the action of aggressive factors, and by preventing the development of dystrophic and necrotic processes, as well as microcirculation disorders and inflammatory reactions, in the stomach walls of white rats [27–30]. The *F. hystrix* extract exhibits an anti-exudative effect (by reducing the exudation degree induced by carrageenan and formalin) and an anti-alterative effect (by limiting tissue damage caused by phlogogenic agents and accelerating the reparative regeneration processes) [31]. The *F. hystrix* extract reduces the platelet aggregation rate, increases bleeding time, prevents thrombosis, has an analgesic and antispasmodic action, stimulates motor–evacuation gastrointestinal tract activity, and it exhibits choleretic activity by increasing the rate of bile secretion, stimulating cholates via bile synthesis and excretion, as well as cholesterol and bilirubin excretion [32]. In vitro, the studied extract inhibits lipid peroxidation (stabilizing the structural and functional properties of the erythrocyte cell membrane lipid bilayer), and it exhibits radical binding activity against reactive molecules.

In vivo, it reduces the malonic dialdehyde (MDA) content and increases the antioxidant's enzyme activity [33,34].

Previous studies have indicated that there is a lack of information concerning *F. hystrix* phytochemistry. The *F. hystrix* plant contains various biologically active substances, such as coumarins, flavonoids, and essential oils [22,35]. Coumarins are central to *F. hystrix* pharmacological activity, and the total content of coumarins in the underground part of the plant can reach 3.9–4.6% [22,35]. Coumarins are effective antitumor, antiviral, antibacterial, and antifungal agents, and they possess anti-inflammatory and antioxidant activities, as well as anticoagulant and vasodilating properties [36–42]. It has been found that natural and synthetic coumarins exhibit gastroprotective [43,44] and anti-inflammatory [45] effects in intestinal diseases. However, no studies have presented a liquid chromatography mass spectrometric (LC–MS) profile of *F. hystrix* coumarins, which prevents its extensive use as a medicinal plant.

Thus, the aim of this study was to examine the coumarin composition of the *F. hystrix* root using chromatographic profiling and to evaluate the gastroprotective and antioxidative properties of skimmin and peucenidin.

2. Materials and Methods

2.1. Chemicals and Plant Material

Roots of *Ferulopsis hystrix* were collected in the Republic of Buryatia (Ivolginsky District, 51°45′55.2″ N, 107°09′34.3″ E), dried in a ventilated oven (50 °C), and stored in a Plant Repository at the IGEB (at 4 °C). Before analysis, roots were ground using an A11 basic analytical mill (IKA®-WerkeGmbh & Co. KG, Staufen, Germany) and sieved using an ERL-M1 sieving machine (Zernotekhnika, Moscow, Russia; particle size 0.5 mm). The reference standards of phytochemicals, and the companies that they were purchased from, are as follows: ANEXIB Chemicals (Richmond Hill, ON, Canada)—libanotin (≥95%; cat. No.: L173004); BioCrick (Chengdu, Sichuan, China)—apterin (≥98%; cat. No.: BCN3910); Biosynth (Compton, UK)—vaginidiol (≥95%; cat. No.: XV163680); ChemFaces (Wuhan, Hubei, PRC)—peujaponiside (≥98%; cat. No.: CFN93412), peucenidin (≥98%; cat. No.: CFN95272); MCE Med Chem Express (Monmouth, NJ, USA)—skimmin (≥98.38%; cat. No.: HY-N2263); and Sigma-Aldrich (St. Louis, MO, USA)—peucedanol (≥90%; cat. No.: PHL84250). Alternatively, they were isolated and characterized in our laboratory as 6″-apiosylskimmin, peucedanol 2′-*O*-(6″-apiosyl)-glucoside, peucedanol 3′-*O*-(6″-apiosyl)-glucoside [46], peucedanol 7-*O*-glucoside, peucedanol 2′-*O*-glucoside, and peucedanol 3′-*O*-glucoside [47]. The bioactivity reagents used in the study were purchased from Sigma-Aldrich, and are as follows: (St. Louis, MO, USA)—2-thiobarbituric acid (>98%; cat. No.: T5500), 5,5′-dithio-bis(2-nitrobenzoic acid) (>98%; cat. No.: 322123), ammonium molybdate (>98%; cat. No.: 277908), tris(hydroxymethyl)aminomethane (>99%; cat. No.: 252859), and hydrogen peroxide (>98%; cat. No.: 1.08597).

2.2. Extract Preparation

Dried and ground roots (100 g) were extracted using 80% methanol (2 L) with triple sonication (40 min, 45 °C, ultrasound power 100 W, frequency 35 kHz), as described in [30]. The methanolic extract was centrifuged (6000 rpm, 30 min), and the supernatant was filtered through a cellulose filter and concentrated in vacuum. The yield of the *F. hystrix* root extract was 31% dry plant weight.

2.3. LC–MS Analysis and Quantification

Profiling of phenolics in the *F. hystrix* root extract, and the quantification of selected compounds, was performed using high-performance liquid chromatography with photodiode array detection and electrospray ionization triple quadrupole mass spectrometric detection (HPLC-PDA-ESI-tQ-MS), using an LC-20 Prominence liquid chromatograph (Shimadzu, Columbia, MD, USA), coupled with a photodiode array detector SPD-M30A (wavelength range 200–600 nm), triple quadrupole mass spectrometer LCMS 8050 (all Shi-

madzu, Columbia, MD, USA), and GLC Mastro column (2.1 × 150 mm, 3 µm; Shimadzu, Kyoto, Japan) using the known conditions described in [48]. Metabolite identification was performed, using retention time, ultraviolet spectra, and mass spectra data, after being compared with reference standards and data in the literature which exploited LabSolution (Shimadzu) workstation software equipped with an internal LC–MS library.

2.4. Animal Study Design

Experiments were performed on 128 Wistar rats that weighed 220–240 g. The animals were kept in standard vivarium conditions, and a regimen observing care, nutrition, light, and temperature conditions was implemented in accordance with GLP rules (Order N. 708H dated 23 August 2010) and the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasburg, 1986). The research report was approved by the Ethics Committee at IGEB SB RAS (No. 4 от 26 January 2017). Before the experiment began, animals that met the inclusion criteria in the experiment were divided into six groups, in accordance with the randomization principle, as follows: non-treated control, negative control, and four experimental groups. Each group included 16 animals. Animals in the I and II experimental groups received skimmin (MCE Med Chem Express, Monmouth, NJ, USA; ≥98.38%) at doses of 1 and 3 mg/kg, respectively, and animals in the III and IV experimental groups received peucenidin (ChemFaces, Wuhan, Hubei, PRC; ≥98%) at doses of 16 and 48 mg/kg, respectively. Individual compounds were dissolved in a small amount of dimethyl sulfoxide (DMSO), and they were adjusted with purified water to 10 mL/kg. Skimmin and peucenidin were administered intragastrically for 7 days; the last administration was carried out 1 h before the use of the ulcerogenic agent. Non-treated control and negative control animals received purified water in a similar mode, with the addition of an appropriate volume of DMSO. On the 7th day, indomethacin and ethanol/steroid gastropathy were modeled in animals of the experimental groups and the negative control. For each model, 50% of the animals from the group were assigned. Indomethacin gastropathy was caused by a single intragastric injection of an aqueous solution of indomethacin at a dose of 60 mg/kg. Ethanol/steroid gastropathy was modelled via the introduction of prednisolone at a dose of 20 mg/kg, and it was dissolved in 80% ethanol. The introduction of an ulcerogenic agent was carried out after 24 h food deprivation and 1 h after the last injection of coumarins. For biochemical studies, blood was taken from the femoral vein in animals 6 h after the administration of indomethacin, and 24 h after the modeling of ethanol/steroid gastropathy. The animals were then decapitated for the stomach wall macroscopic examination. All manipulations with animals were performed under the influence of ether anesthesia.

2.5. Macroscopic Study of the Gastric Mucosa

The stomach was removed from the abdominal cavity, cut along the greater curvature, washed with saline, and a macroscopic visual assessment of the mucosal conditions was performed under a binocular loupe MC-1 (Micromed, Moscow, Russia). In the gastric mucosa, the number of destructions was determined, and they were differentiated in accordance with size into point erosions (1–2 mm), large erosions (2–5 mm), and strip-like erosions (≥5 mm). The Pauls' index was calculated for each damage type, in accordance with the following formula: $X = (A \times B)/100\%$. Here, A is the average number of ulcerative defects in this group, and B is the number of animals (%) with an ulcerative defect [49].

2.6. Biochemical Study

2.6.1. Determination of Malondialdehyde Content

The intensity of lipid peroxidation (LPO) processes was assessed using the MDA content in blood serum [50]. Endogenous aldehyde was measured using a colorimetric method in which a 0.5% solution of 2-thiobarbituric acid was reacted with MDA in an acid medium at a temperature of 95 °C, for 30 min. The optical density of the resultant solution was measured at 532 nm. The MDA content was expressed in µmol/L.

2.6.2. Catalase Activity Determination

Catalase activity was determined in blood serum using the method proposed by Goth et al. [51]. Before the enzymatic reaction, the hemoglobin content in the samples was estimated, the level of which should not exceed 100 mg/L [52]. Enzymatic activity was expressed in mkat/L.

2.6.3. Determination of Reduced Glutathione Content

The content of the glutathione tripeptide systems was determined using the Shaik method [53], expressed in μmol/L.

2.7. Statistical Analysis

Statistical data processing was performed using BioStat 7.7. software (AnalystSoft Inc., Walnut, CA, USA). For the analyzed traits, compliance with the normal distribution law was preliminarily assessed using the Shapiro–Wilk test. Data that did not obey the normal probability distribution law (destructions number) were evaluated using the nonparametric Mann–Whitney test; results are presented as median (Me) and interquartile range (Q1; Q3). Fisher's exact test was used to compare the frequency of occurrence of lesions in the comparison groups. To evaluate biochemical parameters which have a normal distribution, the Student's parametric *t*-test was used; data are presented as an arithmetic mean (M) and arithmetic mean error (m). Differences were considered significant at an achieved significance level of $p < 0.05$.

3. Results

3.1. LC–MS Profiling of the F. hystrix Root Extract

The chromatographic separation of the *F. hystrix* root extract identified 42 compounds (including various derivatives of umbelliferone, vaginidiol, and peucedanol [46–48,54–57]), and the mass spectra showed which typical ions were produced via the protonation and loss of fragments of hexose (m/z 162), pentose (m/z 132), and acetyl (m/z 42). Moreover, a cascade of adduct ions were produced using lithium, sodium, and potassium, which is typical for coumarins [48] (Figure 2, Table 1). Five umbelliferones were known glucosides, including skimmin (umbelliferone 7-*O*-glucoside, **3**) and 6′′-apiosylskimmin (umbelliferone 7-*O*-(6′′-*O*-apiosyl)-glucoside, **1**) [48]. Both coumarins are present in Apiaceous plants, and they were found in *F. hystrix* for the first time in this study. Compound **2** exhibited a protonated ion with m/z 457, and it was identified as an isomer of 6′′-apiosylskimmin [46]. Two acylated derivatives of umbelliferone *O*-pentosyl-*O*-hexoside were identified as mono- (**6**) and diacetates (**11**), as evidenced by the loss of one and two acetyl fragments, respectively [58].

Table 1. Chromatographic (t) and mass-spectrometric (ESI–MS) data and the contents of compounds **1**–**42** found in the *F. hystrix* root extract.

No.	t, min	ESI-MS, *m/z*	Compound [Ref.]	IL [a]	Content, mg/g ± S.D.
1	5.51	495 $[M+K]^+$, 479 $[M+Na]^+$, 463 $[M+Li]^+$, 457 $[M+H]^+$, 325 $[(M+H)\text{-Api}]^+$, 163 $[(M+H)\text{-Api-Glc}]^+$	6′′-Apiosylskimmin (umbelliferone 7-*O*-(6′′-apiosyl)-glucoside) [46]	1	0.85 ± 0.02
2	5.70	495 $[M+K]^+$, 479 $[M+Na]^+$, 463 $[M+Li]^+$, 457 $[M+H]^+$, 325 $[(M+H)\text{-Pent}]^+$, 163 $[(M+H)\text{-Pent-Hex}]^+$	Umbelliferone *O*-pentosyl-*O*-hexoside [46,48]	2	0.26 ± 0.00
3	6.08	363 $[M+K]^+$, 347 $[M+Na]^+$, 325 $[M+H]^+$, 163 $[(M+H)\text{-Glc}]^+$	Skimmin (umbelliferone 7-*O*-glucoside) [48]	1	22.57 ± 0.45
4	6.33	595 $[M+K]^+$, 579 $[M+Na]^+$, 563 $[M+Li]^+$, 557 $[M+H]^+$, 425 $[(M+H)\text{-Pent}]^+$, 263 $[(M+H)\text{-Pent-Hex}]^+$	Vaginidiol *O*-pentosyl-*O*-hexoside [46,47]	2	0.38 ± 0.01
5	6.45		Vaginidiol *O*-pentosyl-*O*-hexoside [46,47]		0.18 ± 0.00
6	6.77	537 $[M+K]^+$, 521 $[M+Na]^+$, 505 $[M+Li]^+$, 499 $[M+H]^+$, 457 $[(M+H)\text{-Ac}]^+$, 325 $[(M+H)\text{-Ac-Pent}]^+$, 163 $[(M+H)\text{-Ac-Pent-Hex}]^+$	Umbelliferone *O*-acetyl-*O*-pentosyl-*O*-hexoside [48]	2	0.41 ± 0.01
7	6.98	597 $[M+K]^+$, 581 $[M+Na]^+$, 565 $[M+Li]^+$, 559 $[M+H]^+$, 427 $[(M+H)\text{-Api}]^+$, 265 $[(M+H)\text{-Api-Glc}]^+$	Peujaponiside (peucedanol 7-*O*-(6′′-apiosyl)-glucoside) [46,47]	1	0.45 ± 0.01
8	7.51	597 $[M+K]^+$, 581 $[M+Na]^+$, 565 $[M+Li]^+$, 559 $[M+H]^+$, 427 $[(M+H)\text{-Api}]^+$, 265 $[(M+H)\text{-Api-Glc}]^+$	Peucedanol 2′-*O*-(6′′-apiosyl)-glucoside [46,47]	1	0.18 ± 0.00
9	7.83		Peucedanol 3′-*O*-(6′′-apiosyl)-glucoside [46,47]		0.15 ± 0.00
10	8.17	463 $[M+K]^+$, 447 $[M+Na]^+$, 431 $[M+Li]^+$, 425 $[M+H]^+$, 263 $[(M+H)\text{-Glc}]^+$	Apterin (vaginidiol 1′-*O*-glucoside) [54]	1	9.14 ± 0.19
11	8.24	579 $[M+K]^+$, 563 $[M+Na]^+$, 547 $[M+Li]^+$, 541 $[M+H]^+$, 499 $[(M+H)\text{-Ac}]^+$, 457 $[(M+H)\text{-2}\times\text{Ac}]^+$, 325 $[(M+H)\text{-2}\times\text{Ac-Pent}]^+$, 163 $[(M+H)\text{-2}\times\text{Ac-Pent-Hex}]^+$	Umbelliferone di-*O*-acetyl-*O*-pentosyl-*O*-hexoside [48,54]	2	0.12 ± 0.00

Table 1. *Cont.*

No.	t, min	ESI-MS, *m/z*	Compound [Ref.]	IL [a]	Content, mg/g ± S.D.
12	8.63	639 $[M+K]^+$, 623 $[M+Na]^+$, 607 $[M+Li]^+$, 601 $[M+H]^+$, 559 $[(M+H)-Ac]^+$, 427 $[(M+H)-Ac-Pent]^+$, 265 $[(M+H)-Ac-Pent-Hex]^+$	Peucedanol *O*-acetyl-*O*-pentosyl-*O*-hexoside [46–48]	2	0.41 ± 0.01
13	8.84	465 $[M+K]^+$, 449 $[M+Na]^+$, 433 $[M+Li]^+$, 427 $[M+H]^+$, 265 $[(M+H)-Glc]^+$	Peucedanol 7-*O*-glucoside [55]	1	0.11 ± 0.00
14	8.90	639 $[M+K]^+$, 623 $[M+Na]^+$, 607 $[M+Li]^+$, 601 $[M+H]^+$, 559 $[(M+H)-Ac]^+$, 427 $[(M+H)-Ac-Pent]^+$, 265 $[(M+H)-Ac-Pent-Hex]^+$	Peucedanol *O*-acetyl-*O*-pentosyl-*O*-hexoside [46–48]	2	<0.01
15	9.08	465 $[M+K]^+$, 449 $[M+Na]^+$, 433 $[M+Li]^+$, 427 $[M+H]^+$, 265 $[(M+H)-Glc]^+$	Peucedanol 2′-*O*-glucoside [46,47]	1	0.02 ± 0.00
16	9.11		Peucedanol 3′-*O*-glucoside [46,47]		0.01 ± 0.00
17	9.61	505 $[M+K]^+$, 489 $[M+Na]^+$, 473 $[M+Li]^+$, 467 $[M+H]^+$, 425 $[(M+H)-Ac]^+$, 263 $[(M+H)-Ac-Glc]^+$	Vaginidiol *O*-acetyl-*O*-hexoside [46–48]	2	0.21 ± 0.00
18	10.88	301 $[M+K]^+$, 285 $[M+Na]^+$, 269 $[M+Li]^+$, 263 $[M+H]^+$	Vaginidiol [46–48]	1	0.12 ± 0.00
19	11.21		Vaginidiol isomer [46–48]	2	0.14 ± 0.00
20	12.20	303 $[M+K]^+$, 287 $[M+Na]^+$, 271 $[M+Li]^+$, 265 $[M+H]^+$	Peucedanol [55]	1	<0.01
21	12.63	315 $[M+K]^+$, 299 $[M+Na]^+$, 283 $[M+Li]^+$, 277 $[M+H]^+$, 263 $[(M+H)-CH_2]^+$	Vaginidiol *O*-methyl ester [46–48,55]	2	<0.01
22	13.01	383 $[M+K]^+$, 367 $[M+Na]^+$, 351 $[M+Li]^+$, 345 $[M+H]^+$, 245 $[(M+H)-C_5H_8O_2]^+$	Vaginidiol 9-*O*-angeloyl/senecioyl ester [46–48]	2	<0.01
23	14.05	329 $[M+K]^+$, 313 $[M+Na]^+$, 297 $[M+Li]^+$, 291 $[M+H]^+$, 277 $[(M+H)-CH_2]^+$, 263 $[(M+H)-2\times CH_2]^+$	Vaginidiol di-*O*-methyl ester [46–48,55]	2	0.08 ± 0.00
24	14.69	329 $[M+K]^+$, 313 $[M+Na]^+$, 297 $[M+Li]^+$, 291 $[M+H]^+$, 263 $[(M+H)-C_2H_4]^+$	Vaginidiol *O*-ethyl ester [46–48,55]	2	<0.01
25	14.71				
26	15.23	383 $[M+K]^+$, 367 $[M+Na]^+$, 351 $[M+Li]^+$, 345 $[M+H]^+$, 245 $[(M+H)-C_5H_8O_2]^+$	Vaginidiol 9-*O*-angeloyl/senecioyl ester [46–48,55]	2	<0.01
27	16.52	343 $[M+K]^+$, 327 $[M+Na]^+$, 311 $[M+Li]^+$, 305 $[M+H]^+$, 245 $[(M+H)-C_2H_4O_2]^+$	Vaginidiol 9-*O*-acetyl ester [55]	2	<0.01
28	15.81	385 $[M+K]^+$, 369 $[M+Na]^+$, 353 $[M+Li]^+$, 347 $[M+H]^+$, 245 $[(M+H)-C_5H_{10}O_2]^+$	Vaginidiol 9-*O*-isovaleroyl/2-methylbutyroyl ester [46–48,56]	2	<0.01
29	15.84				
30	16.31	371 $[M+K]^+$, 355 $[M+Na]^+$, 339 $[M+Li]^+$, 333 $[M+H]^+$, 245 $[(M+H)-C_4H_8O_2]^+$	Vaginidiol 9-*O*-isobutyroyl ester [46–48,56]	2	<0.01
31	16.81	385 $[M+K]^+$, 369 $[M+Na]^+$, 353 $[M+Li]^+$, 347 $[M+H]^+$, 287 $[(M+H)-C_2H_4O_2]^+$, 245 $[(M+H)-C_2H_2O]^+$	Vaginidiol 9,1′-di-*O*-acetyl ester [46–48]	2	0.72 ± 0.02
32	17.14	413 $[M+K]^+$, 397 $[M+Na]^+$, 381 $[M+Li]^+$, 375 $[M+H]^+$, 315 $[(M+H)-C_2H_4O_2]^+$, 245 $[(M+H)-C_2H_4O_2-C_4H_6O]^+$	Vaginidiol 9-*O*-acetyl-1′-*O*-isobutyroyl ester [46–48]	2	0.51 ± 0.01
33	17.42	427 $[M+K]^+$, 411 $[M+Na]^+$, 395 $[M+Li]^+$, 389 $[M+H]^+$, 329 $[(M+H)-C_2H_4O_2]^+$, 245 $[(M+H)-C_2H_4O_2-C_5H_8O]^+$	Vaginidiol 9-*O*-acetyl-1′-*O*-isovaleroyl/2-methylbutyroyl ester [46–48]	2	<0.01
34	18.26				
35	18.72	425 $[M+K]^+$, 409 $[M+Na]^+$, 393 $[M+Li]^+$, 387 $[M+H]^+$, 327 $[(M+H)-C_2H_4O_2]^+$, 245 $[(M+H)-C_2H_4O_2-C_5H_6O]^+$	Vaginidiol 9-*O*-acetyl-1′-*O*-angeloyl ester [46–48,57]	2	1.02 ± 0.02
36	19.11	425 $[M+K]^+$, 409 $[M+Na]^+$, 393 $[M+Li]^+$, 387 $[M+H]^+$, 327 $[(M+H)-C_2H_4O_2]^+$, 245 $[(M+H)-C_2H_4O_2-C_5H_6O]^+$	Peucenidin (vaginidiol 9-*O*-acetyl-1′-*O*-senecioyl ester) [46–48]	1	170.35 ± 3.42
37	19.77	425 $[M+K]^+$, 409 $[M+Na]^+$, 393 $[M+Li]^+$, 387 $[M+H]^+$, 287 $[(M+H)-C_5H_8O_2]^+$, 245 $[(M+H)-C_5H_8O_2-C_2H_2O]^+$	Libanotin (vaginidiol 9-*O*-angeloyl-1′-*O*-acetyl ester) [46–48]	1	8.52 ± 0.17
38	21.02	427 $[M+K]^+$, 411 $[M+Na]^+$, 395 $[M+Li]^+$, 389 $[M+H]^+$, 287 $[(M+H)-C_5H_{10}O_2]^+$, 245 $[(M+H)-C_5H_{10}O_2-C_2H_2O]^+$	Vaginidiol 9-*O*-isovaleroyl/2-methylbutyroyl-1′-*O*-acetyl ester [46–48]	2	4.25 ± 0.09
39	21.21	465 $[M+K]^+$, 449 $[M+Na]^+$, 433 $[M+Li]^+$, 427 $[M+H]^+$, 327 $[(M+H)-C_5H_8O_2]^+$, 245 $[(M+H)-C_5H_8O_2-C_5H_6O]^+$	Vaginidiol 9,1′-di-*O*-angeloyl/senecioyl ester [46–48]	2	6.27 ± 0.12 [b]
40	21.34				
41	21.64	441 $[M+K]^+$, 425 $[M+Na]^+$, 409 $[M+Li]^+$, 403 $[M+H]^+$, 315 $[(M+H)-C_4H_8O_2]^+$, 245 $[(M+H)-C_4H_8O_2-C_4H_6O]^+$	Vaginidiol 9,1′-di-*O*-isobuturoyl ester [46–48]	2	1.14 ± 0.02
42	21.73	469 $[M+K]^+$, 453 $[M+Na]^+$, 437 $[M+Li]^+$, 431 $[M+H]^+$, 329 $[(M+H)-C_5H_{10}O_2]^+$, 245 $[(M+H)-C_5H_{10}O_2-C_5H_8O]^+$	Vaginidiol 9,1′-di-*O*-isovaleroyl/2-methylbutyroyl ester [46–48]	2	0.82 ± 0.02
		Total umbelliferone content			24.21
		Total peucedanol content			1.33
		Total vaginidiol content			203.85
		Total coumarin content			229.39

[a] Identification levels: (1) identified compounds after a comparison of UV, mass-spectral data, and retention time with reference standards; (2) putatively annotated compounds after a comparison of the UV and mass-spectral data with data from the literature. [b] Calculated as a sum. Abbreviations: Ac—acetyl; Api—apiose; Glc—glucose; Hex—hexose; Pent—pentose.

Peucedanol (**20**) was identified in the *F. hystrix* root after its mass-spectral pattern was compared with that of a reference standard, and eight peucedanol glucosides were characterized by the presence of an aglycone ion with *m/z* 265 [47]. Three monoglycosides of peucedanol (i.e., 7-*O*-glucoside (**13**), 2′-*O*-glucoside (**15**), and 3′-*O*-glucoside (**16**)), and three diglycosides (i.e., O-(6″-apiosyl)-glucosides of peucedanol, linked at the 7-*O*-, (peujaponiside, **7**), 2′-*O*- (**8**), and 3′-*O*-positions (**9**)), and they were identified in *F. hystrix* for the first time. Moreover, they have been previously found in *Phlojodicarpus sibiricus* (Fisch.) Koso-Pol. [48] and *Peucedanum morisonii* Besser ex Schult. [47]. Two monoacetyl derivatives of peucedanol O-pentosyl-*O*-hexoside, **12** and **14,** do not have analogues among the known peucedanols.

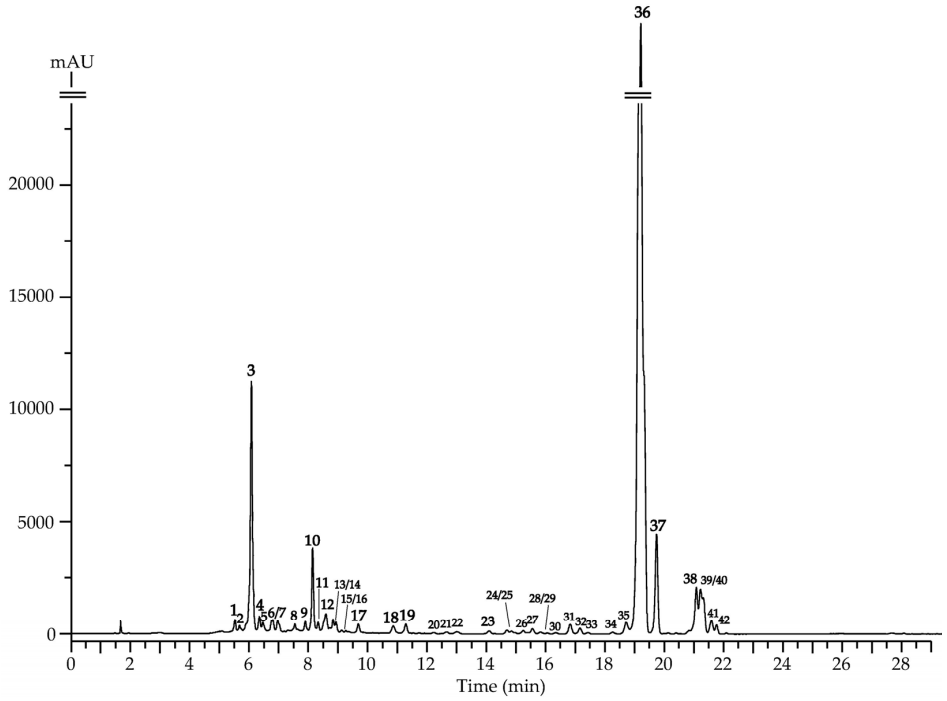

Figure 2. High-performance liquid chromatography data of the *F. hystrix* root extract after electrospray ionization triple quadrupole mass-spectrometric detection (positive ionization, base peak chromatogram). Compounds are numbered as listed in Table 1.

The largest coumarin group found in the *F. hystrix* root (i.e., vaginidiol derivatives) includes 28 members. Unsubstituted vaginidiol (**18**) was identified after a comparison of its spectral parameters with that of the reference substance, and it has been previously found in an *F. hystrix* of Mongolian origin [22]. Two unknown vaginidiol *O*-pentosyl-*O*-hexosides, **4** and **5**, with *m*/*z* 557 [47], the known glycoside apterin (vaginidiol 1′-*O*-glucoside, **10**) [54], and the unknown vaginidiol *O*-acetyl-*O*-hexoside (**17**) with m/z 467, are new phytocomponents of *F. hystrix*. The remaining vaginidiol derivatives are mono- and diesters of acetic, angelic/senecic, and isovaleric/2-methylbutyric acids, methanol, and ethanol, and they are known to exist in apiaceous plants. We have previously reported the specific mass-spectral patterns of coumarins with neighboring hydroxyls [48], which allowed us to determine the most likely structure of these compounds. Among the various vaginidiol esters, two were identified, using reference standards, as peucenidin (vaginidiol 9-*O*-acetyl-1′-*O*-senecioyl ester, **36**) [48] and libanotin (vaginidiol 9-*O*-angeloyl-1′-*O*-acetyl ester, **37**) [22]. Both coumarins were found in the Mongolian samples of *F. hystrix*. Mono- (**21**) and dimethyl esters (**23**) that were found with *m*/*z* 277 and 291, respectively, as well as a monoethyl ester (**24**, **25**) with *m*/*z* 291, are rare coumarins, and they are identified as being derived from the apiaceous plant for the first time. Monoesters of acids included 9-*O*-angeloyl/senecioyl esters (**22**, **26**; *m*/*z* 345), 9-*O*-acetyl esters (**27**; *m*/*z* 305), 9-*O*-isovaleroyl/2-methylbutyroyl esters (**28**, **29**; *m*/*z* 347), and 9-*O*-isobutyroyl esters (**30**; *m*/*z* 333); only the vaginidiol 9-*O*-2-methylbutyroyl ester has been previously found in *F. hystrix* [22]. Diesters of vaginidiol included the 9,1′-di-*O*-acetyl ester (**31;** *m*/*z* 347), 9-O-acetyl-1′-O-isobutyroyl ester (**32**; *m*/*z* 375), 9-O-acetyl-1′-O-isovaleroyl/2-methylbutyroyl esters (**33**, **34**; *m*/*z* 389), 9-*O*-acetyl-1′-*O*-angeloyl ester (**35**; *m*/*z* 387; isomer of **36**), 9-*O*-isovaleroyl/2-methylbutyroyl-1′-*O*-acetyl ester (**38**; *m*/*z* 389), 9,1′-di-*O*-angeloyl/senecioyl esters (**39**, **40**; *m*/*z* 427), 9,1′-di-*O*-isobuturoyl ester (**41**; *m*/*z* 403), and 9,1′-di-*O*-isovaleroyl/2-methylbutyroyl ester (**42**; *m*/*z* 431). Coumarins with a similar structure have not been found in *F. hystrix*.

Quantitative data showed a high coumarin content in the *F. hystrix* root extract (229.39 mg/g). The content of vaginidiol derivatives was the largest (203.85 mg/g). The content of the next largest coumarin group, umbelliferones, was 24.21 mg/g. Peucedanols exhibited the lowest

content (1.33 mg/g). Two basic compounds included peucenidin (170.35 mg/g) and skimmin (22.57 mg/g), which, in combination, accounted for 84% of coumarins in the extract. This suggests that these compounds are responsible for bioactive effects.

3.2. Gastroprotective and Antioxidant Activities of Skimmin and Peucenidin

Table 2 shows that the total number of erosions in the animal gastric mucosa in the experimental groups was 2–3 times lower than that of the negative control group. Point erosions were observed in 100% of animals in the experimental groups, and their Paul's indices were 22–48% lower than those of the animals in the negative control group. The lowest number of point erosions, and accordingly, the Paul's indices, were noted in animals treated with skimmin at a dose of 3 mg/kg. Large erosions were observed in six of eight animals treated with skimmin at a dose of 3 mg/kg, and with peucenidin at a dose of 48 mg/kg (experimental groups II and IV, respectively), compared with two and four animals in experimental groups I and III, respectively. The number of erosions in animals treated with skimmin at a dose of 1 mg/kg, and peucenidin at a dose of 16 mg/kg, was 8.0 times lower than that in animals in the negative control group, and 2.7 and 2.0 times lower than that in animals in experimental groups II and IV, respectively.

Table 2. Destructive processes in the Wistar rat's gastric mucosa, which concern gastropathy, and are induced by indomethacin.

Parameters	Non-Treated Control (H_2O), n = 8	Negative Control (Indomethacin + H_2O), n = 8	Experimental Group I (Indomethacin + Skimmin, 1 mg/kg), n = 8	Experimental Group II (Indomethacin + Skimmin, 3 mg/kg), n = 8	Experimental Group III (Indomethacin + Peucenidin, 16 mg/kg), n = 8	Experimental Group IV (Indomethacin + Peucenidin, 48 mg/kg), n = 8
Erosions total number, Me (Q1–Q3)	0	13 (10–14.5) $p \leq 0.01$ **	5.5 (3–6.5) $p \leq 0.01$ *	4 (2.5–7.5) $p \leq 0.01$ *	5 (1.5–9) $p \leq 0.01$ *	6 (4–8) $p \leq 0.01$ *
Point erosions (1–2 mm)						
Number of animals, %	0	100 $p \leq 0.01$ **	100	100	100	100
Number of erosions, Me (Q1–Q3)	0	6(3.5–9) $p \leq 0.01$ **	4.5 (2–8)	2.5 (1.5–5) $p \leq 0.05$ *	4.5 (1.5–7.5)	4 (1.5–7)
Pauls' index	0	6.3	4.9	3.3	4.5	4.3
Large erosions (2–5 mm)						
Number of animals, %	0	100 $p \leq 0.01$ **	25 $p \leq 0.05$ *	75	50 $p \leq 0.05$ *	75
Number of erosions, Me (Q1–Q3)	0	4(3–5) $p \leq 0.01$ **	0 (0–1) $p \leq 0.01$ *	1.5 (0.5–2) $p \leq 0.01$ *	0.5 (0–1.5) $p \leq 0.01$ *	2 (1–2.5) $p \leq 0.01$ *
Paul's index	0	4.0	0.13	0.98	0.38	1.3
Strip-like erosions (≥5 mm)						
Number of animals, %	0	50 $p \leq 0.01$ **	37.5	50	0 $p \leq 0.05$ *	0 $p \leq 0.01$ *
Number of erosions, Me (Q1–Q3)	0	2(0–3) $p \leq 0.01$ **	0 (0–1)	0.5 (0–1)	0	0
Paul's index	0	1.0	0.15	0.25	0	0

*—vs. negative control group animals; **—vs. non-treated control group animals.

The supplementation of animals with peucenidin limits the development of strip-like erosions in the gastric mucosa; none of the animals in experimental groups III and IV showed such erosions. Single strip-like erosions were noted in three and four of eight animals in experimental groups I and II, respectively. In animals supplemented with skimmin at doses of 1 and 3 mg/kg, the Paul's indices for strip-like erosions were 6.7 and 4.0 times lower than that of the negative control group, respectively.

When modeling ethanol/steroid gastropathy, it was found (Table 3) that in all experimental group animals (except in experimental group II animals), the total damage in the gastric mucosa was 2.0 times lower, on average ($p < 0.01$), than that in the gastric mucosa of the negative control group animals. Skimmin at a dose of 3 mg/kg (experimental group II) reduced this indicator by only 21%, as compared with the negative control. In six of eight experimental group animals, large erosions were noted; in two animals, strip-like erosions were observed. Moreover, their Paul's indices were lower than the negative control group by 53% and 78%, respectively.

Table 3. Destructive processes in the Wistar rats' gastric mucosa upon administration of prednisolone, dissolved in 80% ethanol.

Parameters	Non-Treated Control (H_2O), n = 8	Negative Control (Ethanol/Prednisolone + H_2O), n = 8	Experimental Group I (Ethanol/Prednisolone + Skimmin, 1 mg/kg), n = 8	Experimental Group II (Ethanol/Prednisolone + Skimmin, 3 mg/kg), n = 8	Experimental Group III (Ethanol/Prednisolone + Peucenidin, 16 mg/kg), n = 8	Experimental Group IV (Ethanol/Prednisolone + Peucenidin, 48 mg/kg), n = 8
Total number of erosions, Me (Q1–Q3)	0	7(6.5–9) $p \leq 0.01$ **	3.5 (2.5–4) $p \leq 0.01$ *	5.5 (2.5–9)	3.5 (2.5–4.5) $p \leq 0.01$ *	3(3–3.5) $p \leq 0.01$ *
Point erosions (1–2 mm)						
Number of animals, %	0	100 $p \leq 0.01$ **	100	100	100	100
Number of erosions, Me (Q1–Q3)	0	3.5 (2.5–4) $p \leq 0.01$ **	2.5 (1.5–3.5)	3.5 (1.5–5)	3 (2.5–3)	2 (1–3.5)
Paul's index	0	3.3	2.5	3.3	2.8	2.3
Large erosions (2–5 mm)						
Number of animals, %	0	100 $p \leq 0.01$ **	75	75	50 $p \leq 0.05$ *	50 $p \leq 0.05$ *
Number of erosions, Me (Q1–Q3)	0	3 (2.5–3) $p \leq 0.01$ **	1 (0.5–1) $p \leq 0.01$ *	2 (1–2.5) $p \leq 0.05$ *	0.5 (0–1.5) $p \leq 0.01$ *	1(0–2) $p \leq 0.01$ *
Paul's index	0	2.8	0.6	1.3	0.4	0.5
Strip-like erosions (≥5 mm)						
Number of animals, %	0	50 $p \leq 0.05$ **	0 $p \leq 0.05$ *	25	0 *	0 $p \leq 0.05$ *
Number of erosions, Me (Q1–Q3)	0	1 (0–2.5)	0	0 (0–1.5)	0	0
Paul's index	0	0.9	0	0.2	0	0

*—vs. negative control group animals; **—vs. non-treated control group animals.

Point erosions were noted in 100% of experimental group I, II, and IV animals; their Paul's indices were lower than the negative control group by 30%, on average. The use of skimmin at a dose of 1 mg/kg, and peucenidin at doses of 16 and 48 mg/kg, prevented deep damage. Thus, in these experimental group animals, there were no strip-like erosions in the gastric mucosa; large erosions were observed in only 50% of the animals receiving peucenidin at doses of 16 and 48 mg/kg, and in 75% of the animals receiving skimmin at a dose of 1 mg/kg. In the negative control group, strip-like erosions were observed in 100% of the animals. The Paul's index for large erosions in these experimental group animals was lower than the negative control group animals by 80%, on average.

The use of individual compounds to treat ulcerogenic effects limited "oxidative stress" development, reduced the intensity of biomacromolecule free radical oxidation processes, and increased the organism's antioxidant status (Table 4). The administration of individual compounds to animals contributed to a 2.0 time reduction of MDA content in blood serum, with indomethacin gastropathy, on average, and when used with ethanol/steroid gastropathy, a 1.5 time reduction was exhibited, on average, compared with the indicators in the negative control group animals. Skimmin at a dose of 1 mg/kg, most significantly affected the inhibition of LPO processes.

Under indomethacin gastropathy conditions, the administration of skimmin at a dose of 3 mg/kg, and peucenidin at a dose of 16 mg/kg, increased the catalase activity in the blood serum by 23%, on average, compared with the negative control group animals. The most pronounced effect on catalase activity was shown by skimmin at a dose of 1 mg/kg and peucenidin at a dose of 48 mg/kg, which increased catalase activity by 34%, on average, compared with the negative control group. Peucenidin at doses of 16 and 48 mg/kg had a pronounced effect on the glutathione link that causes indomethacin damage to the gastric mucosa, as indicated by increases in GSH content of 42% and 55%, respectively, as compared with the negative control group. The use of skimmin doses increased this indicator by 39%, compared with the negative control group.

Table 4. Effect of coumarins on the state of pro- and antioxidant systems of Wistar rats, with indomethacin and ethanol/steroid gastropathy.

Animal Groups	MDA, µmol/L	Catalase, mkat/L	GSH, µmol/L
Indomethacin gastropathy			
Non-treated control (H_2O)	6.5 ± 0.39	17.2 ± 0.94	926.2 ± 36.47
Negative control (Indomethacin + H_2O)	23.2 ± 1.08 $p \leq 0.01$ **	10.4 ± 0.74 $p \leq 0.01$ **	584.7 ± 38.06 $p \leq 0.01$ **
Experimental group I (Indomethacin + skimmin, 1 mg/kg)	9.5 ± 0.50 $p \leq 0.01$ *	13.8 ± 0.61 $p \leq 0.01$ *	805.7 ± 66.60 $p \leq 0.01$ *
Experimental group II (Indomethacin + skimmin, 3 mg/kg)	11.0 ± 0.88 $p \leq 0.01$ *	12.7 ± 0.77	812.4 ± 61.85 $p \leq 0.01$ *
Experimental group III (Indomethacin + peucenidin, 16 mg/kg)	12.3 ± 1.15 $p \leq 0.01$ *	12.9 ± 0.42 $p \leq 0.05$ *	832.4 ± 66.63 $p \leq 0.01$ *
Experimental group IV (Indomethacin + peucenidin, 48 mg/kg)	11.3 ± 0.89 $p \leq 0.01$ *	13.9 ± 0.42 $p \leq 0.01$ *	906.1 ± 53.92 $p \leq 0.01$ *
Ethanol/steroid gastropathy			
Non-treated control (H_2O)	5.9 ± 0.40	18.3 ± 0.71	973.1 ± 39.64
Negative control (ethanol/prednisolone + H_2O)	20.2 ± 0.95 $p \leq 0.01$ **	10.3 ± 0.63 $p \leq 0.01$ **	671.7 ± 66.60 $p \leq 0.01$ **
Experimental group I (ethanol/prednisolone + skimmin, 1 mg/kg)	12.5 ± 0.68 $p \leq 0.01$ *	13.1 ± 0.62 $p \leq 0.01$ *	932.9 ± 82.46 $p \leq 0.05$ *
Experimental group II (ethanol/prednisolone + skimmin, 3 mg/kg)	14.1 ± 0.70 $p \leq 0.01$ *	12.8 ± 0.41 $p \leq 0.01$ *	845.9 ± 56.30
Experimental group III (ethanol/prednisolone + peucenidin, 16 mg/kg)	14.2 ± 0.63 $p \leq 0.01$ *	14.8 ± 0.51 $p \leq 0.01$ *	926.2 ± 42.57 $p \leq 0.01$ *
Experimental group IV (ethanol/prednisolone + peucenidin, 48 mg/kg)	13.1 ± 0.86 $p \leq 0.01$ *	12.9 ± 0.30 $p \leq 0.01$ *	959.7 ± 47.57 $p \leq 0.01$ *

*—vs. negative control group animals; **—vs. non-treated control group animals.

4. Discussion

NSAIDs are widely used in the treatment of patients suffering from various diseases [59,60]. Though in recent decades, there has been a tendency to reduce the use of over-the-counter NSAIDs, they remain some of the most common causes of lesions in various parts of the gastrointestinal tract, and gastroduodenal zone ulcerative lesions are among the most frequent and dangerous [61]. The long-term use of NSAIDs that block cyclooxygenase (COX) activity disrupts the arachidonic acid metabolism, which inhibits prostaglandin synthesis in the gastric mucosa and duodenum [59,62–66]. A reduction in the synthesis of PGE2 in the blood and gastric mucosa contributes to a reduction in the secretion of bicarbonates and mucus, as well as an increase in acid production, which increases the imbalance of "protection" and "aggression" factors and causes the development of erosive and ulcerative lesions in the gastric mucosa [67,68]. Prostaglandins synthesized in the wall blood vessels act as platelet aggregation inhibitors in humans and animals, and their deficiency impairs blood flow in the stomach wall, which is an important pathogenetic link in the development of NSAID gastropathy [69]. Regional blood flow impairment and increased neutrophil adhesion and activation occur because of the suppression of nitric oxide (NO) synthesis; this is caused by the inhibition of NO synthetase [70]. According to some authors, indomethacin induces NF-κB activation, and thus, promotes pro-inflammatory cytokine expression (TNF-α, IL-1β, and IL-6), stimulating the migration of neutrophils into the gastric mucosa [71–73]. The accumulation and activation of neutrophils enhance superoxide molecule production in the stomach tissues, ultimately leading to impaired microcirculation and increased free radical formation [74–76].

An increase in the acidity and peptic activity of gastric juice is the cause of erosion development in the gastric mucosa upon glucocorticoid use, and when glucocorticoids are combined with NSAIDs or alcohol, peptic ulcers can develop [77–80]. The ethanol ulcerogenic effect occurs because ethanol increases hydrochloric acid secretion and gastrin production, it slows gastric emptying, promotes the release of endogenous mediators (histamine, serotonin, thromboxane, and leukotrienes) that cause microcirculation disorders in the gastric mucosa, inhibits mucus formation in the stomach, increases vascular permeability, enhances surface epithelial cell desquamation, and inhibits regeneration processes [81].

Microcirculation disorders and hypoxic processes underlying the gastropathy pathogenesis contribute to the formation of reactive oxygen species and an increase in cell membrane lipid peroxidation processes. In addition, the improper functioning of antioxidant defense systems contributes to biological membrane damage and oxidative stress [82].

Our results show that *F. hystrix* roots from the Baikal region are a rich source of coumarins, and most of them have not previously been described. The known data demonstrate that the Mongolian population of *F. hystrix* accumulates various furocoumarins (such as angelicin, columbianetin and its esters, oroselol, and vaginidiol and its esters [22]) that significantly differ from those of the Baikal population of *F. hystrix*, which contains glycosides and esters of umbelliferone, vaginidiol, and peucedanol. This difference may be associated with the climatic conditions that distinguish the Mongolian and Baikal regions. We found that coumarins from the *F. hystrix* roots exhibit a gastroprotective effect with regard to indomethacin and ethanol/steroid gastropathy. The most pronounced gastroprotective effect, which helped to limit pronounced erosive–necrotic development in the gastric mucosa, was caused by skimmin at a dose of 1 mg/kg and peucenidin at doses of 16 and 48 mg/kg. The gastroprotective action of the studied individual compounds is attributed to their ability to inhibit lipid peroxidation processes and increase the antioxidant system activity.

Similar results have been obtained by Cruz et al. [83], who showed that umbelliferon exhibits a pronounced antiulcerogenic effect in ethanol gastropathy. According to these authors, the umbelliferon gastroprotective effect is attributed to its antioxidant action. In other experimental studies, the conjugate of diclofenac with umbelliferon (which has fewer ulcerogenic side effects) showed pronounced anti-inflammatory and antioxidant properties [84]. Numerous studies indicate that various coumarins have pronounced antiradical properties with respect to the 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl stable free radical ($DPPH^{\bullet}$), superoxide anion (O_2^{-}), hydroxyl radical ($OH^{\bullet}$), and $ABTS^{\bullet+}$ radical. Umbelliferon prevents β-carotene oxidation in a model system with linoleic acid, and it can inhibit peroxidation induced by 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), which is a producer of free radicals. Some coumarins also exhibit their antioxidant activity through iron ion chelation. Iron is an essential element and cofactor responsible for the electron transport chain function and catalytic processes. However, excessive iron accumulation can lead to the overproduction of reactive oxygen species (ROS), which can lead to cell membrane damage, necrosis, and possibly cell death. Among the coumarin derivatives, umbelliferone and scopoletin are iron-binding chelators that inhibit iron-catalyzed ROS formation and protect cells from damage [85–88].

Owing to the coumarin antioxidant properties, vascular endothelium cell membrane integrity is preserved, which prevents the desquamation of endotheliocytes, their fragments, and cell layers. In addition, coumarins (which exhibit antiaggregatory properties owing to the inhibition of thromboxane A2 and B2 synthesis [89,90]) can inhibit the adhesion and aggregation of platelets and erythrocytes in places where the endothelium base membrane is exposed [88,91–93]. By influencing the reduction of intracellular calcium concentration in the smooth myocytes of microvasculature vessels, coumarins have a vasodilating effect [89]. The ability of coumarins to inhibit platelet aggregation and improve blood rheological properties [94], in combination with its vasodilating effect, prevents angiospasm and increases local blood supply [88,94]. In turn, effective microcirculation is an important condition for the normal functioning of the gastric mucosa's protective factors. Thus, an increase in blood flow in the gastric mucosa is accompanied by a parallel increase in acidity, and the gastric mucosa is more resistant to acid in the acid production zone than in other locations [95–98]. Coumarins affect vascular endothelial growth factor expression and fibroblast growth factors, thereby controlling growth and new vessel formation, and affecting proliferation and fibroblast migration for synthesizing collagen and glycosaminoglycans in the extracellular matrix; this leads to increased regeneration and the accelerated formation of granulation tissue [99].

In accordance with the literature, scopoletin and umbelliferone (which exhibit a dual inhibitory activity against COX-2 and 5-LO [100]) suppress the synthesis of inflammatory mediators (histamine and serotonin), pro-inflammatory cytokines (IL-1β, TNF-α), leukotrienes, and other biological substances, which decreases the microvasculature vessel permeability and prevents blood plasma exudation, local edema development, blood cell migration, and infiltrated cell development [88,89,101–103]. In addition, coumarins reduce myeloperoxidase concentration and suppress neutrophil chemotaxis [92,104]. The reduced gastric mucosa inflammation is accompanied by an increase in somatostatin secretion and a reduction in gastrin secretion. By controlling the level of gastrin, somatostatin also regulates hydrochloric acid secretion [105], accelerating ulcer healing and reducing relapse risk.

Thus, skimmin and peucenidin from *F. hystrix* roots are promising gastroprotective agents. The observed coumarin gastroprotective action is attributed to their abilities to inhibit lipid peroxidation processes and activate the antioxidant system of the body.

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