

Article

Phytochemical Research and Screening of Pharmacological Activity in *Eryngium planum* L. Herb Extracts

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Abstract: Species of the genus *Eryngium* L. of the Apiaceae family are successfully used in folk medicine in various countries worldwide, but they are hardly implemented in official medicinal and pharmaceutical practices. Therefore, it is advisable to conduct phytochemical and pharmacological research in *E. planum* L. herb extracts to develop and implement new phytomedicines based on this raw material. Purified water, and 40% and 70% ethanol were used for obtaining soft extracts. A total of seven hydroxycinnamic acids, six flavonoids, and three tannin metabolites were identified and quantified in the *E. planum* extracts by HPLC. These extracts were characterized as practically non-toxic medicines (V toxicity class, LD₅₀ > 5000 mg/kg). The hepatoprotective activity of the *E. planum* extracts has been established. They were affected to reduce serum thiobarbituric acid (TBA) levels by 29.3%, 31.5%, and 32.4%, respectively, compared to untreated animals and in liver homogenate by 59.5%, 65.4%, and 66.8%, respectively. The alanine transaminase (ALT) activity decreased by 26.9%, 30.8%, and 33.8%, respectively. The aspartate transaminase (AST) activity decreased by 23.9%, 25.7%, and 30.5%, respectively. The sedative activity of *E. planum* extracts was studied for the first time. The 70% ethanol extract has the most pronounced sedative effect due to a significant decrease in motor activity (by 2.4 times compared to the control group) in the «Open Field test» and reduction in concern in the «Cube tests», «Raised Cross Maze», and «Black and White Camera». This extract also demonstrates a moderate antibacterial effect against gram-positive microorganisms (8–10 mm), including staphylococci, streptococci, and bacilli. These results provide a foundation for further preclinical and clinical studies of the dry extract of *E. planum* herb (extractant: 70% ethanol) to integrate it into modern medical practice and develop new supplements.

Keywords: sea holly; biologically active substances; phenolic substances; hepatoprotective activity; antimicrobial activity; sedative activity



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1. Introduction

The species of the genus *Eryngium* L. (commonly known as sea holly) from the family Apiaceae are perennial, biennial, or annual herbs distributed in the temperate climatic zones of the northern and southern hemispheres. Many species of this genus are both edible and ornamental plants. While they are not officially recognized as medicinal raw materials

in medical practice, some species are successfully used in traditional medicine in various countries worldwide [1–3]. Most species of the genus *Eryngium* are wild plants. They are distributed in Europe, North and South America, Southwest and Central Asia, Australia, and North Africa [4]. Eight species of the genus (*E. fluminense*, *E. corniculatum*, *E. viviparum*, *E. atlanticum*, *E. alpinum*, *E. maroccanum*, *E. variifolium*, and *E. galioides*) are included in the IUCN Red List of Threatened Species [5]. In Ukraine, three species of the genus *Eryngium* are distributed: *Eryngium planum* L. (flat sea holly), *Eryngium campestre* L. (field eryngo), and *Eryngium maritimum* L. (sea holly) (Figure 1) [6]. Raw materials cultivated in Ukraine have not been studied, although *E. planum* is the most common species.



Figure 1. Species of the genus *Eryngium* growing in Ukraine ((A)—flat sea holly (*Eryngium planum*), (B)—field eryngo (*Eryngium campestre*), and (C)—sea holly (*Eryngium maritimum*)).

The aerial and underground parts of species in the genus *Eryngium* contain a significant amount of biologically active substances (BASs). Flavonoids such as rutin, kaempferol, isoquercetin, luteolin, and apigenin, as well as caffeic, rosmarinic, ferulic, and chlorogenic acids, among other compounds capable of pharmacological activity, were identified in *E. planum* [7]. Polyphenols and pectic substances in the aerial parts of *E. planum*, *E. campestre*, and *E. maritimum* were studied [8]. Isoquercetin, quercetin, kaempferol, chlorogenic acid, caffeic acid, *p*-coumaric acid, and ferulic acid were identified in all *Eryngium* species. These phenolics have significant antioxidant activity that might cause hepatoprotective and neuroprotective effects. The pectin content extracted with hot water varied significantly among the species, with the highest level found in *E. maritimum* (8.8%), a moderate level in *E. planum* (4.65%), and the lowest in *E. campestre* (1.7%) [8]. The primary components of the leaves and stems of essential oils of *E. planum* were monocyclic monoterpenes (limonene, α -, β -pinene) and sesquiterpenes. The essential oil derived from the inflorescences of *E. planum* contained 43.2% chrysantenyl acetate and oxygenated sesquiterpenes [9]. The roots of *E. planum* contain carbohydrates such as fructose, glucose, and sucrose; organic acids, including malic, citric, glycolic, and oxalic acids; essential oil (0.13%); and triterpenoid saponins: eryngium saponins A, B, C, and D. Additionally, the roots contained polyacetylene compounds, such as faltarione, faltarinolone, and faltarinol, as well as phenolic carboxylic acids and their derivatives, including rosmarinic and chlorogenic acids [10,11]. The flowers of *E. planum* contained carbohydrates; organic acids (malic, citric, glycolic, and oxalic acids); and flavonoids, such as quercetin and kaempferol [8]. The fruits of *E. planum* are characterized by the following chemical composition: carbohydrates (fructose, glucose, and sucrose), oxalic acid, flavonoids (quercetin and kaempferol), and fatty acids (27–29%) such as palmitic, stearic, oleic, linoleic, and petroselinic acids [12–15]. The chemical composition of raw materials can vary significantly depending on their growth locations. Since Ukrainian samples of *E. planum* have not been studied at all, it is of particular interest to determine their phytochemicals to expand knowledge about the regional influence on the accumulation of BASs, as the phytochemical composition of plants is closely related to their

pharmacological activity. Based on these data, we predict that the *E. planum* extracts may exhibit hepatoprotective, antimicrobial, and sedative activities.

The Pakistani laboratory “Kamal Laboratories” produces homoeopathic remedies under the names “ERYNGIUM Ø” and “ERYNGIUM 30”, based on *E. aquaticum*. These are indicated for issues with the urinary system, such as difficulty with and frequent urination, pain and spasms in the urinary tract, and renal colic [16]. *E. aquaticum* L. is listed in the *American Homoeopathic Pharmacopoeia* and the *Homoeopathic Pharmacopoeia of India* [17,18]. In 2021, the French company SEPPIC introduced a new active cosmetic ingredient based on *E. maritimum*. Their development, patented under “CELTOSOME™ *Eryngium maritimum*”, is a concentrated active ingredient derived from sea holly, containing a specific composition of phenolic acids and flavonols for skin restoration and elasticity [19]. In traditional medicine, the *Eryngium* species treats dropsy, sexual disorders, stomach and heart pain, insomnia, rheumatism, toothache, cavities, and bronchial asthma. The roots were considered an antidote for mushroom poisoning. Externally, *Eryngium* is applied in baths for phytodermatoses and arthritis [20]. Ethanolic extracts of *E. planum* from basal leaves have demonstrated anti-amoebic activity [21]. Extracts of *E. planum* and *E. campestre* are used as remedies for inflammatory dental diseases such as periodontitis, cavities, and toothache [22]. *E. planum* extract has an inhibitory effect on α -amylase and α -glucosidase, making it beneficial in treating type II diabetes mellitus [7]. Preclinical in vivo studies indicate that a 70% ethanolic extract of *E. planum* improved long-term memory in rats [23]. Extracts of *E. billardieri* induced apoptosis in cancer cells [24]. In modern scientific literature, there are no data on the effects of *E. planum* extracts on the functional state of the liver and nervous system, which is undoubtedly a gap in the study of this plant. Thus, numerous studies confirm that *Eryngium* species are promising medicinal plants. Based on the experience of using *E. planum* raw materials in folk medicine in many countries and the current state of research on its chemical composition, especially phenolics, the hypothesis of our studies was that extracts from this plant might possess significant hepatoprotective and sedative activities, which could be utilized in the development of modern phytochemical products.

The aim of this research was to study the main biologically active substances and antimicrobial, hepatoprotective, and neurotropic activities of aqueous and ethanol–aqueous *E. planum* herb extracts to expand scientific knowledge and provide evidence for the potential use of this plant in modern medical and pharmaceutical practice. The sedative and hepatoprotective activities of *E. planum* were studied for the first time.

2. Materials and Methods

2.1. Plant Raw Materials

The wild growing *E. planum* herbs were harvested during the peak flowering period in the middle of July 2020 in the meadows on the outskirts of the village Pidluzhzhya, Tysmenytsia District, Ivano-Frankivsk Region (48°56′29″ N, 24°46′16″ E). The collection adhered to the fundamental rules for harvesting medicinal raw materials, with care taken to preserve the local flora [25]. Before harvesting, the plant was identified [26].

The flowering tops (25–40 cm) of the plant were cut using a knife in dry weather. Immediately after collection, the raw materials were dried in the shade outdoors, avoiding direct sunlight. A total of 2.0 kg of dry raw material was obtained. The plant’s identity was confirmed based on the botanical catalogue with the consulting assistance of Professor A.R. Grytsyk from Ivano-Frankivsk National Medical University (IFNMU) [26]. Voucher specimens No. 583–585 were deposited at the Department of Pharmaceutical Management, Drug Technology, and Pharmacognosy at Ivano-Frankivsk National Medical University.

2.2. Extracts Preparation

The objects of the study were soft extracts of the *E. planum* L. herb, obtained using purified water, and 40% and 70% ethanol. Since, traditionally, galenic preparations are obtained from plant raw materials in both traditional and official medicine, such as infusions or decoctions (using water) and tinctures (using a 70% ethanol solution), and considering that in everyday practice vodka (a 40% ethanol solution) is often used for home-made beverages, we selected these extractants specifically for their availability to the average consumer. The *E. planum* herb was pre-ground to a particle size of 0.5–2 mm. A total of 500 g of raw material was extracted using the fractional maceration method with purified water, 40% ethanol, or 70% ethanol, yielding soft extracts labelled EP1, EP2, and EP3, respectively. Triple extraction was performed using the following sequential raw material-to-solvent ratios: 1:5, 1:3, and 1:2. The aqueous–alcoholic extracts were infused for 12 h at each stage at room temperature, after which the extracts were drained and combined. For the aqueous extract, the mixture at each stage was boiled with a new portion of the solvent for 15 min and then infused for 3 h. The combined extracts were filtered, and the solvent was evaporated under vacuum using a Buchi Rotavapor R-300 (Buchi Labortechnik AG, Flawil, Switzerland) under the following conditions: vacuum 150 mbar, rotation 80 rpm, and heating bath at 80 °C, until the final moisture content did not exceed 25%. The yields of the soft extracts EP1, EP2, and EP3 were $24.6 \pm 0.9\%$, $23.1 \pm 1.1\%$, and $22.1 \pm 0.8\%$, respectively.

2.3. Phytochemical Research

The flavonoids were rigorously analyzed using HPLC on a chromatograph Agilent Technologies 1200. The mobile phase was precisely composed of acetonitrile (A) and a 0.1% formic acid solution in water (B). A systematic gradient elution was employed under the following conditions: at 0 min, A was set at 30% and B at 70%; by 20 min, A was increased to 70% while B decreased to 30%; at 22 min, A reached 100% and B dropped to 0%; and at 30 min, A remained at 100% and B at 0%. Separation was effectively achieved on a Zorbax SB-C18 chromatographic column (3.5 μ m, 150 $\times$ 4.6 mm) from Agilent Technologies (Santa Clara, CA, USA), maintaining a flow rate of 0.25 ml/min. A sample injection volume of 100 μ L was utilized, and the column was consistently maintained at 25 °C. Detection was conducted using a diode-array detector, recording signals at 280 and 365 nm, with spectra comprehensively captured in the range of 210–270 nm [27,28].

The assay of hydroxycinnamic acids was carried out using the chromatograph Agilent Technologies 1200. The mobile phase served methanol (A) and 0.1% formic acid in water (B). The gradient elution parameters were precisely set at 0 min—A (25%): B (75%); at 25 min—A (75%): B (25%); at 27 min—A (100%): B (0%); and at 35 min—A (100%): B (0%). Separation was effectively achieved on a Zorbax SB-Aq column (4.6 mm $\times$ 150 mm, 3.5 μ m) (Agilent Technologies, USA) with a flow rate of 0.5 ml/min, a column temperature maintained at 30 °C, and an injection volume of 4 μ L. Detection was conducted with a diode-array detector, with signals meticulously monitored at 250 and 275 nm, and absorption spectra consistently recorded within the range of 210–270 nm [29–31].

The analysis of tannin metabolites was conducted using the chromatograph Agilent Technologies 1200. The mobile phase effectively comprised methanol (A) and 0.1% formic acid in water (B). The gradient elution conditions were defined as follows: at 0 min—A (20%): B (80%); at 25 min—A (75%): B (25%); at 27 min—A (100%): B (0%); and at 35 min—A (100%): B (0%). The separation process employed a Zorbax SB-C18 chromatographic column (3.5 μ m, 150 $\times$ 4.6 mm) from Agilent Technologies, USA, operating with a flow rate of 0.25 mL/min, a thermostat set to a precise 35 °C, and an injection volume of 4 μ L. The

diode-array detector reliably recorded signals at 250 and 275 nm, while absorption spectra were systematically captured across the range of 210–270 nm [32,33].

The substance identification and quantification were based on comparisons with standard reference materials from Sigma-Aldrich (Burlington, MA, USA). The quantitative analysis of the total flavonoid content, expressed as rutin, was carried out spectrophotometrically according to the *Ukrainian State Pharmacopoeia* (SPHU) 2.0 [28,34]. The quantitative determination of tannins and total amounts of polyphenols in terms of pyrogallol was carried out using the spectrophotometric method in accordance with the pharmacopoeial method SPUH 2.0 [34–36]. The quantitative content of polysaccharides was determined according to the methodology described in the monograph “Plantago major leaves” in the SPUH 2.0, Vol. 3 [35].

2.4. Pharmacological Research

The pharmacological and toxicological properties of the *E. planum* herb extracts were studied according to the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, 1986) and other laws based on it [37–39]. This research was approved by the Bioethics Commission of the Ivano-Frankivsk National Medical University (protocol No. 125/22 from 25 May 2021).

2.4.1. Acute Toxicity

To determine the acute toxicity, the methodology for the preclinical safety studies of medicinal products was used. *E. planum* extracts were administered intragastrically to non-line white mice using a probe at a dose of 5000 mg/kg. The animals were divided into four groups, with six individuals in each group. Animals in the first three groups received EP1, EP2, and EP3 extracts of *E. planum*, respectively. The fourth group consisted of intact (control) animals. The animals were observed for 14 days. The following parameters were assessed: general condition, including mortality rate, breathing patterns, motor activity, skin, tail, ear, and mucous membrane coloration, salivation, and gastrointestinal state (characteristics and coloration of excretions). The body weight was monitored on days 3, 7, and 14. On the 15th day, the animals were decapitated under general anesthesia, organs were weighed, and blood samples were collected for biochemical analysis. This study aimed to evaluate the potential toxic effects and systemic impact of the *E. planum* extracts on various physiological and biochemical parameters [40].

2.4.2. Antimicrobial Activity

This study of the antimicrobial activity of *E. planum* herb extracts on the growth of pure cultures of bacteria and yeasts was conducted at the Department of Microbiology, Virology, and Immunology of IFNMU with the consultative assistance of the department head, Doctor of Medical Sciences, Professor R.V. Kutsyk. The selection of microorganisms for analysis was based on the recommendations of the WHO [41] and Ukraine [40] and the *European Pharmacopoeia* [34], as well as their prevalence in clinical cases. This research was performed on clinical isolates of antibiotic-sensitive and antibiotic-resistant microorganisms. Bacterial strains were identified through the biochemical microtests “STAPHY test 16”, “ENTERO test 24”, and “NEFERMENT test 24” (Lachema, Brno, Czech Republic), along with a combination of morphological and cultural characteristics in accordance with the guidelines provided in the 9th edition of *Bergey’s Manual of Determinative Bacteriology*. The identification of yeast-like fungal cultures was conducted using 40 biochemical tests with the VITEK 2 system and VITEK 2 YST ID cards (bioMérieux, Crapon, France) [42].

The screening of the antimicrobial activity of the plant extracts was performed using the agar diffusion micromethod. This method is characterized by a high sensitivity and discriminatory power, allowing for reliable differentiation between active and inactive

extracts. Petri dishes placed on a strictly horizontal and even surface were filled with 30 mL of agar. After the medium solidified, wells with a diameter of 4.0 mm were created using a specialized punch with uniform edges. The agar was evenly inoculated with a test-culture suspension (concentration of 1×10^7 CFU/mL). The experimental wells were filled with 20 μ L of plant extracts (100 mg/mL), while the control wells received 20 μ L of solvents (40% and 70% aqueous ethanol). After 24 h of incubation, the diameters of the growth inhibition zones for bacterial test cultures were measured. The fungistatic activity was recorded after 2 days of cultivation, and the fungicidal activity was recorded after 4 days [40]. Digital images of the agar plates were captured, and the diameters of the growth inhibition zones were established using the UTHSCSA ImageTool 3.0 software (The University of Texas Health Science Center in San Antonio, ©1995–2002) [43,44].

The activity of the *E. planum* herb extracts was established against the following microorganisms: *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Enterococcus faecalis*, α -hemolytic streptococci *Streptococcus oralis*, *Streptococcus gordonii*, β -hemolytic streptococci of group B *Streptococcus agalactiae*, β -hemolytic streptococci of group G *Streptococcus dysgalactiae* ssp. *equisimilis*, *Bacillus subtilis*, *Escherichia coli*, *Escherichia fergusonii*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida tropicalis*, *Candida lipolytica*, *Candida lusitanae*, and *Candida kefyr* [42].

2.4.3. Sedative Activity

The study of sedative activity was conducted on 35 non-linear sexually mature white mice weighing 18–20 g. The animals were divided into 5 groups of 7 animals each. Animals in groups I, II, and III received intraperitoneal injections of *E. planum* herb extracts EP1, EP2, and EP3, respectively, at a dose of 50 mg/kg body weight. Group IV received a reference drug, motherwort extract, at an effective dose of 50 mg/kg body weight. Group V consisted of intact animals that received an equivalent volume of purified water. The administration of phytopreparations began 5 days before the study, with the final dose administered 30 min before the experiment on the fifth day. The tests used included the “Elevated Plus Maze”, “Cube”, “Black-and-White Box”, and “Open Field”, which are a part of behavioral methods for studying the pharmacological activity of psychotropic agents. In the “Elevated Plus Maze” test, the apparatus consisted of two arms in the shape of a cross, one enclosed by walls. The dark arm allowed the test animals to hide and avoid light and open spaces, exhibiting defensive behavior. Entry into the open arm was considered an indication of exploratory behavior and, correspondingly, a lower level of fear of the unknown [32,40].

2.4.4. Hepatoprotective Activity

The study of the hepatoprotective activity of *E. planum* herb extracts was conducted using a model of acute carbon tetrachloride-induced hepatitis [40,45]. The domestic hepatoprotective drug “Silibor” was used as a reference (at a dose of 25 mg/kg) [46]. Non-line male white rats (0.18–0.22 kg) were used in the research. Six animals were in each group. Groups EP1, EP2, and EP3 received the *E. planum* herb extracts (extractant: water, 50% ethanol, and 70% ethanol, respectively). The extracts and the reference drug were given orally through intragastric gavage as a water solution or suspension, freshly prepared right before administration. The control group consisted of animals that were subcutaneously injected with a 50% oil solution of carbon tetrachloride and did not receive any treatment. The intact animals were given only water. The intensity of pathological changes in the liver was assessed by measuring thiobarbituric acid reactive substances (TBA-RS) in blood serum and liver homogenates. The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) was measured in blood

serum [40,45]. ALT and AST activities were determined using the unified dinitrophenylhydrazine method of Reitman and Frankel with reagent kits from "Philisit-Diagnostics" (Ukraine) [47,48]. The ALP activity was determined photometrically by the intensity of the red dye formed by the oxidative coupling of phenol with 4-aminoantipyrine. The level of lipid peroxidation product (TBA-RS) was assessed through its reaction with 2-thiobarbituric acid spectrophotometrically, using the method of E.N. Korobeynikova and biochemical kits from the domestic manufacturer "Philisit-Diagnostics" (Dnipro, Ukraine) [49].

2.5. Statistical Analysis

The average value was calculated for at least three measurements in the phytochemical study and six ones in the pharmacological study. The values of the confidence interval were calculated using the Student's criterion limit. The data are presented as the mean $\pm$ SD [34,50].

3. Results

Extracts EP1, EP2, and EP3 from the *E. planum* herb are soft, viscous masses of a dark-brown to dark-brown-green color with a characteristic odor.

3.1. Phytochemical Analysis of Extracts

The qualitative composition of biologically active compounds (BACs) in the soft extracts of *E. planum* was studied using identification reactions and thin-layer chromatography (TLC). The soft extracts of the *E. planum* herb contain amino acids, simple phenols, coumarins, flavonoids, hydroxycinnamic acids, tannins, and saponins.

The HPLC method was used to identify and quantify phenolic compounds in the soft extracts of the *E. planum* herb (Table 1).

Table 1. Content of phenolic compounds in the soft extracts of the *Eryngium planum* herb.

№	Compound	Content in Dense Extract, µg/g		
		EP1	EP2	EP3
Hydroxycinnamic acids *				
1	Chlorogenic acid	3945.81 ± 174.12	4931.72 ± 215.32	4843.06 ± 92.07
2	Caffeic acid	1268.87 ± 94.14	1492.33 ± 87.12	1319.78 ± 79.16
3	Syringic acid	176.09 ± 13.07	234.98 ± 19.06	312.86 ± 15.37
4	<i>p</i> -Coumaric acid	418.61 ± 21.16	531.85 ± 16.43	591.77 ± 28.92
5	<i>trans</i> -Ferulic acid	729.98 ± 31.88	910.11 ± 29.19	937.32 ± 51.06
6	Sinapic acid	3011.84 ± 156.18	3532.33 ± 141.82	3319.96 ± 138.46
7	<i>trans</i> -Cinnamic acid	2358.07 ± 93.61	2841.49 ± 105.04	2909.52 ± 135.47
Flavonoids *				
8	Rutin	1382.762 ± 102.04	2412.37 ± 92.08	2631.62 ± 108.37
9	Quercetin-3-O-glucoside	509.71 ± 25.07	917.74 ± 37.05	993.52 ± 47.08
10	Naringenin	2944.71 ± 172.73	4430.62 ± 201.42	4873.07 ± 212.92
11	Neohesperidin	989.72 ± 39.09	2091.52 ± 87.41	2313.43 ± 93.36
12	Quercetin	697.92 ± 29.93	1393.55 ± 65.12	1493.72 ± 72.17
13	Kaempferol	919.23 ± 58.39	1801.17 ± 11.03	1945.91 ± 74.32
Tannin metabolites **				
14	Epicatechin	1507.72 ± 82.06	1420.08 ± 73.93	1274.58 ± 59.37
15	Epicatechin gallate	431.25 ± 15.03	368.42 ± 10.96	331.09 ± 21.12
16	Gallocatechin	1607.71 ± 57.86	1525.03 ± 65.41	1318.62 ± 45.16

Table 1. Cont.

№	Compound	Content in Dense Extract, µg/g		
		EP1	EP2	EP3
Content of compound groups, % ***				
	Total polyphenols (%)	14.92 ± 0.48	17.52 ± 0.61	21.84 ± 0.97
	Tannins (%)	9.13 ± 0.29	8.72 ± 0.21	8.17 ± 0.23
	Flavonoids (%)	3.11 ± 0.14	5.71 ± 0.26	6.19 ± 0.13
	Polysaccharides (%)	21.14 ± 0.95	7.73 ± 0.36	-

Notes: EP1—water extract, EP2—40% ethanolic extract, and EP3—70% ethanolic extract. * There is no statistically significant difference between the content in the extracts EP1, EP2, and EP3 ($p > 0.05$). ** The statistically significant difference exists between extracts EP1-3 ($p < 0.01$). *** The contents in extracts EP1-3 are statistically different ($p < 0.01$).

In total, 16 phenolic compounds were identified in the soft extracts of the *E. planum* herb. The dominant hydroxycinnamic acids in the extracts were chlorogenic acid, sinapic acid, and *trans*-cinnamic acid. Six flavonoids were identified and quantified in the soft extracts, with naringenin being the most abundant. Among the tannin metabolites, the dominant compounds were epicatechin and galocatechin.

3.2. Pharmacological Research

3.2.1. Acute Toxicity

To assess the acute toxicity, we employed the established protocol for the preclinical safety evaluation of medicinal products. *E. planum* extracts were administered to animals intragastrically via a probe at a robust dose of 5000 mg/kg body weight. Immediately following the administration and throughout the experimental period, the overall condition of the animals was consistently stable. Normal salivation was observed in the group that consumed EP1. However, in the EP2 and EP3 groups, we noted the moistening of fur around the mouth within the first 24 h. All the groups exhibited calm and unlabored breathing. The animals were active, demonstrating preserved coordination, and no seizures occurred. Their fur remained dry, white, and smooth, while their tails and paws showed light pink coloration. Throughout the study, both the control and experimental groups experienced an increase in body weight, with no significant differences observed. On day 14, we evaluated the influence of *E. planum* extracts on the biochemical parameters of blood serum, specifically AST (0.48 ± 0.01 µmol/hour·mL) and ALT (0.29 ± 0.01 µmol/hour·mL) levels. These results demonstrated a significant difference ($p \leq 0.05$) when compared to the control group values of 0.42 ± 0.02 and 0.24 ± 0.01 , respectively. Thus, we affirm that the biochemical parameters of the mice's blood, under the influence of *E. planum* herb extracts, remained well within normal limits.

3.2.2. Antimicrobial Activity

The aqueous extract EP1 and the hydroalcoholic extract EP2 demonstrated negligible antimicrobial activity, whereas the *E. planum* herb extract EP3 exhibited moderate antimicrobial activity. The results are presented in Figure 2.

The levels of the extracts' antimicrobial activity are significantly lower than ones for the standard antibiotic Gentamicin and an antifungal agent Fluconazole. For example, the activity of Gentamicin against *Staphylococci* ranged from 10 to 28 mm, *Escherichia coli* showed an activity of 22 mm, and *Escherichia fergusonii* exhibited 12 mm. The activity of Fluconazole against yeast-like fungi of the genus *Candida* reached 19–20 mm [51].

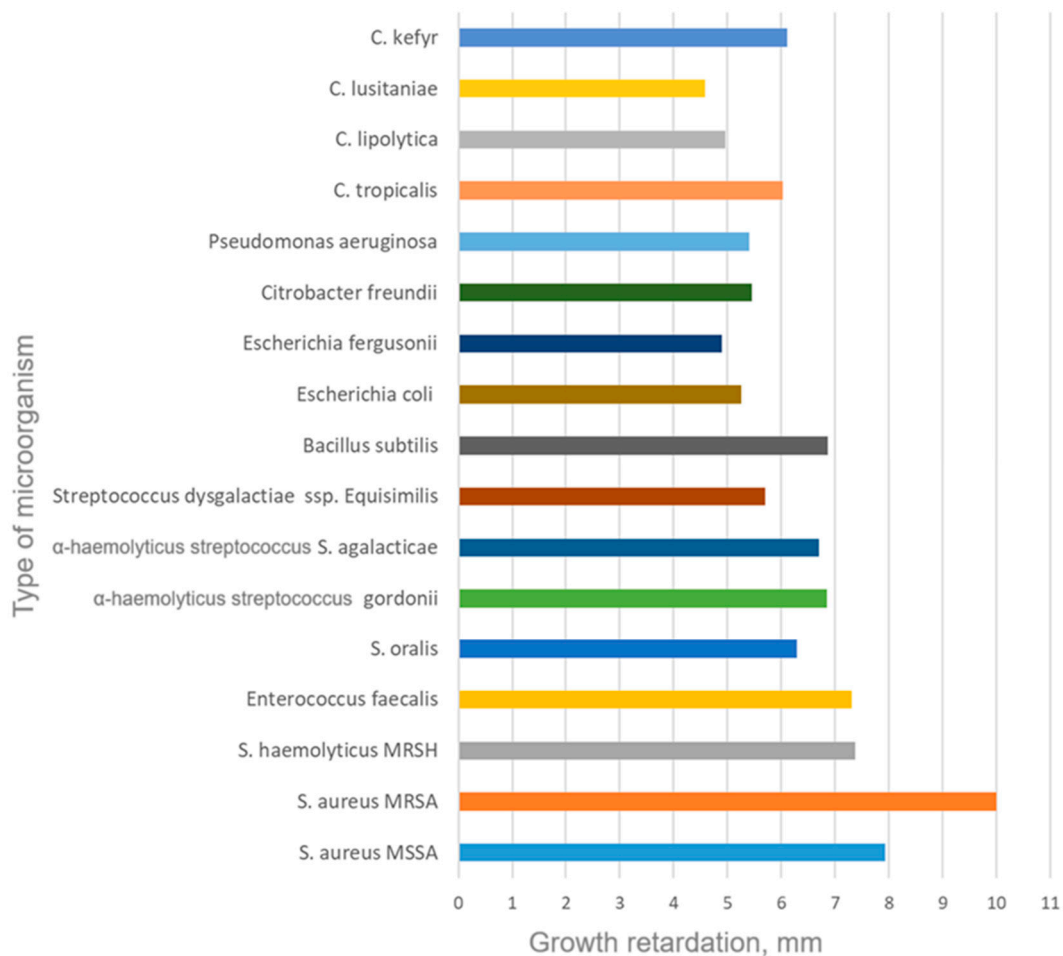


Figure 2. Antimicrobial activity of *Eryngium planum* herb extract EP3.

3.2.3. Sedative Activity

The results of the behavioral activity studies of animals in the “Elevated Plus Maze” test are presented in Table 2.

Table 2. Behavior of animals in the “Elevated Plus Maze” test.

Animal Group	Closed Arm	Time Spent, % Open Arm	In Center	Number of Downward Glances
I group (EP1)	60.9 ± 3.10 *	34.2 ± 1.71 *	4.9 ± 0.22 *	5.7 ± 0.29 *
II group (EP2)	53.1 ± 2.85 *	40.8 ± 1.25 *	6.1 ± 0.29 *	6.8 ± 0.30 *
III group (EP3)	48.3 ± 2.41 *	44.5 ± 1.21 *	7.2 ± 0.31 *	6.5 ± 0.35 *
IV group (control)	44.5 ± 2.40	49.7 ± 1.20	5.8 ± 0.28	7.8 ± 0.35
V group (intact)	78.8 ± 2.89 *	16.2 ± 0.82 *	5.0 ± 0.19 *	3.5 ± 0.12 *

Note: *—the deviation of the indicator is significant compared to the data of the control group ($p < 0.05$). EP1—water extract, EP2—40% ethanolic extract, and EP3—70% ethanolic extract.

In the “Open Field” test, the effects of the extracts on the central nervous system were examined to evaluate the sedative effect (Figure 3).

The data presented in Table 2 indicate that the animals in groups III and IV spent more time in the open arms and the center of the maze compared to the closed arms, suggesting high exploratory activity and low fear levels. The indicators for the group III animals, which received *E. planum* extract EP3, were comparable to those of the control group, which received motherwort extract, a proven sedative. The time spent by animals in the maze’s center was minimal across all the groups and did not differ significantly. An important indicator of stress resilience is the number of downward glances by the animals.

This indicator for groups I–III was nearly double that of the intact group. The significant reduction in activity indicators for the experimental animals (Figure 3) over five minutes indicates the pronounced sedative activity of the tested extracts. The locomotor activity of animals in group III decreased significantly by 2.4 times compared to the control group.

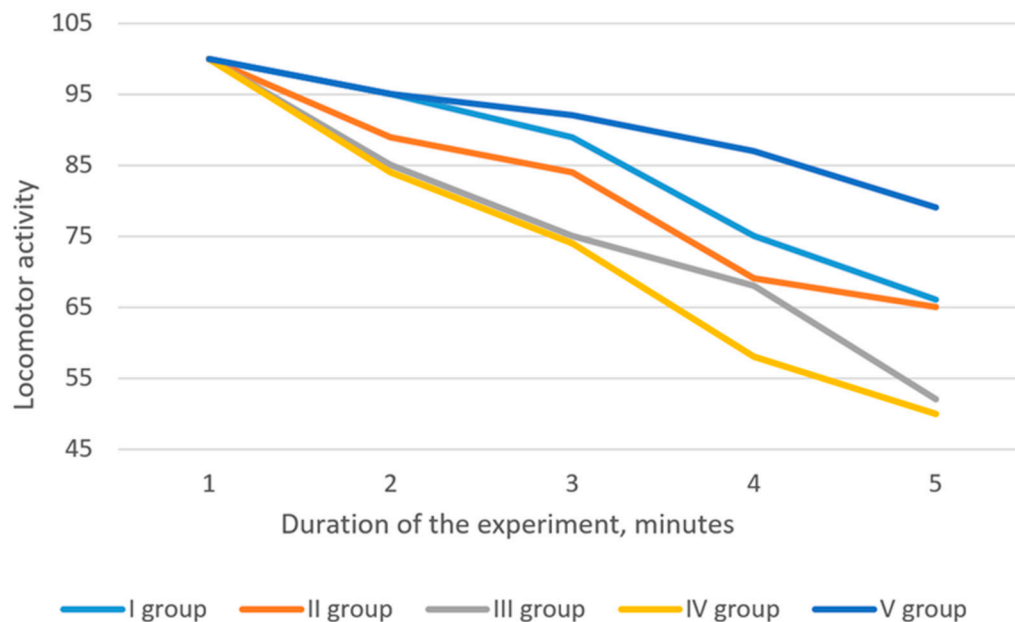


Figure 3. Locomotor activity of animals in the “Open Field” test.

The “Cube” test, designed to study cognitive responses and fear levels toward a new object, provided further insights. The results of these studies are presented in Table 3.

Table 3. Behavior of animals in the “Cube” test.

Group	Time of First Exit (s)	Number of Exits	Total Duration of Exits (s)
I group (EP1)	53.1 ± 2.15 *	2.5 ± 0.01 *	37.2 ± 1.18 *
II group (EP2)	28.6 ± 1.41 *	2.7 ± 0.01 *	36.2 ± 1.41 *
III group (EP3)	19.0 ± 1.12 *	3.2 ± 0.02 *	63.8 ± 2.09 *
IV group (control)	14.3 ± 0.92	5.0 ± 0.02	78.0 ± 2.14
V group (intact)	75.0 ± 3.16 *	1.8 ± 0.01 *	16.1 ± 0.98 *

Note: *—the deviation of the indicator is significant compared to the data of the control pathology group ($p < 0.05$). EP1—water extract, EP2—40% ethanolic extract, and EP3—70% ethanolic extract.

The data presented in Table 3 indicate that *E. planum* extracts and motherwort extract influenced the locomotor activity of mice toward a novel object. The first approach to the cube was performed the fastest by mice in groups III and IV (19.0 s and 14.3 s, respectively). The total duration of the approaches by the mice in group III was nearly four times greater than that of the intact animals, indicating increased exploratory activity due to the EP3 extract and a lack of anxiety. For the “Black-and-White Box” test, a two-chamber apparatus was used, with one chamber shielded from light and the other illuminated. Mice were placed in the dark compartment, and the latency of the first exit to the light chamber, the number of exits, and the duration of time spent in the light compartment were recorded. The results of these studies are presented in Table 4.

Table 4. Behavior of animals in the “Black-and-White Box” test.

Group	Time of First Exit (s)	Number of Exits	Total Duration of Exits (s)
I group (EP1)	23.0 ± 0.98 *	6.5 ± 0.15	33.2 ± 1.22
II group (EP2)	12.7 ± 0.51	10.2 ± 0.20	48.2 ± 1.25
III group (EP3)	10.3 ± 0.12	7.5 ± 0.15	63.6 ± 1.65
IV group (control)	8.3 ± 0.10	9.8 ± 0.15	55.3 ± 1.50
V group (intact)	28.2 ± 0.26	3.8 ± 0.15	13.2 ± 0.25

Note: *—the deviation of the indicator is significant compared to the data of the control pathology group ($p < 0.05$).

The time of the first exit from the dark compartment (Table 4) is a critical indicator characterizing the animals’ fear level. The first exit times for the animals in groups II and III were 12.7 s and 10.3 s, respectively, close to the times observed in the control group (8.3 s) that received motherwort extract. The number of exits from the dark chamber to the light chamber varied between 3 and 11 across the groups; however, this parameter did not significantly affect the total duration of time spent in the light chamber. The longest duration in the light chamber was observed in the group III animals (63.6 s). These results suggest that *E. planum* extracts EP2 and EP3 reduced feelings of fear and anxiety in the experimental animals.

3.2.4. Hepatoprotective Activity

The results of the effects of *E. planum* extracts on the biochemical parameters of blood serum and liver homogenates in the model of acute carbon tetrachloride-induced hepatitis are presented in Figure 4.

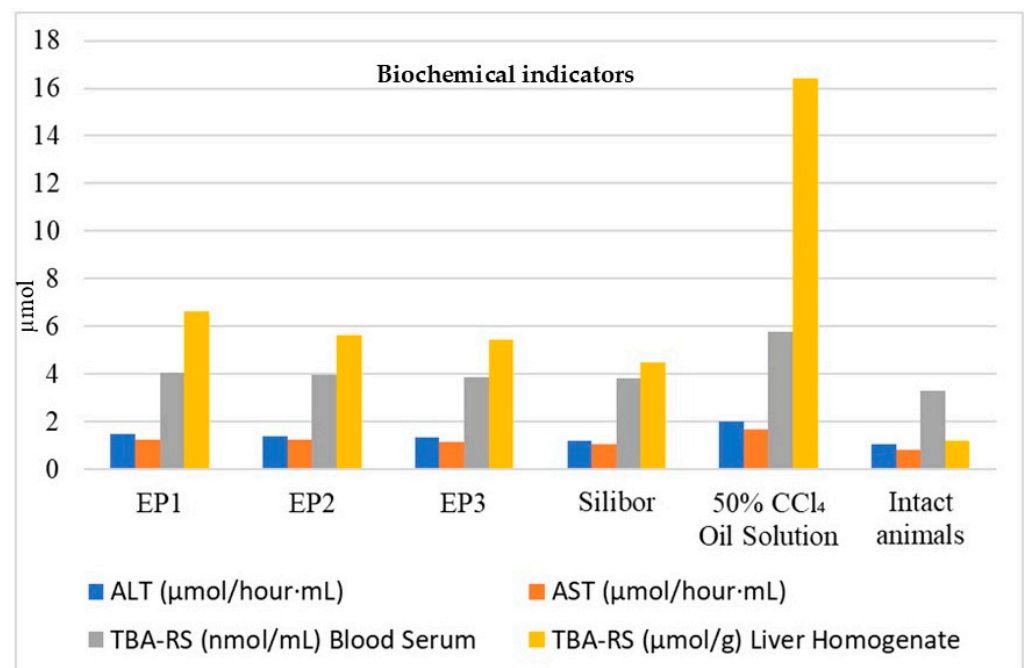


Figure 4. Effects of *Eryngium planum* extracts on biochemical parameters of blood serum and liver condition in acute hepatitis. EP1—water extract, EP2—40% ethanolic extract, and EP3—70% ethanolic extract.

The results (Figure 4) demonstrated that the administration of carbon tetrachloride caused liver damage, as evidenced by the altered ALT, AST, and ALP activities and an accumulation of TBA-reactive substances (TBA-RS) compared to the intact animals. The ALT and AST levels in the untreated animals approximately doubled compared to those in

the intact animals. The ALP and TBA-RS levels in the blood serum of the untreated animals increased by 20.7% and 75.3%, respectively, relative to the intact animals. The TBA-RS levels in liver homogenates increased approximately 13-fold. The use of *E. planum* herb extracts and the reference drug “Silibor” in experimental hepatitis was accompanied by a noticeable reduction in pathological manifestations. This was reflected in the decreased TBA-RS levels in blood serum and liver homogenates, as well as the reduced ALT and AST activities in the blood serum, compared to the untreated group of animals. At the same time, the values of these indicators are comparable. The levels of ALT and AST were, on average, 10% higher in the group receiving *E. planum* herb extracts compared to the reference drug “Silibor”, while the TBA-RS values did not show significant differences. All of this indicates that *E. planum* extracts exhibit hepatoprotective activity comparable to that of the reference drug “Silibor”.

4. Discussion

The phytochemical analysis of the thick extracts of the *E. planum* herb identified the qualitative composition and quantitative content of the main groups of biologically active substances, including flavonoids, hydroxycinnamic acids, tannins, and polysaccharides. Using HPLC, 16 phenolic compounds were identified and quantified in the dense extracts (Table 1). Among all the identified flavonoids, naringenin was dominant (33.97%), while chlorogenic acid (34.05%), sinapic acid (24.37%), and *trans*-cinnamic acid (19.62%) were the most abundant hydroxycinnamic acids. Previously, it was reported that the extracts of *E. planum* contain mainly flavonoids, especially rutin and isoquercitrin [7,8], but in our samples, naringenin was the dominant compound.

This study conclusively demonstrated that the intragastric administration of *E. planum* extracts at a dose of 5000 mg/kg does not cause fatalities in animals. There were no changes in behavior or vital signs observed, confirming the absence of toxic effects at this dosage. This establishes the extracts as effectively non-toxic (toxicity class V, LD50 > 5000 mg/kg). As illustrated in Figure 2, body weight consistently increased in all the experimental animals. Macroscopic evaluations of the internal organs across all groups revealed no pathological signs. The organs exhibited an anatomically normal color, shape, size, and position. The heart muscle displayed the correct form, characterized by a dark-red cut surface, while the lungs remained unaffected. The liver, pancreas, and kidneys showed uniform and smooth surfaces, and the spleen was elastic and filled with blood. The *E. planum* extract EP3 (utilizing 70% ethanol as the solvent) demonstrated moderate antimicrobial activity (as evidenced in Figure 2) against gram-positive microorganisms, including staphylococci, streptococci, and bacilli. Prior research confirmed that *E. amethystinum* and *E. alpinum* possess a broad spectrum of antibacterial and antifungal activity, with minimum inhibitory concentrations (MIC) of 1.944 mg/mL and 1.11 mg/mL, respectively. The antibacterial efficacy of *E. caeruleum* remedies against six bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptomyces scabies*, *Erwinia amylovora*, *Xanthomonas axonopodis* pv. *citri*, and *Klebsiella* sp.) has been conclusively validated in vitro [52]. Thus, the data supporting the antimicrobial activity of *E. planum* extracts are both original and significant.

The sedative activity of *E. planum* was studied for the first time. It was found that EP3 extract (extractant—70% ethanol) has the most pronounced sedative effect due to a significant decrease in motor activity in the «Open Field test» and reduction in concern in the «Cube tests», «Raised Cross Maze», and “Black and White Camera”. We used the standard behavioral tests, which do not allow us to draw conclusions about the mechanisms of the sedative action of the extracts. This requires further research. It may involve conducting molecular docking to assess the affinity of the main identified compounds in the extracts to the primary receptors of the nervous system. Previously, the molecular docking analysis

revealed a high probability of γ -aminobutyric acid type A (GABAA) and N-methyl-D-aspartate (NMDA) receptor antagonism with some of the identified compounds.

The results of this study (Figure 4) showed that the administration of *E. planum* extracts EP1, EP2, and EP3 to animals reduced ALT activity by 26.9%, 30.8%, and 33.8%, respectively, and AST activity by 23.9%, 25.7%, and 30.5%, respectively. The extracts also reduced TBA-reactive substances (TBA-RS) levels in blood serum by 29.3%, 31.5%, and 32.4%, respectively, and in liver homogenates by 59.5%, 65.4%, and 66.8%, respectively, compared to untreated animals. These findings indicate that using *E. planum* herb extracts in acute toxic liver damage significantly reduces the toxic effects of carbon tetrachloride, providing hepatoprotective activity that is nearly comparable to the reference drug. Among the extracts, EP3 at a dose of 25 mg/kg body weight demonstrated the most intensive and effective action in acute hepatitis compared to EP1 and EP2. The hepatoprotective activity of the *E. planum* extracts is comparable to that of the reference drug “Silibor”. The hepatoprotective activity of *E. planum* extracts has also been studied for the first time.

5. Conclusions

Eryngium planum extracts are promising substances for use in medical practice particularly for disorders of the liver and nervous system. The main biologically active substances were identified and quantified in the soft *E. planum* L. herb extracts, which is the basis for their further standardization. The soft extracts of *E. planum* were practically non-toxic substances ($LD_{50} > 5000$ mg/kg) with pronounced sedative and hepatoprotective effects, which were discovered at first for this plant. The most promising substance was the soft *E. planum* extract obtained using a 70% aqueous ethanol solution. Its further pre-clinical and clinical studies might lead to its implementation in clinical medicine.

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Institutional Review Board Statement: The pharmacological and toxicological properties of *E. planum* herb extracts were studied according to the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, 1986). The research was approved by the Bioethics Commission of the Ivano-Frankivsk National Medical University (protocol №125/22 from 25 May 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the results of this study can be obtained from the corresponding authors upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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