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Sanicula europaea L. Herb and Rhizomes with Root Extracts with Hemostatic, Wound Healing, Anti-Inflammatory and Antimicrobial Activity: Phytochemical and Pharmacological Research

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Featured Application

Based on the results of phytochemical analysis and pharmacological screening, herb extracts of *Sanicula europaea* L. demonstrated pronounced hemostatic, wound-healing, anti-inflammatory, and antimicrobial activities and may be used for the development of novel plant-based medicinal products.

Abstract

Sanicula europaea L. (Apiaceae), commonly known as European sanicle, has long been used in traditional medicine as a hemostatic agent, for accelerating wound healing, and for treating inflammatory and dermatological conditions. However, scientific evidence supporting these uses remains limited. Comprehensive phytochemical and pharmacological screening of extracts from the herb and rhizomes with roots of *S. europaea* holds promise, as aqueous and hydroethanolic extracts were obtained from its aerial and underground parts. Phytochemical analysis identified 16 phenolic compounds, including tannins, flavonoids (3.61–5.46% in the herb extracts; 0.13–0.21% in the rhizome-root extracts), hydroxycinnamic acids, and coumarin. The total phenolic content in the extracts ranged from 11.08% to 15.02%. Rosmarinic acid was the most abundant among the hydroxycinnamic acids. Quercetin and apigenin emerged as the leading flavonoids, and epicatechin gallate and gallocatechin were identified as the predominant tannin-related compounds. All tested extracts demonstrated anti-inflammatory activity in a formalin-induced paw oedema model. Hemostatic properties were assessed using the Duke bleeding time method, and the herb extracts significantly reduced bleeding time. The use of herb extracts also accelerated wound healing. Both herb and rhizome-root extracts exhibited inhibitory effects against *P. aeruginosa*, *E. coli*, *P. vulgaris*, *S. aureus*, and *S. epidermidis* in the agar diffusion method with paper discs. Based on the results of pharmacological screening, herb extracts of *S. europaea* demonstrated pronounced hemostatic, wound-healing, anti-inflammatory, and

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antimicrobial activities. Future studies should focus on expanding the experimental model and conducting clinical trials to assess safety, optimal dosage, and long-term efficacy.

Keywords: *Sanicula europaea*; herb; rhizomes with roots; extract; phenolic compounds; hemostatic activity; wound-healing activity; anti-inflammatory activity; antimicrobial activity

1. Introduction

Among more than 440 families of flowering plants, the Apiaceae (Umbelliferae) family ranks sixth in terms of the number of taxa and seventh in terms of species diversity [1]. The highest species richness of this family is observed in moderately warm and subtropical areas of the Northern Hemisphere, while in tropical countries, species are mainly concentrated in mountainous regions [2–4]. Many food, fodder, medicinal, and aromatic plants belong to the Apiaceae family and are utilised across various countries. Certain Apiaceae species play a significant role in shaping vegetation cover, acting as dominant components of herbaceous communities, particularly in mountainous and arid regions [1,5]. Of particular interest in this context are plants of the genus *Sanicula*, which comprises approximately 50 species, with only *Sanicula europaea* L. occurring in Ukraine. This species is widely distributed across forested regions of Europe, Western Asia, and Northern Africa, and is predominantly found in deciduous and mixed forests [6,7]. *S. europaea* has long been used in traditional medicine for stopping bleeding, wound healing, and the treatment of inflammatory and dermatological conditions [8]. However, the number of scientifically substantiated studies supporting these uses remains limited. Its broad distribution in the European region, substantial natural resources, and traditional medicinal applications make this plant and its preparations a promising candidate for integration into pharmaceutical and medical practice.

S. europaea is characterised by a chemical diversity of biologically active compounds, which underlie its broad spectrum of pharmacological and biological activities. Species of the genus *Sanicula* contain a wide range of bioactive constituents, including tannins, flavonoids, organic acids, saponins, polysaccharides, coumarins, and essential oils [9–11]. Both the underground and aboveground parts of *S. europaea* accumulate organic acids; triterpenoid saponins (acidic and neutral monodesmosides, saniculosides A, B, C, D); nitrogen-containing compounds; hydroxycinnamic acids; vitamins K and C; flavonoids; tannins, bitter substances; and essential oils [12–14]. The rhizomes with roots of *S. europaea* contain organic acids (malic, citric, malonic, and oxalic), triterpenoid saponins (3.3–5.5%), the nitrogen-containing compound allantoin, as well as hydroxycinnamic acids and their derivatives: chlorogenic acid (0.8%) and rosmarinic acid (1.4%). The leaves are characterised by the presence of organic acids (malic, citric, malonic, and oxalic), triterpenoid saponins (2.8–3.6%), vitamin C, hydroxycinnamic acids and their derivatives: chlorogenic acid (0.6%) and rosmarinic acid (1.7%), as well as flavonoids including quercetin, isoquercetin, rutin, and astragalin. In the flowers and fruits, organic acids (malic, citric, malonic, and oxalic), triterpenoid saponins (3.0–3.6%), and hydroxycinnamic acids with their derivatives: chlorogenic acid (0.95% and 1.05%) and rosmarinic acid (3.1% and 1.1%), respectively, have been identified [12]. However, all these data were obtained quite a long time ago and are primarily found in reference-based popular scientific literature. In recent years, there has been a noticeable lack of contemporary publications concerning the investigation of raw materials and medicinal products derived from *S. europaea*. Meanwhile, the advancement of modern phytochemical research methods offers the potential to expand current knowledge

about their phytochemical composition and forecasted therapeutic applications. Further comparative analysis of the chemical composition of aerial and underground parts of *S. europaea* will provide a foundation for the scientific justification of their use in the production of phytopharmaceuticals and for elucidating the biochemical mechanisms underlying their pharmacological activity.

In Ukraine, *S. europaea* is not officially recognised as a medicinal plant, yet it is used in folk medicine and homoeopathy. This plant is not included in the State Pharmacopoeia of Ukraine [15], nor is it listed in the official register of medicinal products or medicinal plants [16]. Furthermore, there is no approved normative documentation regulating its quality parameters for pharmaceutical use. In traditional medicine, infusions and decoctions are used to treat various types of bleeding, bronchitis, pneumonia, and gastrointestinal disorders. Externally, the plant is used for treating wounds, burns, and frostbite, as well as for gargling in cases of stomatitis and tonsillitis. The rhizome tincture is traditionally regarded as a remedy that enhances male sexual function [17–19]. In folk medicine across various countries, the multifaceted use of *S. europaea* has been documented. In the Czech Republic, it is applied for chronic wounds; in the Caucasus region, for respiratory diseases; in Bulgaria, for dermatological conditions; in Romania, as an expectorant and sedative; in the Netherlands, for internal bleeding; and in Germany, as an astringent and antimicrobial agent [20–22]. Since phenolic compounds and saponins have previously been identified in *S. europaea* raw material, it is likely that these constituents are responsible for the types of biological activity exhibited by galenic extracts traditionally used in folk medicine. Therefore, one of the hypotheses of our study is to experimentally validate these ethnopharmacological claims, given the limited availability of scientific evidence supporting them. In future studies, it would be appropriate to identify and isolate individual compounds or groups of compounds responsible for these activities. However, the present work is focused on investigating whole galenic extracts derived from these types of *S. europaea* raw materials.

Recent studies confirm the anti-inflammatory and antiviral activity of *S. europaea*. Researchers from Austria have demonstrated that its extracts inhibit the formation of inflammatory mediators [23], while scientists from Turkiye and Japan have shown that aqueous leaf extracts exhibit activity against influenza and parainfluenza viruses [24,25].

In homoeopathy, *S. europaea* is included in the formulation of RICURA® spag. Peka N., which is effective in the treatment of acute rhinosinusitis. In Ukraine, the company "Nutrimed" (Kyiv, Ukraine) has developed the phytocomplex "Kamavit," used for managing urological and sexual disorders in men [26]. Thus, *S. europaea* exhibits a broad spectrum of pharmacological properties and represents a promising candidate for further scientific investigation and integration into medical practice.

The aim of this study was to identify the key bioactive constituents of *S. europaea* herb and rhizome-with-root extracts, and to evaluate their antimicrobial, anti-inflammatory, haemostatic, and wound-healing properties, as previously reported in folk medicine. This study presents, for the first time, specific aspects of the phytochemical composition of aqueous and aqueous-ethanolic extracts of *S. europaea* herb and rhizomes with roots, and provides new in vitro evidence of their bacteriostatic effects, along with experimental in vivo confirmation of haemostatic and wound-healing activities, thereby supporting the ethnobotanical use of *S. europaea* raw material.

2. Materials and Methods

2.1. Chemicals and General Experimental Conditions

All reagents used in the experimental protocols were of analytical or HPLC grade, unless otherwise specified. Deionised water (Milli-Q, MilliporeSigma, Burlington, MA, USA) with a resistivity of 18.2 MΩ·cm was used in all experiments. Ethanol (≥96.0%),

acetonitrile (≥99.9%, HPLC grade), formic acid (≥98%, analytical grade), glacial acetic acid (≥99.7%, analytical grade), and ethyl acetate (≥99.5%, analytical grade) were purchased from VWR Chemicals (Radnor, PA, USA). Orthophosphoric acid (≥85%, analytical grade) and trifluoroacetic acid (≥99%, HPLC grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Phenolic standards, including quercetin, kaempferol, apigenin, coumarin, chlorogenic, rosmarinic, caffeic and ferulic acids (≥95–98%, analytical grade), were acquired from Carl Roth GmbH (Karlsruhe, Germany). Aluminium chloride and additional standards used for HPLC profiling, gallocatechin, catechin, epicatechin, catechin gallate, epicatechin gallate, ellagic and gallic acids, epigallocatechin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Carrageenan (food-grade, refined powder) and agar (microbiological grade) were obtained from Merck KGaA (Darmstadt, Germany).

2.2. Plant Raw Materials

The plant raw materials: herb and rhizomes with roots of *S. europaea* (0.5 kg of each kind) were collected in the Ivano-Frankivsk region (Viktoriv village, Halych district, 49.050° N 24.628° E) during the phases of peak flowering (June–July 2024) and senescence of the aerial parts (October–November 2024), respectively. Standard protocols for harvesting medicinal plant raw materials were strictly followed, with particular attention paid to preserving the surrounding flora [27]. Prior to collection, the plant species was accurately identified. The identification was confirmed based on a botanical catalogue [28], with consultative support from Professor A. R. Hrytsyk of Ivano-Frankivsk National Medical University (IFNMU). Voucher specimens No. 421–423 of *S. europaea* were deposited at the Department of Pharmaceutical Management, Drug Technology, and Pharmacognosy at IFNMU. The above-ground part of the plant (up to 20 cm) was harvested using a knife or pruner in dry weather, after the dew had evaporated and following 3–5 consecutive days without precipitation. The plant material was dried in a well-ventilated room in the shade, spread in a thin layer on paper, and periodically turned to ensure uniform drying. Rhizomes with roots of *S. europaea* were harvested by carefully digging the plants out of the soil. The collected raw material was cleaned of soil, washed with running water, and any damaged or rotten parts were removed. The cleaned rhizomes with roots were air-dried for several hours, followed by drying in a shaded, well-ventilated room, where they were spread in a thin layer and periodically turned to ensure even drying. The plant material was dried in the shade in a well-ventilated room, spread in a thin layer on paper. The dried raw material was stored in paper bags and used for experimental purposes within one year of the date of collection.

2.3. Extract Preparations

Complexes of biologically active compounds (BACs) from the herb and rhizomes with roots of *S. europaea* were obtained by remaceration, infusing the raw materials at a temperature of 50–60 °C with periodic stirring. The raw materials were ground using a Laboratory Grinder PG500 (Labtehservice, Odesa, Ukraine) to achieve particle sizes ranging from 1.0 to 2.5 mm. Purified water and 70% ethanol solution were used as extractants, as these solvents are most commonly employed in traditional medicine for the preparation of teas, decoctions, or tinctures, respectively, and provide a high level of BACs extraction.

To obtain the extract (solvent–purified water), 0.2 kg of the dried herb or 0.2 kg of the rhizomes with roots of *S. europaea* were infused with 2.80 L or 2.66 L of purified water, respectively. The raw material-to-solvent ratio was 1:10, taking into account the water absorption coefficient, which was 4.0 for the herb and 3.3 for the rhizomes with roots. The mixture was left to infuse for 1 h at room temperature. After swelling, the material was extracted for 30 min at a temperature of 50–60 °C. The resulting extract was cooled and

decanted, while the plant residue was re-extracted twice with fresh portions of solvent (2.0 L) under the same conditions. The obtained extracts (SEH0 and SER0, respectively) were combined, allowed to settle, and filtered.

To obtain the extract (solvent–70% ethanol), 0.2 kg of the dried herb or 0.2 kg of the rhizomes with roots of *S. europaea* were infused with 2.64 L or 2.44 L of 70% ethanol, respectively. The raw material-to-solvent ratio was 1:10, taking into account the ethanol absorption coefficient, which was 3.2 for the herb and 2.2 for the rhizomes with roots. The mixture was left to infuse for 1 h at room temperature. After swelling, the material was extracted for 30 min at a temperature of 50–60 °C. The resulting extract was cooled and decanted, while the plant residue was re-extracted twice with fresh portions of solvent (2.0 L) under the same conditions. The obtained extracts (SEH70 and SER70, respectively) were combined, allowed to settle, and filtered.

After decantation and filtration, the obtained extracts were dispensed into sterile 200 mL vials and subjected to lyophilisation. Initially, the vials were frozen in alcohol baths at a temperature not exceeding −40 °C for 30 min. Subsequently, the frozen extracts were tempered and stored in a refrigerator at a temperature not exceeding −30 °C for 12 h prior to loading into the freeze-drying apparatus. Lyophilisation was performed using a KS-30 sublimation unit (Frigera plant, Brno, Czech Republic). During the initial phase of drying, the pressure in the sublimator was reduced from $1 \cdot 10^{-1}$ to $1 \cdot 10^{-5}$ mmHg, and the temperature of the frozen extracts was lowered from −35 °C to −50 °C. After 2–2.5 h, heating was initiated, and over the next 12–16 h, the temperature was gradually increased from subzero to positive values. In the final stage of drying, the product temperature did not exceed +40 °C. The total duration of the drying process was 28–32 h.

2.4. Phytochemical Analysis

The phenolic content was analysed using a high-performance liquid chromatography system (Agilent 1200 3D LC System Technologies, Santa Clara, CA, USA), equipped with a UV-Vis diode-array (G1315C) and refractive index (G1362A) detectors, a vacuum degasser (G1322A), a four-channel low-pressure gradient pump (G1311A), an autosampler (G1329A), a column thermostat (G1316A). The system was operated in conjunction with a personal computer running Agilent ChemStation software, version B.04.03 (Agilent Technologies, Santa Clara, CA, USA). Phenolic compounds were isolated using reversed-phase chromatography with a Discovery C18 column (250 × 4.6 mm), packed with silica gel modified with octadecyl groups and a particle size of 5 μm.

For the separation of hydroxycinnamic acids, 0.005 N orthophosphoric acid (eluent A) and acetonitrile (eluent B) were used as the mobile phase. Chromatographic parameters were as follows: eluent flow rate—0.7 mL/min; operating pressure—10,000–12,000 kPa; column thermostat temperature—25 °C; sample volume—5–10 μL; total chromatography time—50 min. The gradient elution programme was: 0 min—5% B; 8 min—8% B; 15 min—10% B; 30 min—20% B; 40 min—40% B; 41–42 min—75% B; 43–50 min—5% B. Scanning time was 0.6 s; detection range—190–400 nm; detection wavelengths—320 and 330 nm.

To separate flavonoids into individual components, the same mobile phase (0.005 N orthophosphoric acid and acetonitrile) was used. Chromatographic parameters included: maximum eluent flow rate—0.8 mL/min; operating pressure—15,600 kPa; column thermostat temperature—25 °C; sample volume—5–10 μL; total chromatography time—60 min. The gradient elution programme was: 0 min—12% B; 30 min—25% B; 33 min—25% B; 38 min—30% B; 40 min—40% B; 41 min—80% B; 48 min—80% B; 49 min—12% B; 60 min—12% B. Scanning time was 0.6 s; detection range—190–400 nm; detection wavelengths—255 and 340 nm.

For the separation of tannins, the mobile phases consisted of a mixture of trifluoroacetic acid (0.1%), acetonitrile (5%), and deionised water (eluent A), and trifluoroacetic acid (0.1%) in acetonitrile (eluent B). Chromatographic parameters were: maximum eluent flow rate—0.1 mL/min; maximum operating pressure—40 kPa; column thermostat temperature—25 °C; sample volume—10 µL; total chromatography time—40 min. The gradient elution programme was: 0 min—0% B; 8 min—12% B; 10 min—12% B; 15 min—25% B; 20 min—25% B; 25 min—75% B; 28 min—75% B; 29 min—0% B; 40 min—0% B. Scanning time was 0.6 s; detection range—190–400 nm; detection wavelength—280 nm [29,30].

The total phenolic content in the studied extracts was determined spectrophotometrically in accordance with the requirements of the State Pharmacopoeia of Ukraine, Edition 2.0 [15]. The results were expressed in terms of pyrogallol equivalents. Quantitative determination of total flavonoids was also performed spectrophotometrically using the same pharmacopoeial procedures, with rutin as the reference standard [31,32]. All analyses were conducted in triplicate to ensure statistical reliability of the results [15,33].

2.5. Pharmacological Research

All pharmacological research was conducted in accordance with general ethical principles for animal experimentation and was approved by the Bioethics Commission of IFNMU (Protocol No. 154/25, dated 22 October 2025).

The study of the specific pharmacological activity of *S. europaea* extracts was conducted with the consultative support of Professor O. H. Popadynets, Head of the Department of Human Anatomy, and Associate Professor V. M. Ivanochko, also from the same department. Biochemical and haematological parameters of the experimental animals' blood were examined at the "Bioelementology Centre" of IFNMU.

The study was conducted using non-linear sexually mature white rats and guinea pigs of both sexes, bred and maintained in the vivarium of IFNMU. The average body weight of the rats was approximately 190–230 g, and that of the guinea pigs was approximately 700–900 g. The animals were standardised according to physiological and biochemical parameters and maintained under vivarium conditions in compliance with sanitary and hygienic regulations, housed in plastic cages, and fed a standard diet in accordance with current norms. Animal care and experimental procedures were carried out in accordance with both national and international guidelines for the humane treatment of laboratory animals, in alignment with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty," and the general ethical principles for animal experimentation adopted by the First National Congress of Ukraine on Bioethics (2001) [34–36].

2.5.1. Anti-Inflammatory Activity

To investigate the effect of *S. europaea* extracts on the exudative phase of inflammation, a rat paw oedema model induced by subplantar injection of a phlogogenic agent was employed. Specifically, 0.1 mL of a 1% carrageenan solution was administered beneath the aponeurosis of the hind paw [37,38]. The experiments were conducted on white outbred male rats weighing 190–230 g. The animals were divided into six groups, each consisting of six rats. The animals were randomly divided into six groups (n = 6 per group): Group I—received an intragastric aqueous extract of *S. europaea* herb (solvent: purified water) at a dose of 100 mg/kg; Group II—received an intragastric hydroalcoholic extract of *S. europaea* herb (solvent: 70% ethanol) at a dose of 100 mg/kg; Group III—received an aqueous extract from the rhizomes with roots of *S. europaea* (solvent: purified water) at a dose of 100 mg/kg; Group IV—received a hydroalcoholic extract from the rhizomes with

roots of *S. europaea* (solvent: 70% ethanol) at a dose of 100 mg/kg; Group V—received the reference plant-based anti-inflammatory agent, quercetin (granules "Quercetin" (0500924), PJSC "Borshchahivskiy Chemical-Pharmaceutical Plant", Kyiv, Ukraine), at a conditionally effective dose of 5 mg/kg; Group VI—served as the control and received the phlogogenic agent only. All treatments were administered one hour before and immediately after the induction of inflammation. Grouping was balanced by sex and body weight. The average body weight of the rats was approximately 190–230 g.

Paw volume was measured using a digital plethysmometer (Ugo Basile 7140; Ugo Basile S.R.L., Gemonio, Italy) prior to the experiment, at 1 h, 3 h, and at the peak of oedema development—5 h after administration of the phlogogenic agent.

The effect of *S. europaea* extracts was assessed based on their ability to suppress paw oedema in rats over time, in comparison with the control group and the reference drug. The obtained data were evaluated using statistical analysis methods.

2.5.2. Wound Healing and Haemostatic Activity

The haemostatic activity of the extracts from the herb and rhizomes with roots of *S. europaea* was studied in sexually mature guinea pigs bred in the vivarium of IFNMU, standardised according to physiological and biochemical parameters. The average body weight of sexually mature guinea pigs of both sexes was approximately 700–900 g.

The haemostatic properties of the tested extracts were evaluated by measuring bleeding time using the Duke method, following the induction of a linear incised wound [39,40]. Bleeding duration was recorded with a stopwatch. The wound was modelled by making an incision through all layers of previously depilated skin on the lateral surface of the hind limb using a scalpel. The standard wound size was 2.5 × 0.3 cm. Prior to incision, local anaesthesia was administered using a 2% solution of novocaine [37,39,40].

The animals were divided into six groups (n = 6 per group): Group I—treated with a quadruple-folded gauze pad soaked in an aqueous extract of *S. europaea* herb (solvent: purified water), applied topically to the wound surface immediately after incision; Group II—treated with a gauze pad soaked in a hydroalcoholic extract of *S. europaea* herb (solvent: 70% ethanol), applied in the same manner; Group III—treated with an aqueous extract of *S. europaea* rhizomes with roots (solvent: purified water); Group IV—treated with a hydroalcoholic extract of *S. europaea* rhizomes with roots (solvent: 70% ethanol); Group V—received the reference preparation "Liquid Extract of Water Pepper" (10222, PJSC "Phytopharm"); Group VI—served as the untreated control group. All treatments were applied immediately after modelling the incised wound. Grouping was balanced by sex and body weight. The average body weight of the guinea pigs was approximately 700–900 g.

The wound-healing activity of the extracts from the herb and rhizomes with roots of *S. europaea* was studied in sexually mature guinea pigs. An aseptic incised wound was modelled by making a 25 mm long and 5 mm deep incision under local anaesthesia on the depilated skin of the lateral surface of the hind limb [29,37]. The animals were divided into six groups (six individuals per group). Following the modelling of an incised wound, animals in the first and second groups received topical applications of aqueous extracts of *S. europaea* herb (solvent–purified water or 70% ethanol) twice daily. Animals in the third and fourth groups were treated with aqueous extracts of *S. europaea* rhizomes with roots (solvent–purified water or 70% ethanol) under the same regimen. The fifth group received the reference preparation "Recutan" (Liquid extract of chamomile flowers (*Flores Chamomillae*) in a ratio of 1:1, UA/5120/01/01, LLC "Pharmaceutical Company Zdorov'ya", Kharkiv, Ukraine), and the sixth group served as the control. Wound planimetry was performed in all experimental groups until complete healing was achieved [41].

2.5.3. Antimicrobial Activity

The antimicrobial activity of *S. europaea* extracts was investigated using the agar diffusion method with paper discs, following the procedure developed by A. M. Chornomyrdik [15,42]. The concentration of the active substance on the discs was 5 mg. A 5% blood agar and 24 h culture broths prepared in 1% sugar broth were used as a universal nutrient medium, with microbial suspensions standardised to a density of 1 billion microbial cells per millilitre. For inoculation, 1 mL of the bacterial suspension was applied to the surface of the 5% blood agar and gently rubbed in. The cultures were incubated at 37 °C for 24 to 72 h, depending on the characteristics of the tested microorganisms. The antimicrobial activity of the extracts was evaluated using standard microbial strains: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 33420, *Staphylococcus aureus* ATCC 25923, and *Staphylococcus epidermidis* ATCC 12228. The activity of the herb and rhizome extracts of *S. europaea* was determined by measuring the diameter (in mm) of the microbial growth inhibition zones around the paper discs saturated with the test substance. For comparison of bacteriostatic activity, paper discs impregnated with standard antibiotics were used: ampicillin at 30 μg/disc and oleandomycin at 30 μg/disc.

2.6. Statistical Analysis

Data are presented as the mean ± standard deviation (SD). For phytochemical evaluations, each data point represents the average of at least three independent determinations. Pharmacological assays were conducted with at least six replicates. Confidence intervals were calculated using critical values from Student's t-distribution [33,43,44]. Statistical analysis was performed using Statistica 10.0 software (StatSoft Inc., Tulsa, OK, USA). One-way ANOVA followed by Tukey's multiple comparison test was applied, with the significance level set at $p \leq 0.05$.

3. Results

S. europaea extracts are hygroscopic powders ranging in colour from light yellow to dark brown. The loss on drying ranges from 3.80% to 4.59%, which complies with the requirements for dry extracts.

3.1. Phytochemical Research

Phenolic compounds in the dry extracts of *S. europaea* herb and rhizomes with roots were determined using HPLC and spectrophotometry (Table 1).

According to the results of HPLC analysis (Table 1), 14 phenolic compounds were identified in the herb of *S. europaea*, and 13 compounds in the rhizomes with roots. These include hydroxycinnamic acids (rosmarinic, chlorogenic, neochlorogenic, caffeic, ferulic, *p*-coumaric), flavonoids (rutin, quercetin), and catechins (halocatechin, epicatechin gallate). The herb is primarily composed of phenolic acids, particularly rosmarinic, chlorogenic, and caffeic, which together account for approximately two-thirds of the total phenolic content. In the rhizomes with roots, the total content of these acids is lower, while the content of catechins, especially halocatechin, is somewhat higher.

The total content of phenolic compounds and flavonoids in the studied extracts of *S. europaea* was higher in 70% ethanol extracts compared to aqueous ones. This pattern is attributed to the greater extraction capacity of aqueous-alcoholic solutions for phenolic compounds of varying polarity. Ethanol at a concentration of 70% provides an optimal balance between hydrophilicity and lipophilicity, facilitating the extraction of both polar hydroxycinnamic acids and less polar flavonoids and catechins

Table 1. Quantitative content of phenolic compounds in *Sanicula europaea* extracts.

Phenolic Compound	Content of BACs (mg/kg)			
	SEH0	**SEH70**	**SER0**	**SER70**
Metabolites of tannins				
Gallocatechin	3473 ± 104	4236 ± 148	3464 ± 116	3893 ± 325
Catechin	933 ± 48	1104 ± 72	325 ± 27	435 ± 14
Epicatechin	532 ± 21	764 ± 51	2123 ± 324	2598 ± 78
Catechin gallate	1075 ± 54	1432 ± 102	-	-
Epicatechin gallate	12,275 ± 422	17,658 ± 679	2015 ± 134	2765 ± 107
Ellagic acid	28 ± 2	37 ± 4	104 ± 12	164 ± 8
Gallic acid	-	-	68 ± 9	109 ± 11
Epigallocatechin	-	-	2161 ± 275	2703 ± 168
Flavonoids				
Quercetin	132 ± 16	196 ± 9	-	-
Kaempferol	20 ± 2	42 ± 3	-	-
Apigenin	120 ± 11	203 ± 15	150 ± 12	217 ± 9
Coumarins				
Coumarin	97 ± 6	193 ± 9	108 ± 5	217 ± 21
Hydroxycinnamic acids				
Chlorogenic acid	108 ± 14	142 ± 24	425 ± 21	612 ± 22
Rosmarinic acid	5476 ± 261	6024 ± 187	3697 ± 128	4737 ± 308
Caffeic acid	319 ± 41	386 ± 24	213 ± 13	362 ± 21
Ferulic acid	99 ± 11	173 ± 9	114 ± 9	238 ± 17
Content of BACs groups in the extract, % (spectrophotometry)				
Total polyphenols	13.54 ± 0.24	15.02 ± 0.19	11.08 ± 0.15	12.24 ± 0.24
Flavonoids	3.61 ± 0.14	5.46 ± 0.23	0.13 ± 0.01	0.21 ± 0.02

Notes: SEH0, SEH70—herb extract (solvent–purified water and 70% ethanol, respectively); SER0, SER70—rhizome with root extracts (solvent–purified water and 70% ethanol, respectively).

3.2. Anti-Inflammatory Activity

The anti-inflammatory and anti-exudative activities of the extracts SEH0, SEH70, SER0, and SER70 were studied using white non-linear rats bred in the vivarium of IFNMU and standardised according to physiological and biochemical parameters. The increase in paw volume and the anti-exudative activity of *S. europaea* extracts are presented in Table 2.

The findings presented in Table 2 indicate that paw oedema in experimental groups increased during the first 3 h following administration of the phlogogenic agent, whereas in the untreated control group, oedema continued to progress for up to 5 h. Maximum inflammation suppression was observed in treated animals at the 5 h mark, although the anti-exudative activity varied depending on the type of administered extract. The anti-exudative effects of the *S. europaea* extracts and quercetin were evident as early as 1 h after treatment initiation, compared to the control group (SEH0—10.70%, SEH70—12.92%, SER0—8.29%, SER70—4.24%, Quercetin—9.16%). Throughout the 5 h experiment, the most pronounced activity was demonstrated by the extracts from the herb of *S. europaea* (SEH0—36.40%, SEH70—32.62%). At the 3 h time point, the anti-exudative effects of SEH0 (26.97%), SEH70 (26.80%) and the reference drug (26.70%) were comparable. However, by hour 5, the activity of the reference drug slightly declined relative to the tested extracts (SEH0—36.40%, SEH70—32.62%, Quercetin—29.40%). The rhizomes with root extracts also exhibited anti-exudative effects compared to the control group, although the activity of SER70 (27.40%) was somewhat lower, and SER0 (29.56%), was comparable to that of quercetin (29.40%).

Table 2. Anti-exudative activity of *Sanicula europaea* extracts.

Animal Group	Investigated Sample	Dose mg/kg	Paw Volume Increase, $\bar{x} \pm \Delta\bar{x}$, $n = 6$ (Inflammatory Response Inhibition Index, %)		
			1 h	3 h	5 h
I	SEH0	100	9.26 ± 0.16 * (10.70)	27.46 ± 0.16 * (26.97)	29.73 ± 0.23 * (36.40)
II	SEH70	100	9.03 ± 0.23 * (12.92)	27.52 ± 0.35 * (26.80)	31.56 ± 0.35 * (32.62)
III	SER0	100	9.51 ± 0.18 * (8.29)	29.96 ± 0.18 * (20.31)	32.99± 0.25 * (29.56)
IV	SER70	100	9.93 ± 0.29 * (4.24)	30.61 ± 0.29 * (18.59)	34.02 ± 0.24 * (27.40)
V	Quercetin	5	9.42 ± 0.21 * (9.16)	27.56 ± 1.13 * (26.70)	33.07 ± 1.11 * (29.40)
VI	-	-	10.37 ± 0.63	37.60 ± 0.91	46.84 ± 1.01

Notes: * statistically significant difference compared to the pathological control group ($p \leq 0.05$); *n*—number of animals in the group.

3.3. Wound Healing and Hemostatic Activity

The haemostatic properties of the tested extracts were evaluated by measuring bleeding time using the Duke method [39]. A linear incised wound was applied to the animals, and the duration of bleeding was recorded using a stopwatch. The results of the study are presented in Table 3.

Table 3. Effect of *Sanicula europaea* extracts on bleeding time.

Animal Group, $n = 6$	Bleeding Time, s	Reduction in Bleeding Time vs. Control Group, %	Reduction in Bleeding Time vs. Reference Drug, %
SEH0	113.33 ± 1.45 */**	49.56	34.37
SEH70	116.17 ± 2.89 */**	48.29	32.72
SER0	179.50 ± 2.42 *	20.11	-
SER70	193.50 ± 2.42 *	13.87	-
Reference drug: Liquid extract of water pepper	172.67 ± 2.90	23.15	-
Control group: untreated animals (pathological control)	224.67 ± 4.45	-	-

Notes: *n*—number of animals in the group; *—statistically significant difference compared to the control group ($p \leq 0.05$); **—statistically significant difference compared to the reference drug ($p \leq 0.05$).

As shown in Table 3, topical application of the reference drug, liquid extract of water pepper, reduced bleeding time by 23.15% compared to the control group. The application of *S. europaea* herb extracts resulted in a more pronounced reduction in bleeding time, by 49.56% and 48.29%, respectively, compared to the control group, and by 34.37% and 32.72%, respectively, compared to the reference drug. The rhizomes with root extracts also demonstrated haemostatic activity, reducing bleeding time by 20.11% and 13.87%, respectively, compared to the control group. Thus, it can be concluded that application of the herb extracts (SEH0 and SEH70) to the wound accelerated haemostasis by 1.98 and 1.93 times compared to the control group, and by 1.52 and 1.48 times compared to the reference drug, respectively. The reference drug itself shortened bleeding time by 1.3 times relative to the control. In contrast, the rhizomes with root extracts (SER0 and SER70) exhibited lower haemostatic activity than the reference drug but still reduced bleeding time

by 1.25 and 1.16 times, respectively, compared to the control group. These findings indicate the presence of local haemostatic activity in the herb extracts of *S. europaea*.

Over a 16-day period, wound planimetry was performed in conjunction with assessments of the animals' general condition and the rate of wound healing. The dynamics of the wound healing process are presented in Table 4.

Table 4. Wound healing dynamics following application of *Sanicula europaea* herb and rhizomes with roots extracts.

Animal Group, $n = 6$	Wound Healing Area, %, Mean ± SD Values							
	Day: 2	4	6	8	10	12	14	16
SEH0	4.69 ± 0.72	15.48 ± 1.83	48.87 ± 2.93	80.58 ± 3.79	100	100	100	100
SEH70	4.37 ± 1.04	14.36 ± 1.07	43.71 ± 4.06	76.55 ± 4.21	100	100	100	100
SER0	3.80 ± 0.46	10.71 ± 1.37	32.00 ± 2.38	54.33 ± 3.47	74.33 ± 0.72	100	100	100
SER70	3.83 ± 0.51	10.04 ± 0.83	31.02 ± 2.75	53.39 ± 2.94	68.16 ± 0.72	95.47 ± 2.12	100	100
Reference drug: Recutan	5.33 ± 0.77	17.34 ± 2.03	39.63 ± 2.01	80.69 ± 2.72	98.76 ± 0.72	100	100	100
Control group: untreated animals (pathological control)	3.40 ± 0.38	8.25 ± 0.96	30.90 ± 1.95	51.80 ± 4.83	68.91 ± 0.72	79.33 ± 4.86	94.98 ± 3.38	100

The results of the study indicate that the application of *S. europaea* herb extracts (SEH0 and SEH70) accelerated the wound healing process by 5.34 and 4.84 days, respectively, compared to the control group, and by 1.5 and 1.0 days relative to the reference drug Recutan. In contrast, the rhizomes with root extracts (SER0 and SER70) shortened the healing period by 2.83 and 2.01 days, respectively, compared to the control group, but were less effective than the reference drug. Thus, *S. europaea* herb extracts exhibit wound-healing activity, positively influencing the wound repair process and demonstrating therapeutic potential for topical treatment of skin injuries.

3.4. Antimicrobial Activity

The antibacterial activity of *S. europaea* raw material extracts was evaluated at the bacteriological laboratory of Military Unit A4520 (Lviv, Ukraine) using the agar diffusion method with paper discs. The results of the study are presented in Table 5.

Table 5. Antimicrobial activity of *Sanicula europaea* extracts.

Investigated Sample	Inhibition Zone Diameter, mm, $\bar{x} \pm \Delta\bar{x}$, $n = 3$				
	Pseudomonas aeruginosa ATCC 27853	*Escherichia coli* ATCC 25922	*Proteus vulgaris* ATCC 33420	*Staphylococcus aureus* ATCC 25923	*Staphylococcus epidermidis* ATCC 12228
SEH0	12 ± 0.38	11 ± 0.34	10 ± 0.36	11 ± 0.37	12 ± 0.38
SEH70	-	15 ± 0.41	-	15 ± 0.40	8 ± 0.29
SER0	15 ± 0.39	9 ± 0.28	7 ± 0.25	7 ± 0.31	11 ± 0.34
SER70	10 ± 0.34	10 ± 0.33	9 ± 0.27	9 ± 0.29	9 ± 0.30
Ampicillin	14 ± 0.39	10 ± 0.30	0	25 ± 1.02	8 ± 0.23

The results of the study demonstrate that both herb and rhizomes with roots extracts of *S. europaea* inhibit the growth of rod-shaped and coccoid microorganisms. Extracts SEH0, SER0, and SER70 exhibited bacteriostatic activity against all tested microbial strains. In contrast, the SEH70 extract showed no activity against *P. aeruginosa* and *P. vulgaris*.

Extract SEH0 showed moderate activity, inhibiting the growth of *P. aeruginosa*, *E. coli*, *S. aureus*, and *S. epidermidis* (12 ± 0.38 mm, 11 ± 0.34 mm, 11 ± 0.37 mm, and 12 ± 0.38 mm, respectively). In contrast, extract SEH70 demonstrated a more pronounced antimicrobial

effect against *E. coli* (15 ± 0.41 mm) and *S. aureus* (15 ± 0.40 mm), exceeding the clinical inhibition threshold (>10 mm). Extract SER0 exhibited inhibitory activity against *P. aeruginosa* (15 ± 0.39 mm), while the activity of extract SER70 was lower or at the clinical inhibition threshold (>10 mm).

Therefore, the ethanol extract of *S. europaea* herb showed the highest antimicrobial activity, which correlates with its higher content of phenolic compounds.

4. Discussion

A total of sixteen phenolic constituents were detected in the dry extracts of *S. europaea* herb and rhizomes with roots. These included one coumarin, four hydroxycinnamic acids, three flavonoids, and eight metabolites derived from tannins. Rosmarinic acid was the most abundant among the hydroxycinnamic acids. Quercetin and apigenin emerged as the leading flavonoids, and epicatechin gallate and gallocatechin were identified as the predominant tannin-related compounds. The results are consistent with recent phytochemical reviews that highlight the presence of phenolic acids, flavonoids, and tannins across *Sanicula* species; however, those studies lacked experimental confirmation of biological activity [45]. Rosmarinic and chlorogenic acids have previously been reported in the extracts of *S. europaea* herb [46]. Among the identified constituents, epigallocatechin gallate (EGCG) [47], caffeic, chlorogenic and rosmarinic acids, quercetin, apigenin, and kaempferol demonstrated the strongest anti-inflammatory potential. The biological activity is largely attributed to their ability to modulate key inflammatory signalling cascades, such as NF-κB, suppress pro-inflammatory cytokine production, and downregulate enzymes involved in inflammation. Specifically, chlorogenic acid is known to inhibit NF-κB activation, a central regulator of inflammatory gene expression, thereby decreasing levels of TNF-α, IL-6, and IL-1β, reducing oxidative stress, and limiting the expression of COX-2 and iNOS [48].

Similarly, caffeic acid exerts anti-inflammatory effects by blocking the synthesis of inflammatory mediators and enzymes, primarily through the inhibition of NF-κB signalling and oxidative pathways [49,50]. EGCG is well known for its strong anti-inflammatory properties, largely due to its influence on critical cellular signalling mechanisms. Numerous in vitro and in vivo experiments have demonstrated that EGCG can effectively inhibit the activation of NF-κB, prevent its translocation into the nucleus, and interfere with AP-1 transcriptional activity [47,51]. Some flavonoids detected in these dry extracts demonstrate significant anti-inflammatory effects through various molecular pathways. Quercetin, in particular, has been found to reduce the expression of major pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α, as well as enzymes such as COX-2. Its mechanism of action primarily involves the inhibition of the JAK1/STAT3/HIF-1α axis and the NF-κB signalling cascade [52,53].

Thus, in the experiment, the studied extracts of *S. europaea* exhibit a pronounced anti-inflammatory effect in the carrageenan-induced oedema model. The SEH0 extract suppresses the inflammatory response more effectively, reducing oedema by 36.40% at the 5th hour of the experiment (compared to quercetin—29.40%). In previous scientific literature, dichloromethane and methanol extracts of *S. europaea* root have been shown to exhibit moderate to strong anti-inflammatory effects in vitro on endothelial cells, reducing the expression of inflammatory mediators IL-8 and E-selectin following stimulation with TNF-α and the bacterial product lipopolysaccharide (LPS) [23,45]. The anti-inflammatory effect may be associated with the ability of flavonoids and phenolic acids to inhibit the synthesis of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and the activity of COX-2 enzymes, as well as to suppress the NF-kB and JAK/STAT signalling cascades, leading to reduced exudation and oedema [47,49,53].The anti-inflammatory activity observed in the *S. europaea* herb extract may also be attributed to its higher concentrations of rosmarinic

and chlorogenic acids, which are known to inhibit pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), suppress COX-2 and iNOS expression, and modulate NF-κB and MAPK signalling pathways. Their antioxidant properties further contribute to the attenuation of inflammatory responses [54,55].

Topical application of herb extracts (SEH0 and SEH70) resulted in cessation of bleeding that was 1.52 and 1.48 times faster, respectively, compared to the reference drug, and 1.98 and 1.93 times faster relative to the control group. The reference drug, liquid extract of water pepper, reduced bleeding time only by 1.3 times compared to the control group. These findings indicate the presence of local haemostatic activity in the herb extracts of *S. europaea*. The roots of *S. europaea* were previously used in Austrian traditional medicine as a haemostatic agent [23,45,46]; however, scientific confirmation through experimental studies has been obtained for the herb extracts for the first time. The observed hemostatic effect of *S. europaea* herb extracts may be attributed to the action of tannins and phenolic compounds, which promote platelet aggregation, enhance fibrin formation, and induce vasoconstriction. These mechanisms contribute to the formation of a faster thrombus and the cessation of bleeding. Recent studies confirm that plant-derived polyphenols can modulate key components of the hemostatic system, including platelet function and coagulation pathways [56–58]. These compounds promote platelet aggregation by enhancing intracellular calcium signalling and activating membrane glycoproteins involved in adhesion. They also stimulate fibrin formation by accelerating thrombin activity, thereby stabilising clot development. Additionally, tannins induce vasoconstriction and reduce capillary permeability, thereby contributing to faster cessation of bleeding [56,57,59]. These mechanisms are consistent with the observed reduction in bleeding time and support the traditional use of *S. europaea* as a topical hemostatic agent.

It was established that complete wound healing occurred on days 8–9 and 9–10 following the application of SEH0 and SEH70 herb extracts, respectively. In comparison, the reference drug Recutan achieved healing on days 10–11, while in the control group, wound closure was observed only on days 15–16. The most pronounced wound-healing effect and favourable healing dynamics were recorded with the SEH0 extract. This effect is attributed to the combined action of its constituents: flavonoids and phenolic acids stimulate fibroblast proliferation and epithelialization, while tannins promote the formation of a robust granulation layer and vasoconstriction. To date, the wound-healing properties of *S. europaea* have been primarily documented in ethnobotanical sources and phytochemical reviews, with limited experimental validation [45,46]. Our study is the first to demonstrate complete wound closure within 8–10 days following topical application of SEH0 and SEH70 herb extracts.

It was found that *S. europaea* extracts inhibit the growth of rod-shaped and coccoid microflora and exhibit bacteriostatic activity against *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *P. vulgaris* ATCC 33420, *S. aureus* ATCC 25923, and *S. epidermidis* ATCC 12228. This activity is attributed to the presence of a wide range of BACs in the extracts, including tannins, flavonoids, and phenolic substances [60]. Tannins are capable of binding to bacterial cell wall proteins, disrupting membrane integrity and limiting microbial growth. Flavonoids, such as quercetin and rutin, inhibit bacterial enzymes and nucleic acid synthesis in pathogens, thereby enhancing the antibacterial efficacy of the extracts [61,62]. The observed bacteriostatic activity of *S. europaea* herb extracts against both Gram-negative (*P. aeruginosa*, *E. coli*, *P. vulgaris*) and Gram-positive (*S. aureus*, *S. epidermidis*) strains aligns with earlier phytochemical reports highlighting the antimicrobial potential of the genus *Sanicula* in traditional folk medicine [45,46]. However, this is the first report demonstrating direct in vitro bacteriostatic effects of *S. europaea* herb extracts against standard ATCC strains, thereby expanding the pharmacological evidence base for this species.

Although the aqueous and ethanolic extracts of *S. europaea* were evaluated separately, the possibility of synergistic interactions between their bioactive fractions warrants further investigation. Aqueous extracts are typically rich in polar compounds such as hydroxycinnamic acids and tannins, while ethanolic extracts more effectively solubilise flavonoids and less polar catechins. Combining these fractions may enhance pharmacological effects through complementary mechanisms, such as simultaneous modulation of inflammatory mediators, improved antimicrobial spectrum, and reinforced hemostatic action. Previous studies on polyphenol-rich plant extracts suggest that such synergy can amplify biological activity beyond the sum of individual effects [56,57]. Future work should explore fraction recombination and assess potential additive or synergistic effects using cellular assays and combination index models.

These findings align with the phytochemical profiles described in recent reviews, which emphasised the presence of phenolic acids, flavonoids, and tannins across Saniculeae genera, but did not provide experimental data on bioactivity [45]. Unlike *S. marilandica* and *S. odorata*, which are primarily known from ethnobotanical records, *S. europaea* herb extracts demonstrate quantifiable biological effects. This positions *S. europaea* as a promising candidate for further pharmacological development and supports its traditional use in European herbal medicine.

Despite the promising results, several limitations should be acknowledged. First, the sample size (n) in biological assays was relatively small, which may affect the statistical power and generalizability of the findings. Second, the study did not include cellular-level assays (e.g., cytotoxicity, anti-inflammatory signalling in vitro, etc.), which would provide deeper mechanistic insights into the observed effects. Third, the acute toxicity and safety profiling of the extracts were not performed, which limits conclusions about their therapeutic applicability. Future studies should address these aspects to validate and expand upon the current findings.

5. Conclusions

The key bioactive constituents of *S. europaea* herb and rhizome-with-root extracts have been identified, and their antimicrobial, anti-inflammatory, hemostatic, and wound-healing properties have been evaluated, thereby scientifically confirming previously reported knowledge from folk medicine. These biological activities correlate with elevated concentrations of phenolic compounds, particularly tannin metabolites, rosmarinic and chlorogenic acids, which are known to modulate inflammatory pathways, coagulation mechanisms, and microbial growth. Compared to rhizome-with-root extracts, the herb fractions, especially the aqueous extract, exhibited superior efficacy across all tested models. Given the preliminary nature of this screening, future research should incorporate cellular assays, toxicity profiling, and larger experimental cohorts to validate therapeutic potential. These findings contribute to the scientific re-evaluation of *S. europaea* as an underutilised European medicinal plant with measurable pharmacological relevance.

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