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Melampyrum nemorosum L. Herb Extracts: Phytochemical Composition and Screening of Pharmacological Activities

Victoria Reznik [1], Andriy Grytsyk [1], Roman Hrytsyk [2], Roman Kutsyk [3], Oksana Yurchyshyn [3], Andriy Kaplaushenko [4], Ain Raal [5] and Oleh Koshovyi [5,*]

[1] Department of Pharmaceutical Management, Drug Technology and Pharmacognosy, Ivano-Frankivsk National Medical University, 76018 Ivano-Frankivsk, Ukraine; vikareznik88@gmail.com (V.R.); grycyk@ukr.net (A.G.)
[2] Department of Oncology, Ivano-Frankivsk National Medical University, 76018 Ivano-Frankivsk, Ukraine; grytsyk95@gmail.com
[3] Department of Microbiology, Virology and Immunology, Ivano-Frankivsk National Medical University, 76018 Ivano-Frankivsk, Ukraine; rkutsyk@ifnmu.edu.ua (R.K.); oiurchyshyn@ifnmu.edu.ua (O.Y.)
[4] Department of Physicocolloid Chemistry, Zaporizhzhia State Medical and Pharmaceutical University, 69035 Zaporizhzhia, Ukraine; kaplaushenko@ukr.net
[5] Institute of Pharmacy, Faculty of Medicine, University of Tartu, 50411 Tartu, Estonia; ain.raal@ut.ee
* Correspondence: oleh.koshovyi@ut.ee; Tel.: +38-0509642706

Abstract

In folk medicine, species of the genus Melampyrum (Orobanchaceae) have traditionally been used to treat dermatological conditions, neuralgia, rheumatism, and wounds. *M. nemorosum* L. possesses a diverse chemical profile that supports its therapeutic potential. This study aimed to investigate its principal biologically active compounds and to evaluate the antimicrobial, anti-inflammatory, haemostatic, and wound-healing activities of aqueous-ethanolic extracts (40% ethanol (MN40) and 70% ethanol (MN70)) of *M. nemorosum* herb. Nineteen phenolic compounds were identified in the extracts, including phenolic acids, hydroxycinnamic acids, flavonoids, and tannin metabolites. At a dose of 100 mg/kg, the extracts exhibited anti-inflammatory activity in the formalin-induced paw oedema model. Haemostatic effects were demonstrated by reductions in bleeding time by 38.5% (MN40) and 45.5% (MN70). Both extracts significantly accelerated wound healing, with MN70 showing the most pronounced effect: achieving 97.8% wound closure by day 11 and complete healing by day 13. Additionally, both extracts demonstrated antimicrobial activity, with MN70 being the most effective across all tested parameters. These findings reported here for the first time for this plant support the potential of *M. nemorosum* herb extracts for further preclinical and clinical development as a multifunctional phytotherapeutic agent.

Keywords: *Melampyrum nemorosum*; wood cow-wheat; phenolic compounds; anti-inflammatory activity; haemostatic effect; antimicrobial activity; wound-healing activity

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1. Introduction

Plants of the genus *Melampyrum* L. of the family Orobanchaceae Ventare traditionally used in folk medicine across various countries. This genus includes 37 species, 10 of which are native to Ukraine. They are commonly found in mountainous regions of both the Eastern and Western Mediterranean, as well as in the temperate zones of Europe, North Africa, and Asia [1–3]. The considerable diversity in the chemical composition of these species accounts for a broad spectrum of pharmacological properties and biological activity [4]. Our analysis

of the available literature indicates that plants of the genus *Melampyrum* are valuable medicinal species widely distributed throughout Ukraine. Considering their geographic distribution, the most promising species in terms of raw material availability include *M. cristatum*, *M. nemorosum*, *M. pratense*, and *M. arvense*. To date, the plant raw materials of *Melampyrum* species have been insufficiently studied. Consequently, further investigation of their raw materials and extracts is warranted, with the aim of developing and introducing novel plant-based medicinal products into clinical practice and pharmaceutical applications. Based on these considerations, *M. nemorosum* (wood cow-wheat) (Figure 1) was selected as the target species for this study.

The medicinal raw materials derived from *Melampyrum* plants are rich in various biologically active compounds (BACs). Carbohydrates (notably sucrose), iridoids (aucubin, catalpol, isocatapol, 8-epiloganin, melampyroside, methyl ester of gardoside, musainoside) [5,6], flavonoids (derivatives of apigenin, luteolin, and quercetin) [6,7], cardenolides, steroid saponins [8], alkaloids, carboxylic acids (particularly caffeic acid), fatty oils, ascorbic acid, carotene, and pectins have been found in these plants [4,9,10]. This complex phytochemical profile underpins the high pharmacological potential of these plants for medicinal use.

Figure 1. *Melampyrum nemorosum* L. in natural conditions. Flowering phase: (**A**) onset of flowering; (**B**) peak (mass) flowering. Photo: V. Reznik.

M. nemorosum L. exhibits a diverse chemical profile encompassing several classes of BAC. The plant contains iridoids (catalpol, isocatapol, aucubin acetate, agnuside), flavonoids (apigenin 7-glucoside, luteolin 7-glucoside, derivatives of apigenin, luteolin, and quercetin) [10,11], saponins, the carbohydrate melampyrite, alkaloids, and tannins (5.8%), along with free amino acids (0.1–0.9%) such as L-glutamic acid, L-proline, and

tyrosine. Organ-specific distribution includes vitamin C and starch in the stem; aucubin, carotenoids (0.14%), starch, vitamin C, and various flavonoids—such as rutin, luteolin, and glycosides of apigenin, luteolin, and quercetin—in the leaves; vitamin C in the flowers; and aucubin, catalpol, dextrin, and starch in the seeds. This phytochemical complexity supports the therapeutic potential of *M. nemorosum* and provides a compelling rationale for further pharmacological investigation [4,12].

In the folk medicine of European countries, *Melampyrum* species are employed in the treatment of various dermatological conditions [8], dizziness, gastrointestinal disorders, neuralgia, rheumatism, wound healing [13], and as sedatives, among other uses [4,6,14,15]. Currently, *Melampyrum* species are not included in any pharmacopoeias and are not utilised in conventional clinical practice [16,17]. Nevertheless, their widespread distribution in Europe, along with the presence of diverse groups of BAC, underscores their promise for further scientific investigation. The current body of scientific literature contains limited validated data on the chemical composition and pharmacological activity of *M. nemorosum*, indicating that this species and its extracts remain largely unexplored within this context. Consequently, research into the anti-inflammatory, antimicrobial, haemostatic, and wound-healing activities of *M. nemorosum* extracts represents a significant and novel contribution to the field. These extracts may serve as a foundation for the development of novel plant-based medicinal products.

The aim of this study was to investigate the principal biologically active compounds, as well as assess the acute toxicity, antimicrobial, anti-inflammatory, haemostatic, and wound-healing activities of aqueous-ethanolic extracts of *Melampyrum nemorosum* herb, with the objective of expanding scientific knowledge and substantiating the potential application of this plant in modern medical and pharmaceutical practice.

2. Materials and Methods

2.1. Chemicals and General Experiments

Deionised water was produced using Millipore Simplicity UV station (Merck Millipore, Burlington, MA, USA). Ethanol, acetonitrile, formic acid, glacial acetic acid, and ethyl acetate were purchased from VWR (Radnor, PA, USA). Chlorogenic acid, *p*-coumaric acid, quercetin, rutin, kaempferol, naringenin, apigenin, pyrogallol, and gallic acid were purchased from Carl Roth (Karlsruhe, Germany). Aluminium chloride and other chemical standards (hydroxyphenylacetic acid, benzoic acid, caffeic acid, *trans*-ferulic acid, *trans*-cinnamic acid, quercetin-3-O-glucoside, neohesperidin, rhamnetin, catechin, epicatechin, and gallocatechin) used for HPLC analysis were purchased from Sigma-Aldrich (Sant Louis, MI, USA).

2.2. Plant Raw Materials

The raw materials (herb) of *M. nemorosum* were harvested in the Ivano-Frankivsk region (Mykytintsi village, Kosiv district, 48°23′44″ N, 25°03′43″ E, around 350 m above sea level) in phases of peak (mass) flowering. During collection, standard protocols for harvesting medicinal plant materials were strictly followed, with careful attention paid to the preservation of the surrounding flora [18]. Prior to harvesting, plant species were accurately identified. The plant's identity was confirmed based on the botanical catalogue [19] with the consulting assistance of Professor A.R. Grytsyk from Ivano-Frankivsk National Medical University (IFNMU). Voucher specimens nos. 578–580 of *M. nemorosum* were deposited at the Department of Pharmaceutical Management, Drug Technology, and Pharmacognosy at IFNMU. The aerial parts (top 20–30 cm) were collected by cutting with a knife under dry weather conditions, ensuring an absence of rainfall for 3–5 days prior. Immediately after harvesting, the raw materials were dried (14 days) in a shaded, well-ventilated area, avoid-

ing direct exposure to sunlight. The plant material was spread in a thin layer approximately 2–3 cm thick and was periodically turned to ensure uniform drying. The raw material was stored in a well-ventilated room in paper bags and was used for extract preparation within three months of collection.

2.3. Extract Preparations

Liquid extracts from dried *M. nemorosum* herb (approximately 1000 g) were obtained using the percolation method with 40% and 70% ethanol solutions as extractants. The plant material was crushed and sieved to obtain fractions with a particle size of 1–3 mm. Based on preliminary calculations, the required volumes of 40% and 70% ethanol solution were prepared. The processed raw material was loaded into a laboratory percolator and moistened with the ethanol solution through the upper nozzle until a liquid "mirror" formed above the plant layer. The raw material was macerated for 24 h. Subsequently, percolation was carried out using a fresh portion of the extractant at a flow rate of 0.31 mL/min. The initial portion of the extract, corresponding to 85% of the raw material mass, was collected and set aside. Extraction continued until a liquid extract with a drug-to-extract ratio (DER) of 1:7 (for 40% ethanol) or 1:5 (for 70% ethanol) was obtained. The collected extract was concentrated using a laboratory rotary vacuum evaporator Buchi B-300 (Buchi AG, Flawil, Switzerland) at a temperature of (55 ± 5) °C until the volume was reduced to 15% of the original raw material mass. The concentrated residue was then dissolved in the previously set-aside first portion of the extract and stored at 10 °C for 48 h. The final product yield was 1:1 relative to the mass of the raw material. The finished extracts were filtered, dispensed into containers, and appropriately labelled. The liquid extracts from *M. nemorosum* herb, obtained using 40% and 70% ethanol solutions, were designated with the conventional names MN40 and MN70, respectively. Three batches of extracts from different raw materials harvested in the same year were prepared to determine their quality indicators.

2.4. Phytochemical Research

Flavonoids and hydroxycinnamic acids (caffeic and chlorogenic acids) were identified using TLC. Chromatographic analysis was carried out using a solvent system consisting of glacial acetic acid R: water R: ethyl acetate R (20: 20: 60). Two reagents were used to detect components on the chromatographic plate: the first was a solution of aminoethyl ester of diphenylboronic acid R with a concentration of 10 g/L in methanol R, which promotes the fluorescence of certain compounds and the second was a solution of macrogol 400 R with a concentration of 50 g/L in methanol R, which was used to enhance the signal. Identification of substances was carried out under ultraviolet light with a wavelength of 365 nm using a special UV lamp, which allowed for clear visualisation of chromatographic zones and determination of the presence of target compounds.

Flavonoid content was analysed using high-performance liquid chromatography (HPLC) on an Agilent Technologies 1200 system (Santa Clara, CA, USA). The mobile phase consisted of acetonitrile (solvent A) and 0.1% aqueous formic acid (solvent B), applied in a gradient mode: 0 min—30% A/70% B; 20 min—70% A/30% B; 22 min—100% A; 30 min—100% A. Separation was performed on a Zorbax SB-C18 column (3.5 µm, 150 × 4.6 mm; Agilent Technologies, Santa Clara, CA, USA) at a flow rate of 0.25 mL/min. The injection volume was 100 µL and the column temperature was maintained at 25 °C. Detection was carried out using a diode-array detector (DAD) at 280 and 365 nm, with spectral data collected in the 210–270 nm range [20,21].

Hydroxycinnamic acids were examined using the same HPLC system. Methanol (A) and 0.1% formic acid in water (B) served as the mobile phase, with gradient elution as

follows: 0 min—25% A/75% B; 25 min—75% A/25% B; 27 min—100% A; 35 min—100% A. The separation was carried out on a Zorbax SB-Aq column (4.6 mm × 150 mm, 3.5 µm; Agilent Technologies, Santa Clara, CA, USA) at a flow rate of 0.5 mL/min, with the column maintained at 30 °C and an injection volume of 4 µL. Detection was performed at 250 and 275 nm, with absorption spectra recorded between 210 and 270 nm [22].

Tannin-related compounds were also analysed using the Agilent 1200 HPLC system. The mobile phase consisted of methanol (A) and 0.1% aqueous formic acid (B), with the following gradient: 0 min—20% A/80% B; 25 min—75% A/25% B; 27 min—100% A; 35 min—100% A. Separation was conducted on a Zorbax SB-C18 column (3.5 µm, 150 × 4.6 mm) at a flow rate of 0.25 mL/min, with the column temperature set to 35 °C and an injection volume of 4 µL. Detection was carried out at 250 and 275 nm, with spectra recorded in the 210–270 nm range [23].

The identification and quantification of compounds were performed by comparing retention times and UV spectra with those of certified reference standards, including quinic, gallic, benzoic, syringic, caffeic, *p*-coumaric, *trans*-ferulic, sinapic, and *trans*-cinnamic acids, as well as rutin, quercetin-3-glucoside, naringin, neohesperidin, quercetin, luteolin, apigenin, naringenin, pyrocatechin, epicatechin, epicatechin gallate, and gallocatechin (Sant Louis, MI, USA). All measurements were performed in triplicate for statistical reliability.

The content of total phenolic compounds in the extracts was quantified using a spectrophotometric method in accordance with the State Pharmacopoeia of Ukraine, edition 2.0, with results expressed in pyrogallol equivalents [24]. Total flavonoid concentration was determined spectrophotometrically following the same pharmacopeial guidelines, with rutin used as the reference standard [16,24]. All analyses were performed in triplicate to ensure statistical reliability.

2.5. Pharmacological Research of the Extracts

The experiments were conducted in accordance with international and national standards for the humane treatment of animals. The study adhered to the provisions of the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" [25], the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [26] as well as the generally accepted ethical principles for animal experimentation approved by the First National Congress of Ukraine on Bioethics and adopted in Strasbourg on 18 March 1986 [25,26]. The research was approved by the Bioethics Commission of the IFNMU (protocol 151/25 dated 10 April 2025).

The animals were bred in the vivarium of the IFNMU and standardised according to physiological and biochemical parameters. They were housed under standard sanitary and hygienic conditions in plastic cages and fed a standard diet.

2.5.1. Anti-Inflammatory Activity

The study of the extracts' anti-inflammatory activity was conducted using the method developed by O.V. Stefanov [27], employing male white rats weighing 130–240 g. The animals were divided into six experimental groups, each consisting of six individuals. Experimental procedures and data analysis were performed in comparison with two reference anti-inflammatory agents: the non-steroidal drug diclofenac sodium (administered at 8 mg/kg) and quercetin (administered at 5 mg/kg) [28,29]. To evaluate the effects of *M. nemorosum* extracts on the exudative phase of inflammation, the rat paw oedema model was used. In this model, 0.1 mL of a 2% aqueous formalin solution was injected subplantarly under the aponeurosis of the hind paw. Rats in groups I and II received oral administration of the respective extracts MN40 and MN70 at a dose of 100 mg/kg body weight, 2 h before and immediately after the injection of the phlogogenic agent. Group III received di-

clofenac sodium at 8 mg/kg (using the injectable solution "Diclofenac-Darnitsa (CP120622)" 25 mg/mL, 3 mL ampoules, manufactured by PrJSC "Darnitsa", Kyiv, Ukraine). Group IV was treated with quercetin at 5 mg/kg (granules "Quercetin (0500924)", manufactured by PJSC "Borshchahivskiy Chemical-Pharmaceutical Plant", Kyiv, Ukraine). Group V (control) received only 0.1 mL of 2% formalin without any treatment. Group VI consisted of intact animals that were not subjected to any intervention. The volume of the rat hind paw was measured using an oncometric method before the experiment and at 1, 3, and 5 h after formalin injection, corresponding to the peak phase of oedema.

The anti-inflammatory efficacy of the *M. nemorosum* extracts was assessed by the degree of inhibition of formalin-induced oedema compared to the untreated control group. The inflammation inhibition index was calculated using the following formula:

$$A = \frac{(V_k - V_d) \times 100}{V_d},$$

where A is the percentage of anti-inflammatory activity; V_k is the increase in paw volume in the control group (arbitrary units); and V_d is the increase in paw volume in the experimental group (arbitrary units).

2.5.2. Wound Healing and Haemostatic Activity

The wound healing and haemostatic effects of *M. nemorosum* herb extracts were evaluated in sexually mature guinea pigs weighing over 300 g, bred at the vivarium of the Clinical and Biological Experimental Base of IFNMU. The animals were pre-standardised based on physiological and biochemical parameters.

Haemostatic activity was assessed by measuring bleeding time using the Duke method, which involved making a linear skin incision and recording the duration of bleeding with a stopwatch [30–32]. A standardised linear incised wound was created by making a scalpel incision through all layers of depilated skin on the lateral surface of the hind limb thigh. The wound dimensions were 1.5 × 0.3 cm. Prior to incision, local anaesthesia was administered using a 2% solution of novocaine (active ingredient—procaine hydrochloride) (2308-011B), Novocaine-Zdorovye (LLC "Pharmaceutical Company Zdorovye", Kharkiv, Ukraine). The study was conducted on 24 guinea pigs grown in the vivarium of the IFNMU in order to evaluate the wound healing and haemostatic activity. Rotokan—a combined herbal preparation—was chosen as a drug for comparison [17]. "Rotokan" (2403-012A, manufacturer: JSC "Lubnypharm", Lubny, Ukraine)—liquid extract (1:1) from medicinal plant raw materials: chamomile flowers (*Flores chamomillae*), calendula flowers (*Flores calendulae*), and yarrow herb (*Herba millefolii*) in a ratio of 2:1:1. Its effectiveness is due to the presence of phytoactive substances that have anti-inflammatory, antimicrobial and antispasmodic effects. In addition, "Rotokan" strengthens blood vessels, accelerates the healing of mucous membranes, and has weak haemostatic properties. The animals were divided into four groups, each consisting of six individuals. After wound induction, Group I and II received MN40 and MN70, respectively. Group III was treated with a reference preparation, liquid extract of *Polygonum hydropiper* (2404-008B, manufacturer: Ternopharm LLC, Ternopil, Ukraine); Group IV served as the untreated control and received no therapeutic intervention. All extracts were applied topically directly onto the wound area once daily. Each wound was treated with 0.2 mL of the respective extract per application.

The wound healing activity of the liquid extracts from *M. nemorosum* herb was studied using an aseptic incised wound model. Under local anaesthesia, a linear incision measuring 15 mm in length and 3 mm in depth was made on the depilated skin of the lateral surface of the hind limb thigh [33,34].

The animals were divided into four groups, each consisting of six guinea pigs. After wound induction, Group I and II received MN40 and MN70, respectively; Group III was treated with the reference preparation "Rotokan"; and Group IV served as the untreated control. Throughout the experimental period, planimetric analysis of the wounds was performed by precisely measuring the wound surface area. In addition, the general physiological condition of the animals was regularly assessed, including activity, appetite, behavioural responses, and external appearance. Special attention was given to the rate of wound closure, observing changes in wound area and stages of tissue regeneration. This comprehensive approach allowed for a detailed evaluation of the therapeutic efficacy of the tested extracts.

2.5.3. Antimicrobial and Antifungal Activity

Experimental studies on the antimicrobial activity of *M. nemorosum* herb extracts against pure cultures of Gram-positive bacteria and yeasts were conducted at the Department of Microbiology, Virology, and Immunology of the IFNMU.

The antimicrobial activity of the extracts was evaluated using clinical isolates of both antibiotic-sensitive and antibiotic-resistant microorganisms. Bacterial cultures were identified using the biochemical microtests ENTEROtest 24 (MLT00008), STAPHYtest 16 (MLT00012), STREPTOtest 16 (MLT00014), and NEFERMENTtest 24 (manufacturer: Lachema, Brno, South Moravia, Czech Republic), taking into account morphological and cultural characteristics, in accordance with the 9th edition of Bergey's Manual of Determinative Bacteriology [35]. Yeast-like fungi were identified using 40 biochemical tests and the VITEK 2 system (2403A), with the VITEK 2 YST (43409-2) ID card (21343, manufacturer: bioMérieux, Marcy-l'Étoile, Rhône, France) [36,37].

Screening of antimicrobial activity was performed using the agar well diffusion method, known for its high sensitivity and ability to clearly distinguish active plant extracts from inactive ones. Petri dishes were filled with 30 mL of agar medium and placed on a flat horizontal surface. After solidification, wells with a diameter of 4.0 mm were made using a sterile punch. The agar surface was evenly inoculated with a suspension of test cultures at a concentration of 1×10^8 CFU/mL.

Wells in the experimental group were filled with 20 µL of plant extracts (concentration: 10 mg/mL), while control wells received 20 µL of the corresponding extractants (40% and 70% aqueous ethanol). After 24 h of incubation, the diameters of the zones where bacterial growth was inhibited were measured. These zones of inhibition indicated the effectiveness of the tested substances in suppressing bacterial development. Measurements were taken using a ruler or a specialised calliper, recording the size of the microbial growth-free areas around the application sites.

The fungistatic activity was assessed after 2 days of incubation and the fungicidal activity after 4 days. All culture plates were digitally documented and image analysis was performed using the UTHSCSA ImageTool 3.0 software (The University of Texas Health Science Centre at San Antonio, ©1995–2002, San Antonio, TX, USA).

2.6. Statistical Analysis

The results are expressed as the mean ± standard deviation (SD). In the phytochemical analysis, each value represents the average of no fewer than three independent measurements, while in the pharmacological experiments, at least six replicates were used. Confidence intervals were determined based on the critical values of Student's t-distribution [16,38,39]. Statistical significance was assessed using one-way ANOVA followed by Tukey's post hoc test.

3. Results

The obtained liquid extracts of *M. nemorosum* herb were transparent, light green to green in colour, odourless, and free from visible mechanical impurities. The dry residues of the MN40 and MN70 extracts that were further studied were 13.65% and 12.22%, respectively.

3.1. Phytochemical Research

Phenolic compounds in the liquid extracts of *M. nemorosum* herb were determined by HPLC and spectrophotometry (Table 1).

Table 1. Content of phenolic compounds in the liquid extracts of *Melampyrum nemorosum* herb.

No.	Compound	Content in the Liquid Extract, μg/mL	
		MN40	MN70
	Phenolic acids		
1	Hydroxyphenylacetic acid	54.49 ± 3.82	53.64 ± 3.79
2	Benzoic acid	1294.43 ± 76.17	1274.69 ± 99.35
	Hydroxycinnamic acids		
3	Chlorogenic acid *	203.66 ± 8.05	218.74 ± 14.79
4	Caffeic acid *	157.79 ± 7.37	166.68 ± 7.82
5	*p*-Coumaric acid *	696.42 ± 45.86	810.55 ± 53.40
6	*trans*-Ferulic acid *	25.66 ± 2.18	32.14 ± 2.69
7	*trans*-Cinnamic acid *	399.40 ± 18.97	524.36 ± 31.04
	Flavonoids		
8	Rutin *	4625.30 ± 183.32	5009.22 ± 144.72
9	Quercetin-3-O-glucoside	532.21 ± 2.18	517.76 ± 3.18
10	Naringin *	927.24 ± 40.13	1042.37 ± 55.72
11	Neohesperidin *	843.98 ± 34.40	993.36 ± 31.04
12	Quercetin *	1217.03 ± 45.12	1416.42 ± 52.67
13	Naringenin *	475.70 ± 18.02	596.21 ± 22.61
14	Apigenin *	145.78 ± 7.64	189.17 ± 9.90
15	Rhamnetin *	917.69 ± 38.77	1026.85 ± 43.38
16	Kaempferol *	345.48 ± 15.15	414.50 ± 18.21
	Tannin metabolites		
17	Catechin *	266.99 ± 14.06	217.39 ± 11.49
18	Epicatechin	567.57 ± 28.53	548.80 ± 27.74
19	Gallocatechin *	165.71 ± 16.11	192.83 ± 20.04
	Content of compound groups in the liquid extract, % (spectrophotometry)		
Total polyphenols (%) *		4.73 ± 0.20	6.77 ± 0.21
Flavonoids (%) *		0.86 ± 0.07	1.01 ± 0.06

Notes: MN40, extract obtained with 40% aqueous-ethanol solution; MN70, extract obtained with 70% aqueous-ethanol solution; *p*-values < 0.05 were considered statistically significant, while values above 0.05 indicated no significant difference between the groups. All calculations were based on independent two-sample t-tests. Asterisks (*) denote statistically significant differences in the content of substances between MN40 and MN70.

A total of 19 phenolic compounds were identified, including 2 phenolic acids, 5 hydroxycinnamic acids, 9 flavonoids, and 3 tannin metabolites.

3.2. Quality Indicators of the Obtained Extracts

The quality assessment of the *M. nemorosum* herb liquid extracts (MN40 and MN70) (three batches of the extracts) was carried out according to the methods of European pharmacopeia [16] and the following parameters (Table 2): identification A (description), identification B (main phenolic compounds by thin-layer chromatography (TLC) method),

quantitative content of flavonoids (not less than 0.8% (for MN40) or 0.9% (for MN70)), heavy metals (not more than 0.002%) [16], dry residue (not less than 12.0%) [16], ethanol content (38–42 or 68–72%, respectively) [16], and microbiological purity (microbiological quality criteria: total aerobic microbial count (TAMC): acceptance criterion: $\leq 10^5$ CFU/g or CFU/mL, maximum allowable limit: 500,000 CFU/g or CFU/mL; total yeast and mould count (TYMC): acceptance criterion: $\leq 10^4$ CFU/g or CFU/mL, maximum allowable limit: 50,000 CFU/g or CFU/mL; bile-tolerant Gram-negative bacteria: acceptance criterion: $\leq 10^4$ CFU/g or CFU/mL; *Escherichia coli*: absence in 1 g or 1 mL; *Salmonella* spp.: absence in 25 g or 25 mL) [16].

TLC analysis of the extracts, conducted in comparison with authentic standards, confirmed the presence of hydroxycinnamic acids (caffeic and chlorogenic) and flavonoids (quercetin and rutin).

Table 2. Results of determining quality indicators in the liquid extracts of *Melampyrum nemorosum* herb.

Quality Indicators	Extracts					
	MN40			MN70		
	Batch 001	Batch 002	Batch 003	Batch 001	Batch 002	Batch 003
Identification A (description)	Meets specs	Meets specs	Meets specs	Meets specs	Meets specs	Meets specs
Identification B (main phenolic compounds (TLC))	Meets specs	Meets specs	Meets specs	Meets specs	Meets specs	Meets specs
Quantitative content of flavonoids, %	0.86	0.85	0.81	1.01	0.98	1.03
Heavy metals (not more than 0.002%)	Meets specs	Meets specs	Meets specs	Meets specs	Meets specs	Meets specs
Dry residue (not less than 12.0%)	13.65	13.90	13.22	12.22	12.08	12.03
Ethanol content, %	41	39	40	68	71	72
Microbiological purity	Meets specs	Meets specs	Meets specs	Meets specs	Meets specs	Meets specs

The studied extracts of *M. nemorosum* herb met the specified numerical parameters and microbiological purity requirements outlined in the proposed draft quality control methods.

3.3. Anti-Inflammatory Activity

From the literature data, it is known that BAC of species of the genus *Melampyrum* exhibit anti-inflammatory properties [9,13]. The study of the anti-inflammatory activity of the *M. nemorosum* herb extracts was carried out on white linear male rats (130–240 g) in accordance with the methodological recommendations of the State Centre of the Ministry of Health of Ukraine [27]. Data characterising the anti-inflammatory activity of the extracts are presented in Table 3.

Table 3. The effect of *Melampyrum nemorosum* herb extracts on the development of limb oedema in rats.

Group of Animals (n = 6)	Dose, mg/kg	Rat Paw Volume Increase, %: $\bar{x} \pm \Delta\bar{x}$			Inflammatory Response Suppression Index, %		
		In 1 h	In 3 h	In 5 h	In 1 h	In 3 h	In 5 h
Group I (MN40)	100	29.45 ± 1.57 **	32.13 ± 3.33 *	29.31 ± 5.92	15.89	33.47	35.36
Group II (MN70)	100	24.95 ± 3.12	27.88 ± 1.98 *	24.38 ± 1.46 *	28.82	42.25	46.24
Group III (diclofenac sodium)	8	21.29 ± 2.23 *	20.18 ± 4.51 *	17.82 ± 4.29 *	39.25	58.21	60.69
Group IV (quercetin)	5	24.51 ± 2.20 *	29.15 ± 1.46 *	28.43 ± 2.3 *	30.07	39.65	37.30
Group V (control animals)	-	35.041 ± 4.14	48.30 ± 5.86	45.35 ± 6.62	-	-	-

Notes: * Statistically significant difference compared to the control group ($p \leq 0.05$); ** statistically significant difference compared to the group of animals treated with diclofenac sodium ($p \leq 0.05$); MN40, extract obtained with 40% aqueous-ethanol solution; MN70, extract obtained with 70% aqueous-ethanol solution; $\bar{x} \pm \Delta\bar{x}$, the results are expressed as the average mean ± standard deviation (SD).

The results presented in Table 3 indicate that, in animals of the control group, the inflammatory process in the paw was accompanied by a sustained increase in its volume, which persisted until the end of the experiment. In contrast, administration of the herb

extracts led to suppression of the inflammatory response of varying intensity compared to the control group, with measurable effects observed as early as 1 h after treatment initiation.

3.4. Wound Healing and Hemostatic Activity

Bleeding time was determined using the Duke method [32]. This method allows for determining how long bleeding lasts after the tissues have been cut. The results of the study of bleeding time (M ± m, s) are given in Table 4.

Table 4. The effect of the liquid extracts from *Melampyrum nemorosum* herb and water pepper on bleeding duration.

Group of Animals	Bleeding Duration, s, n = 6	Reduction in Bleeding Time, Relative to theControl Group, %
Group I (MN40)	89.50 ± 13.48 *	38.49
Group II (MN70)	79.33 ± 9.28 *	45.48
Group III (liquid extract of *Polygonum hydropiper*)	61.83 ± 3.38 *	57.51
Group IV (control animals)	145.5 ± 12.24	-

Notes: * Statistically significant difference compared to the control group, $p \leq 0.05$. MN40, extract obtained with 40% aqueous-ethanol solution; MN70, extract obtained with 70% aqueous-ethanol solution. Values are presented as mean ± SD; n = 6. Statistical significance was assessed using one-way ANOVA followed by Tukey's post hoc test.

After determining the bleeding time, the wound healing dynamics were studied using the same groups of animals (Table 5).

Table 5. Dynamics of the wound healing process with the use of the liquid extracts from *Melampyrum nemorosum* herb.

Group of Animals	Percentage (%) of Wound Area Healing Compared to the Initial State in Dynamics (Days)							
	2	3	5	7	9	11	13	15
Group I (MN40)	5.93 *	17.78 *	34.44 *	57.04 *	77.04 *	92.96 *	98.89 *	100 *
Group II (MN70)	4.81 *	19.26 *	40.37 *	71.85 *	89.63 *	97.78 *	100 *	100 *
Group II (Rotokan)	5.19 *	18.52 *	30.37 *	53.17 *	75.93 *	87.78 *	95.93 *	100 *
Group IV (control animals)	2.59	7.78	28.52	37.41	51.11	73.33	85.93	96.30

Notes: MN40, extract obtained with 40% aqueous-ethanol solution; MN70, extract obtained with 70% aqueous-ethanol solution. Asterisks (*) indicate statistically significant difference vs. control group ($p \leq 0.05$). Values are presented as a percentage of wound area reduction over time (days). Statistical significance assessed using repeated measures ANOVA.

The data presented in Table 5 show that in animals of the control group, the wound healing process progressed relatively slowly: by day 9, the wound closure area reached only 51.11%, and complete healing was observed only on day 16. In contrast, animals in the experimental groups exhibited a faster healing process.

3.5. Antimicrobial and Antifungal Activity

Data on antimicrobial and antifungal activity of the *M. nemorosum* herb extracts are given in Table 6.

Both *M. nemorosum* herb extracts MN40 and MN70 demonstrated antimicrobial properties. The MN40 extract inhibited the growth of *Staphylococcus aureus* and *Enterococcus faecalis*. The MN70 extract showed an exceptionally broad spectrum of activity, demonstrating the ability to inhibit the growth of numerous clinically significant microorganisms isolated from various biological materials. Overall, the extract exhibited greater efficacy against Gram-positive bacteria, including various *Staphylococcus* spp., *Streptococcus* spp. (β-haemolytic groups A, B, and G; α-haemolytic), *Enterococcus faecalis*, and *Bacillus subtilis*. Activity was also observed against selected Gram-negative strains, notably *Escherichia coli* (including haemolytic and ESBL-producing variants) and *Acinetobacter baumannii*. Furthermore, the extract demonstrated notable antifungal activity, particularly against fluconazole-resistant

Candida spp. (*C. albicans*, *C. tropicalis*, *C. glabrata*, *C. lusitaniae*, *C. lipolytica*), as well as *Aspergillus fumigatus*, *Rhodotorula mucilaginosa*, and *Geotrichum candidum*.

Table 6. Antimicrobial and antifungal activity of *Melampyrum nemorosum* herb extracts (at a concentration of 10 mg/mL).

Types of Microorganisms	Clinical Material	Resistance	Diameters of Growth Inhibition Zones, mm			
			Reference Extractant		MN40	MN70
			Ethanol 40%	Ethanol 70%		
Staphylococci						
Staphylococcus aureus	ATCC 25923	S	13.00 ± 0.54	18.10 ± 1.50	Growth	22.17 ± 1.15 *
Staphylococcus aureus	Pharynx	BSSA	Growth	20.35 ± 1.05	11.63 ± 0.08 *	32.02 ± 1.60 *
Staphylococcus aureus	Skin	S	Growth	16.68 ± 0.49	10.32 ± 0.60 *	24.71 ± 1.55 *
Staphylococcus aureus	Wound	BSSA, MLS	Growth	22.06 ± 0.82	12.59 ± 0.57 *	33.00 ± 3.67 *
Staphylococcus aureus	Wound	MRSA	Growth	16.62 ± 0.94	10.57 ± 0.50 *	23.99 ± 1.34 *
Staphylococcus epidermidis	Skin	MLS, Ind+	Growth	22.75 ± 1.01	Growth	22.96 ± 0.57
Staphylococcus haemolyticus	Sputum	MRSH	Growth	14.25 ± 0.49	Growth	22.99 ± 2.17 *
Enterococci						
Enterococcus faecalis	Urethra	Tet, FQin	Growth	11.73 ±0.43	11.92 ± 0.64 *	21.12 ± 1.29 *
Enterococcus faecalis	Urethra	Tet, FQin	Growth	Growth	13.99 ± 1.33 *	16.80 ± 0.54 *
β-Haemolytic streptococci						
Streptococcus pyogenes (β Str A)	Pharynx	S	14.60 ± 2.28	16.39 ± 0.82	Growth	14.73 ± 2.08
Streptococcus pyogenes (β Str A)	Pharynx	S	Growth	10.43 ± 0.42	Growth	13.45 ± 1.30 *
Streptococcus dysgalactiae (β Str G)	Pharynx	S	Growth	Growth	Growth	12.18 ± 1.17 *
Streptococcus agalactiae (β Str B)	Vaginal mucus	S	Growth	17.45 ± 1.29	Growth	18.89 ± 2.32 *
α-Haemolytic streptococci						
Streptococcus anginosus	Pharynx	AMO, Tet, MLS	15.51 ± 1.28	13.63 ± 1.20	Growth	14.59 ± 0.76
Streptococcus gordonii	Oral cavity	S	11.53 ± 0.98	13.04 ± 0.21	Growth	13.07 ± 0.67
Streptococcus oralis	Oral cavity	S	Growth	14.67 ± 0.56	9.67 ± 0.63 *	20.44 ± 1.07 *
Streptococcus sanguinis	Oral cavity	S	Growth	19.54 ± 0.87	Growth	20.65 ± 1.02
Streptococcus pneumonia	Sputum	S	Growth	Growth	Growth	Growth
Streptococcus pneumonia	Sputum	β-Lac, Tet	Growth	Growth	Growth	17.40 ± 0.21 *
Enterobacteria						
Escherichia coli	Wound	S	Growth	8.70 ± 0.52	Growth	Growth
Escherichia coli	Wound	S	Growth	Growth	Growth	12.89 ± 0.56 *
Escherichia coli	Wound	AMO Tet, FQin	Growth	Growth	Growth	11.79 ± 0.69 *
Escherichia coli hly+	Faecal sample	AMO, MLs	Growth	Growth	Growth	13.20 ± 0.95 *
Escherichia fergusonii	Faecal sample	ESβL	Growth	Growth	Growth	Growth
Providencia rettgeri	Urine sample	ESβL	Growth	Growth	Growth	Growth
Morganella morganii	Urine sample	ESβL	Growth	Growth	Growth	Growth
Non-fermenting bacteria						
Pseudomonas aureginosa	Wound	ESβL	Growth	Growth	Growth	Growth
Pseudomonas aureginosa	Pus from the wound	ESβL	Growth	Growth	Growth	Growth
Acinetobacter baumani	Sputum	ESβL	Growth	Growth	Growth	10.61 ± 1.03 *
Bacilli						
Bacilus subtilus	ATCC 6051	S	Growth	11.89 ± 0.40	Growth	17.75 ± 1.37 *

Table 6. *Cont.*

Types of Microorganisms	Clinical Material	Resistance	Diameters of Growth Inhibition Zones, mm			
			Reference Extractant		MN40	MN70
			Ethanol 40%	Ethanol 70%		
Fungi						
Candida albicans	Oral cavity	FCZ-R	Growth	16.35 ± 1.3	Growth	22.22 ± 1.25 *
Candida albicans	Sputum	FCZ-R	Growth	21.86 ± 1.22	Growth	31.64 ± 2.24 *
Candida albicans	Urine sample	FCZ-R	Growth	10.82 ± 0.34	Growth	15.53 ± 0.64 *
Candida albicans	Oral cavity	FCZ-S	Growth	11.15 ± 0.58	Growth	15.24 ± 0.29 *
Candida tropicalis	Sputum	FCZ-R	Growth	10.02 ± 0.44	Growth	16.34 ± 0.80 *
Candida glabrata	FRS 585	FCZ-S	Growth	19.59 ± 1.89	Growth	21.29 ± 1.20
Candida lusitaniae	Oral cavity	FCZ-R	Growth	10.13 ± 1.47	Growth	19.95 ± 0.97 *
Candida lusitaniae	Oral cavity	FCZ-S	Growth	20.96 ± 1.22	Growth	26.88 ± 0.55 *
Candida lipolytica	Oral cavity	FCZ-R	Growth	10.29 ± 0.81	Growth	15.16 ± 0.60 *
Candida kefyr	Oral cavity	FCZ-S	10.59 ± 0.99	15.60 ± 0.43	Growth	26.55 ± 2.30 *
Aspergillus fumigatus	Sputum	FCZ-R	Growth	Growth	Growth	17.04 ± 3.20 *
Rhodotorula mucilaginosa	Pus from the wound	FCZ-S	Growth	25.68 ± 0.99	Growth	32.32 ± 1.25 *
Geotrichum candidum	Faecal sample	FCZ-S	Growth	10.73 ± 0.59	Growth	20.22 ± 0.87 *

Notes: 1. MRSA, methicillin-resistant *S. aureus*; BSSA, borderline methicillin-resistant *S. aureus* (β-lactamase hyperproduction); EsβL, extended-spectrum β-lactamases; MLS resistance, resistance to macrolides and lincosamides; Tet, tetracycline; FQin, fluoroquinolones; S, antibiotic-sensitive strains; FCZ-R, fluconazole-resistant; FCZ-S, fluconazole-sensitive. 2. $p < 0.05$ compared to the control (solvent). Asterisks (*) indicate statistically significant difference vs. control group ($p \leq 0.05$).

4. Discussion

In the liquid extracts of *M. nemorosum* herb, nineteen phenolic compounds were identified, including two phenolic acids, five hydroxycinnamic acids, nine flavonoids, and three tannin metabolites. Among the phenolic acids, benzoic acid was predominant. The main hydroxycinnamic acids were *p*-coumaric acid and *trans*-cinnamic acid. Rutin and quercetin were identified as the dominant flavonoids. Epicatechin was the major tannin metabolite. The most potent anti-inflammatory compounds among the identified compounds are chlorogenic acid, caffeic acid, quercetin, rutin, naringin, naringenin, and apigenin. These substances act via modulation of signalling pathways like NF-kB, inhibition of cytokines, and reduction of inflammatory enzyme expression. Chlorogenic acid exhibits notable anti-inflammatory properties by inhibiting the activation of nuclear factor kappa B (NF-kB), a key transcription factor involved in the expression of pro-inflammatory cytokines; reducing the production of inflammatory mediators such as TNF-α, IL-6, and IL-1β; modulating oxidative stress and suppressing the expression of COX-2 and iNOS, enzymes involved in inflammation [40]. Caffeic acid also exhibits strong anti-inflammatory effects by inhibiting pro-inflammatory cytokines (e.g., TNF-α, IL-6) and enzymes such as COX-2 and iNOS. Its mechanism of action involves the suppression of NF-kB activation and oxidative stress [41,42].

Several identified flavonoids in these liquid extracts also exhibit pronounced anti-inflammatory activity through diverse molecular mechanisms. Quercetin has been shown to inhibit key pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, as well as enzymes like COX-2, primarily by suppressing the JAK1/STAT3/HIF-1α and NF-kB signalling pathways [43,44]. Rutin exerts similar effects by downregulating TNF-α, IL-1β, COX-2, and iNOS expression, and modulating NF-kB, MAPK, and Nrf2/HO-1 pathways [45,46]. Naringin has demonstrated the ability to suppress IL-6 and TNF-α production and inhibit NF-kB and MAPK/ERK signalling, contributing to its anti-inflammatory and antioxidant effects [47,48]. Naringenin, a related flavanone, reduces the expression of inflammatory me-

diators and oxidative stress by activating Nrf2 and inhibiting NF-kB, making it a promising candidate for managing chronic inflammation [49,50]. Lastly, apigenin has been reported to inhibit IL-1β, IL-6, TNF-α, and COX-2, while modulating NF-kB, MAPK, and Nrf2 pathways, thereby exhibiting both anti-inflammatory and neuroprotective properties [51,52].

Many of the identified phenolic acids and flavonoids also exhibit significant wound-healing and haemostatic activities. Chlorogenic acid, caffeic acid, *p*-coumaric acid, and *trans*-ferulic acid are known for their antioxidant and anti-inflammatory properties, which are critical in promoting tissue repair and reducing inflammation during wound healing [53–55]. Flavonoids such as quercetin, rutin, and kaempferol have demonstrated accelerative effects on wound closure, primarily through modulation of oxidative stress and enhancement of collagen synthesis [56–58]. Quercetin and its derivatives, including quercetin-3-O-glucoside and rhamnetin, also exhibit haemostatic effects by promoting platelet aggregation and vasoconstriction [59,60]. Additionally, catechins and epicatechin have been reported to improve wound healing by enhancing angiogenesis and modulating inflammatory responses [61–63]. Overall, these compounds contribute to wound repair via antioxidant, anti-inflammatory, and haemostatic mechanisms, supporting their potential therapeutic use in wound management.

The MN40 extract demonstrated moderate anti-inflammatory activity (Table 3), with inhibition increasing progressively from 15.89% at 1 h to 35.36% at 5 h, approaching the efficacy of quercetin. In contrast, the MN70 extract exhibited higher activity at all time points compared to MN40. The maximum anti-inflammatory effect of MN70 (46.24%) was observed 5 h after administration of the phlogogenic agent, surpassing the activity of quercetin (37.30%) and approaching that of diclofenac sodium (60.69%). Importantly, the differences between MN70 and MN40 were statistically significant at 3 h ($p < 0.05$) and 5 h ($p < 0.05$), as evidenced by lower rat paw volume increases for MN70 (27.88 ± 1.98% and 24.38 ± 1.46%) compared to MN40 (32.13 ± 3.33% and 29.31 ± 5.92%). These findings reinforce the superior anti-inflammatory potential of MN70, likely attributable to its richer composition of phenolic and flavonoid compounds, including quercetin and hydroxycinnamic acids, which may synergistically enhance its pharmacological activity. These findings indicate that liquid extracts of *M. nemorosum* herb, administered at a dose of 100 mg/kg body weight, exert anti-inflammatory effects of varying intensity in the formalin-induced paw oedema model. The extract MN70 (70% ethanol as extractant) showed greater efficacy than MN40 (40% ethanol), with MN70 outperforming quercetin and approaching the activity level of diclofenac sodium by the fifth hour of the experiment. These results are consistent with previous studies demonstrating the anti-inflammatory potential of ethanol-based plant extracts in formalin-induced paw oedema models; however, extracts of *M. nemorosum* herb were investigated in this context for the first time. For example, tannin-rich and flavonoid-containing preparations have shown significant oedema inhibition and modulation of myeloperoxidase activity in similar experimental models [64]. The enhanced activity of MN70 may be attributed to the greater solubility and extraction efficiency of bioactive polyphenols in 70% ethanol, as reported in comparative studies of ethanolic extracts from *Bougainvillea buttiana* and *Sanghuang* species [65,66]. Statistical analysis of the content of the main identified phenolic compounds (Table 1) revealed significant differences in the levels of chlorogenic acid, caffeic acid, *p*-coumaric acid, *trans*-ferulic acid, *trans*-cinnamic acid, rutin, naringin, neohesperidin, quercetin, naringenin, apigenin, rhamnetin, kaempferol, catechin, and gallocatechin in the extracts MN40 and MN70. Likewise, the content of flavonoids and total polyphenols in the liquid extract, as determined by spectrophotometry, differed significantly. Their concentrations were higher in the MN70 extract. The superior performance of MN70 compared to quercetin aligns with findings that whole-plant extracts often exert synergistic effects due to the combined action of multiple bioactive constituents

such as rutin, apigenin, and caffeic acid, which are known to modulate key inflammatory pathways, including NF-kB and COX-2 [43,67,68].

The results of the study (Table 4) demonstrate that topical application of liquid extracts MN40 and MN70 from *M. nemorosum* herb led to a reduction in bleeding time by 38.49% and 45.48%, respectively, compared to the control group. However, when compared to the reference drug, water pepper liquid extract, the haemostatic activity of the *M. nemorosum* herb extracts was less pronounced, as evidenced by an increase in bleeding time by 44.75% (MN40) and 28.30% (MN70) relative to the water pepper group. Water pepper liquid extract reduced bleeding time by 57.51% compared to the control group. Thus, the liquid extracts of *M. nemorosum* herb exhibit haemostatic activity when applied topically to a wound, reducing bleeding time by 1.62-fold (MN40) and 1.83-fold (MN70) compared to the control. The MN70 extract demonstrated the most pronounced effect, exceeding the activity of MN40 by 11.36% in terms of bleeding time reduction. These findings are in line with previous studies on the haemostatic potential of ethanol-based plant extracts; however, extracts of *M. nemorosum* herb were investigated in this context for the first time. For instance, ethanol extracts of *Euonymus fortunei* significantly shortened bleeding and clotting times in animal models, likely due to increased intracellular calcium levels in platelets and enhanced coagulation factor activity [69]. Similarly, *Chromolaena odorata* ethanol extracts demonstrated potent haemostatic effects in vivo, reducing bleeding time in rabbit ear artery models and promoting wound healing [70]. The superior performance of MN70 may be attributed to the higher extraction efficiency of active polyphenolic compounds in 70% ethanol, which is consistent with findings from other plant-based haemostatic agents [71]. Furthermore, the presence of flavonoids and tannins, known to enhance platelet aggregation and vascular constriction, likely contributes to the observed activity [72].

The results of the study (Table 4) confirm that topical application of the liquid extracts from *M. nemorosum* herb (MN40 and MN70) significantly accelerates wound surface healing compared to the control group. The most pronounced effect was observed with the MN70 extract: by day 7, the wound healing area reached 71.85%, by day 11 it increased to 97.78%, and complete wound closure was recorded by day 13. The MN40 extract also promoted tissue regeneration, though its efficacy was slightly lower, with complete healing observed on day 15. The reference drug, Rotokan, demonstrated better wound healing activity than the control but was less effective than both the *M. nemorosum* herb extracts. In Group II (MN70), complete healing occurred by day 13, while in Group I (MN40), the healing rate reached 98.89%, and in Group III (Rotokan), 95.93%. By day 15, all experimental groups achieved 100% wound closure. Thus, the liquid extracts of *M. nemorosum* herb exhibit a pronounced wound-healing effect when applied topically, surpassing the efficacy of the reference drug Rotokan. The MN70 extract demonstrated the highest activity. No signs of irritation or adverse reactions were observed following topical application of either MN40 or MN70 extracts, indicating a favourable safety profile for dermal use. The information was added to the manuscript.

These findings are consistent with previous studies on the wound-healing potential of ethanol-based plant extracts; however, extracts of *M. nemorosum* herb were investigated in this context for the first time. For example, ethanol extracts of *Costus speciosus* leaves and seeds significantly enhanced wound contraction and epithelialization in excision wound models, with leaf extracts showing faster granulation and reepithelialisation than seed extracts [73]. Similarly, *Madhuca longifolia* leaf and bark ethanol extracts demonstrated wound closure rates comparable to standard treatments like Betadine, supporting the role of polyphenolic compounds in tissue regeneration [74]. The superior performance of MN70 may be attributed to the higher extraction efficiency of flavonoids and phenolic acids in 70% ethanol, which are known to promote angiogenesis, collagen synthesis, and modulation of inflammatory mediators involved in wound repair [75,76]. Furthermore, the synergistic

action of compounds such as rutin, apigenin, and caffeic acid, identified in *M. nemorosum*, likely contributes to the enhanced healing response observed in the MN70 group.

Both *M. nemorosum* herb extracts MN40 and MN70 demonstrated antimicrobial properties. The comparative analysis of MN40 and MN70 extracts reveals a clear enhancement in antimicrobial efficacy associated with MN70, particularly against resistant strains such as methicillin-resistant *S. aureus* (MRSA) and *Candida albicans*. The inhibition zone for MRSA increased from 10.57 ± 0.50 mm with MN40 to 23.99 ± 1.34 mm with MN70, while for *C. albicans* (sputum isolate), MN70 exhibited 31.64 ± 2.24 mm compared to 21.86 ± 1.22 mm with MN40. These findings are consistent with the elevated concentrations of bioactive compounds in MN70, as indicated in the phytochemical profile (Table 1). MN70 demonstrated significantly higher levels of flavonoids such as rutin (5009.22 vs. 4625.30 µg/mL), quercetin (1416.42 vs. 1217.03 µg/mL), and naringin (596.21 vs. 475.70 µg/mL), all of which are known to exert antimicrobial effects via membrane disruption and inhibition of microbial enzymatic systems [77,78]. Moreover, the content of hydroxycinnamic acids, including *p*-coumaric (810.55 vs. 696.42 µg/mL) and *trans*-cinnamic acid (524.36 vs. 399.40 µg/mL), may further contribute to the observed activity through oxidative stress induction and microbial metabolic interference [79,80]. The increased total polyphenol content in MN70 (6.77% vs. 4.73%) also suggests a broader spectrum of synergistic phytochemicals enhancing antimicrobial potency. Overall, the more pronounced biological activity of MN70 is likely attributable to its richer and more diverse composition of phenolic and flavonoid compounds, reinforcing its potential for further development as a phytotherapeutic agent.

Importantly, the extracts retained antimicrobial activity in many cases against strains of micro-organisms resistant to commonly used antibiotics, highlighting their potential in addressing antibiotic resistance.

Limitations and Future Perspectives. While the current study offers encouraging evidence of pharmacological activity, several limitations warrant acknowledgement. The pharmacological investigations conducted were primarily of a preliminary, screening nature. Crucially, no histological analyses were performed and chronic or long-term exposure models were not included. Additionally, the biological effects of the extract have yet to be evaluated in human-derived cells, which restricts translational relevance. Considering the promising results, future research should aim to establish dose-dependent relationships and elucidate the underlying mechanisms of action across more advanced experimental systems.

5. Conclusions

Melampyrum nemorosum herb extracts are promising substances with significant haemostatic and wound-healing potential for medical applications. A total of 19 major phenolic compounds were identified and quantified in the liquid extracts, forming the basis for their standardisation. The liquid extracts of *M. nemorosum* herb were found to exhibit pronounced anti-inflammatory, antimicrobial, haemostatic, and wound-healing activities, reported here for the first time for this plant. The most promising preparation was the liquid extract obtained using 70% aqueous ethanol. Further preclinical and clinical studies may support its potential implementation in clinical practice.

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