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Chemical Composition, Acute Oral Toxicity and Wound-Healing Activity of the Herbal Blend "Lorseptil-Etd" based on Quercus Robur L., Hypericum Perforatum L. and Calendula Officinalis L. (1:1:1)

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ABSTRACT:

Background. Inflammatory diseases of the oral cavity — gingivitis, stomatitis and periodontitis — affect a large share of the world population and create a continuing demand for effective, safe and affordable topical preparations. Multi-component herbal blends combining astringent, anti-inflammatory and antiseptic raw materials are a rational source of such preparations, but blends composed of locally available raw material are rarely characterised chemically and pharmacologically in a single study.

Aim. To characterise the chemical composition of the herbal blend "Lorseptil-ETD", consisting of *Quercus robur* L. bark, *Hypericum perforatum* L. herb and *Calendula officinalis* L. flowers in a 1:1:1 ratio, and to evaluate the acute oral toxicity and the wound-healing activity of its aqueous extract.

Methods. The blend was prepared from botanically authenticated raw material meeting the requirements of the State Pharmacopoeia of the Republic of Uzbekistan. The metabolite profile was determined by nano-flow LC–ESI-Q-TOF-MS/MS and the amino acid profile by RP-HPLC of phenylthiocarbamyl derivatives with DAD detection. The preclinical part of the study was performed between 5 and 26 January 2026 at the Scientific Centre for Standardisation of Medicines (Tashkent), a facility certified for Good Laboratory Practice (certificate No. GLP-03:2025, O'zDSt 2762:2018) and accredited to O'z DSt ISO/IEC 17025:2019 (national register No. O'ZAK.SL.0314). Acute oral toxicity of the purified aqueous extract (1:10) was assessed in 12 white mice (19–22 g) at 20 and 25 mL/kg intragastrically. Wound-healing activity was studied in 18 outbred male rats (180–220 g) allocated to three groups of six using a full-thickness excision wound

model (1 cm diameter, interscapular region), with Rotokan® (JSC Tverskaya Pharmaceutical Factory, Russia) as the reference preparation.

Results. Thirteen chromatographic peaks were registered, eleven of which were assigned; the profile included flavonoids (quercetin-3-*O*-α-L-rhamnopyranoside 16.03%, isoquercitrin 7.55%, hesperidin 5.38%, quercetin 3.57%, catechin 1.83% of total peak area), the phenolic acid chlorogenic acid (2.92%), the polyphenol selaginpulvilin T (7.96%).Twenty amino acids were identified with a total content of 23.855 mg/g, essential amino acids accounting for 32.15%; proline (6.285 mg/g) and histidine (2.352 mg/g) predominated, and hydrophobic amino acids made up 51.50% of the total. No mortality occurred at either dose, indicating an intragastric LD_{50} above 25 mL/kg of the 1:10 extract. In the excision model, both treated groups showed earlier clearance of the wound surface, reduced exudation and accelerated epithelialisation compared with untreated controls; complete healing was observed on day 21 in the Lorseptil-ETD group and on day 20 in the Rotokan group, versus day 24 in controls.

Conclusion. The blend is a chemically complex, polyphenol- and amino-acid-rich material with low acute enteral toxicity, and its extract accelerated the macroscopic course of wound healing to an extent comparable with the reference preparation. These findings support its further development as a topical phytopreparation for oral-cavity disorders, and warrant confirmation in a quantitative, planimetrically controlled study.

1. INTRODUCTION

Protecting public health and developing effective methods of prevention and treatment remain priority tasks worldwide. Diseases of the oral cavity — gingivitis, stomatitis and periodontitis among them — are among the most widespread pathologies and have a substantial impact on general health and quality of life. According to the World Health Organization, between 3.5 and 3.7 billion people experience oral health problems of varying severity, which creates a persistent demand for effective, safe and economically acceptable medicines [1].

Alongside the synthetic agents used in the treatment of oral-cavity diseases, phytopreparations derived from natural sources are of particular importance. Medicines based on medicinal plants are characterised by a comparatively low incidence of adverse effects, a complex therapeutic action and good biological compatibility. The creation of effective phytopreparations from plant raw material, together with their standardisation on a scientific basis, is therefore an important direction of pharmaceutical research.

The three species combined in the present blend have long-standing and partly evidence-based use in disorders of the oral mucosa and in wound care. Oak bark (*Quercus robur* L.) is rich in gallotannins, ellagitannins and catechin-type proanthocyanidins, which are responsible for its astringent taste and for anti-inflammatory and antibacterial effects; bark of *Q. robur* is recommended for symptomatic treatment of minor inflammation of the oral mucosa and is a traditional component of herbal mixtures intended for the mouth and teeth [2]. Extracts of *Q. robur* bark show pronounced antibacterial and antifungal activity, including activity against a broad panel of Gram-positive and Gram-negative bacteria [3, 4].

Hypericum perforatum L. is one of the most extensively studied wound-healing medicinal plants; its activity is attributed to the combined action of hypericin, hyperforin and flavonoids, which modulate the inflammatory phase, stimulate fibroblast migration and support re-epithelialisation [5]. Its antibacterial activity, including activity against oral bacteria and biofilm formation, has also been documented [6].

Calendula officinalis L. is traditionally applied for skin injuries and inflammation of the oral and pharyngeal mucosa. Systematic and narrative reviews of the experimental evidence report faster resolution of the inflammatory phase and increased granulation tissue formation in calendula-treated animals, while the clinical evidence remains heterogeneous and of variable quality [7, 8].

Although each of the three species has been characterised individually, data on their combined use in a fixed 1:1:1 ratio are scarce, and the metabolite and amino acid profile of such a blend prepared from raw material of Uzbek origin has not previously

been reported. The aim of the present work was therefore to study the chemical composition, acute oral toxicity and specific (wound-healing) activity of the herbal blend "Lorseptil-ETD", recommended for diseases of the oral cavity.

2. Materials and Methods

2.1. Plant material and preparation of the blend

The object of the study was the herbal blend "Lorseptil-ETD", consisting of the medicinal plant raw materials *Quercus robur* L. (bark), *Hypericum perforatum* L. (herb) and *Calendula officinalis* L. (flowers) in a 1:1:1 ratio. The chemical investigations were performed at the A. S. Sadykov Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan, and the preclinical investigations at the Scientific Centre for Standardisation of Medicines (Tashkent).

The raw material was obtained from botanically authenticated plants collected at the appropriate vegetation periods and dried in accordance with established collection and drying instructions [9–11]. Compliance of each raw material with the requirements of the corresponding regulatory documents was verified before use [12]. The raw materials were then comminuted, weighed in the required proportion, mixed and sieved through a C30/50 sieve [13].

2.2. Preparation of the extract

Distilled water was used as extractant. The water was boiled for 15 min and cooled to room temperature (20–25 °C). Extraction was carried out at the standard pharmacognostic raw material-to-solvent ratio of 1:10 (mass of plant material to volume of extractant), a ratio adopted in pharmacognostic practice as producing reproducible and pharmacologically active aqueous extracts without excessive dilution.

A weighed portion of the comminuted blend was placed in a sterile glass flask with a tightly closing lid and covered with the calculated volume of boiled water previously cooled to room temperature. The contents were stirred with a glass rod until the plant material was completely wetted and the extractant evenly distributed. The vessel was then hermetically sealed and kept at 4 °C for 24 h; during infusion the contents were carefully stirred three times to intensify diffusion and improve the recovery of biologically active substances.

The resulting infusion was filtered sequentially: first through a double layer of sterile gauze to remove coarse mechanical impurities, then through a paper filter, and finally through a membrane filter with a pore diameter of 0.45 μm, yielding a purified extract suitable for pharmacological investigation. The purified extract was stored in tightly closed dark glass containers at 4 °C and used no later than 48 h after preparation.

2.3. LC–MS analysis

Compounds present in the blend were determined by LC–MS using an Agilent 1200 nano-flow LC system coupled to a reversed-phase nano-LC–MS/MS 6520V series CHIP-Q-TOF mass spectrometer (Agilent Technologies, USA). Mobile phase: A — 0.1% formic acid + 5% acetonitrile; B — acetonitrile + 0.1% formic acid + 10% deionised water. Sample loading was performed on an Agilent Technologies 1260 series Cap Pump at a flow rate of 4 μL/min; elution was carried out on an Agilent Technologies 1260 Nano Pump at 0.6 μL/min. Gradient of solution B: 0% at 3 min, 60% at 12–18 min, 0% at 20 min. Solutions were degassed on an Agilent Technologies 1260 μ-degasser. Samples of 2 μL were introduced onto the column using an Agilent Technologies Micro WPS autosampler.

Mass-spectrometric analysis of the eluted fractions was performed under the following conditions: ionisation source ESI+; drying gas flow 4 μL/min; drying gas temperature 350 °C; skimmer cone voltage 65 V; fragmentor voltage 175 V; mass range 50–3000 m/z in MS mode and 50–2500 m/z in MS/MS mode; capillary voltage 1800–2500 V; positive ionisation mode [14, 15].

Compound assignment was based on accurate mass and MS/MS fragmentation compared with the available analytical data. Relative quantities are expressed as the percentage of total peak area of the chromatogram and do not represent absolute mass fractions.

2.4. Amino acid analysis

For the separation of amino acids, proteins and peptides were precipitated from the aqueous extract: 20% trichloroacetic acid was added to 1 mL of the test sample and, after 10 min, the precipitate was separated by centrifugation at 8000 rpm for 15 min.

A 0.1 mL aliquot of the supernatant was taken and lyophilised. The hydrolysate was evaporated and the dry residue was dissolved in a triethylamine–acetonitrile–water mixture (1:7:1) and dried; the procedure was repeated twice to neutralise the acid. Phenylthiocarbamyl (PTC) derivatives of the amino acids were obtained by reaction with phenylisothiocyanate (Edman reagent) [16–18].

Identification of amino acids was carried out by HPLC on an Agilent Technologies 1200 chromatograph with a DAD detector under the following conditions: column 75 × 4.6 mm Discovery HS C_{18}; solution A — 0.14 M sodium acetate + 0.05% triethylamine, pH 6.4; solution B — acetonitrile; flow rate 1.2 mL/min; detection at 269 nm; gradient (%B/min): 1–6%/0–2.5 min; 6–30%/2.51–40 min; 30–60%/40.1–45 min; 60–60%/45.1–50 min; 60–0%/50.1–55 min.

The amino acid score (AAS) was calculated as the ratio of the content of each essential amino acid in the sample to the corresponding FAO reference value, expressed as a percentage.

2.5. Animals, facility and ethical approval

The preclinical part of the study was carried out between 5 and 26 January 2026 at the Scientific Centre for Standardisation of Medicines (Oybek St. 45, Tashkent 100015, Republic of Uzbekistan). The testing laboratory of the Centre holds a Good Laboratory Practice certificate issued under O'zDSt 2762:2018 (certificate No. GLP-03:2025) and is accredited by the Uzbek Accreditation Centre to O'z DSt ISO/IEC 17025:2019 (national register No. O'ZAK.SL.0314, valid until 6 September 2028). The study was conducted under the supervision of the Head of the Research Department and performed by the responsible investigator; an unscheduled quality inspection was carried out on 26 January 2026.

Acute toxicity was studied in 12 white mice of body weight 19–22 g, allocated to two groups of six. Animals were kept in standard plastic cages with sawdust bedding in a separate room at an air temperature of 20–25 °C and relative humidity of 40–70%; temperature and humidity were recorded daily. Food and water were available *ad libitum*. All animals used were clinically healthy and free of physiological abnormalities.

Wound-healing activity was studied in 18 outbred white male rats of body weight 180–220 g, allocated to three groups of six. Before the start of the experiment all animals underwent acclimatisation for 5 days. Rats were housed under standard vivarium conditions at 22–24 °C, relative humidity 55–65%, on a 12-hour light–dark cycle, with free access to food and water.

2.6. Acute oral toxicity

On the day of the experiment the mice were deprived of food until administration of the preparation. Two doses were selected on the basis of previously registered levels of acute enteral toxicity of similar preparations, taking into account the expected pharmacokinetics and safety margins of the test substance. The purified aqueous extract (1:10) was administered as a single intragastric dose:

- Group I (n = 6): 20 mL/kg;
- Group II (n = 6): 25 mL/kg.

Animals were observed for signs of acute toxicity and mortality. LD_{50} was estimated from mortality data.

2.7. Excision wound model

A comparative preclinical study of the effect of the preparations on the rate and quality of healing of superficial skin wounds was carried out in laboratory rats using a full-thickness excision wound model. Animals were allocated to three groups of six:

- **Group I (control) —** animals with a reproduced full-thickness skin wound receiving no treatment;
- **Group II (Lorseptil-ETD) —** animals with a reproduced full-thickness skin wound treated with the purified extract of the herbal blend "Lorseptil-ETD";
- **Group III (reference) —** animals with a reproduced full-thickness skin wound treated with Rotokan® (extract for oral and topical use, 50 mL, series 130623, exp. 07.2026; JSC Tverskaya Pharmaceutical Factory, Russia).

Under general anaesthesia the operative field was treated with 70% ethanol and a circular full-thickness skin fragment 1 cm in diameter was excised from the previously shaved interscapular region of the back. The wound surface was left open without

suturing, which ensured a natural course of healing and allowed an objective assessment of the efficacy of the preparations studied.

The preparations were applied strictly according to group allocation. Extracts were applied directly onto the wound surface and into the wound cavity by irrigation with a sterile syringe, ensuring complete and uniform wetting of the entire wound surface. The first application was performed immediately after the wound was created; subsequent applications were carried out once daily until complete healing.

2.8. Assessment of healing and data analysis

The course of the wound process was assessed macroscopically at fixed intervals over 24 days. The following features were recorded for each group: intensity of exudation, presence and character of inflammation, condition of the wound edges, formation of granulation tissue, and the extent of epithelialisation. The reduction of the wound area was estimated visually and expressed as an approximate percentage relative to the initial defect; the day of complete healing was recorded for each group.

No planimetric measurement of wound area and no statistical analysis were performed. Accordingly, the results in Section 3.5 are presented as qualitative and semi-quantitative observations, and no claim of statistical significance is made.

3. Results

3.1. Metabolite profile (LC–MS)

The LC–MS chromatogram of the blend is shown in Figure 1; the assigned compounds and their relative peak areas are given in Table 1 and Figure 2.

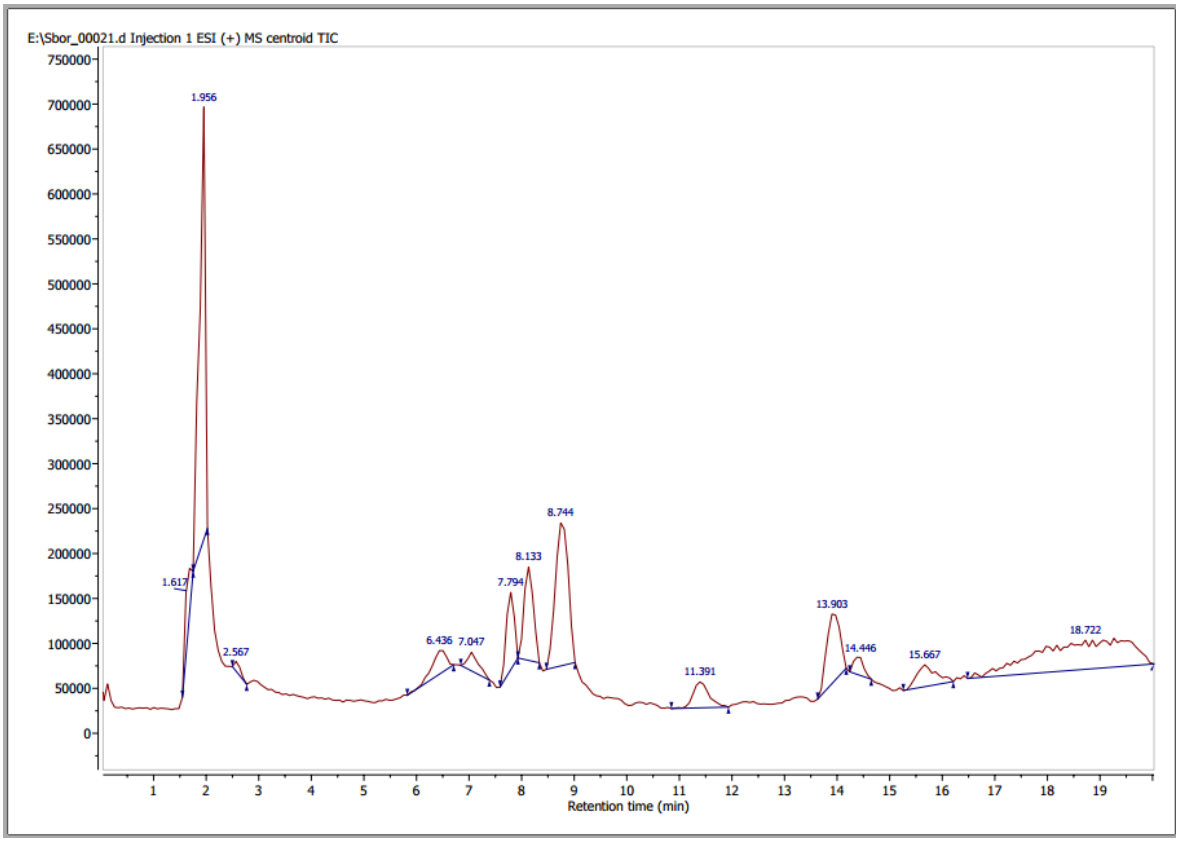

Figure 1. LC–MS total ion chromatogram (ESI+) of the herbal blend "Lorseptil-ETD" (*Quercus robur* L., *Hypericum perforatum* L., *Calendula officinalis* L., 1:1:1). Peak labels show retention times, min.

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Table 1: Results **of LC–MS analysis of the herbal blend "Lorseptil-ETD".**

Peak	RT, min	Relative peak area, %	Compound	Chemical class
1	1.62	2.86	Not identified	–
2	1.96	22.19	L-Isoleucine or L-leucine	Amino acid
3	2.57	0.54	L-Phenylalanine	Amino acid
4	6.44	2.92	Chlorogenic acid	Phenolic acid
5	7.05	1.83	Catechin	Flavonoid
6	7.79	5.38	Hesperidin	Flavonoid
7	8.13	7.55	Isoquercitrin	Flavonoid
8	8.74	16.03	Quercetin-3-*O*-α-L-rhamnopyranoside (quercitrin)	Flavonoid
9	11.39	3.57	Quercetin	Flavonoid
10	13.90	7.96	Selaginpulvilin T	Polyphenolic compound
11	15.67	3.64	*N*-Acetyl-L-proline	Amino acid derivative
12	18.72	23.99	Not identified	–
		100.00	**Total**	

RT — retention time. Percentages are relative peak areas of the total chromatogram and do not represent absolute mass fractions.

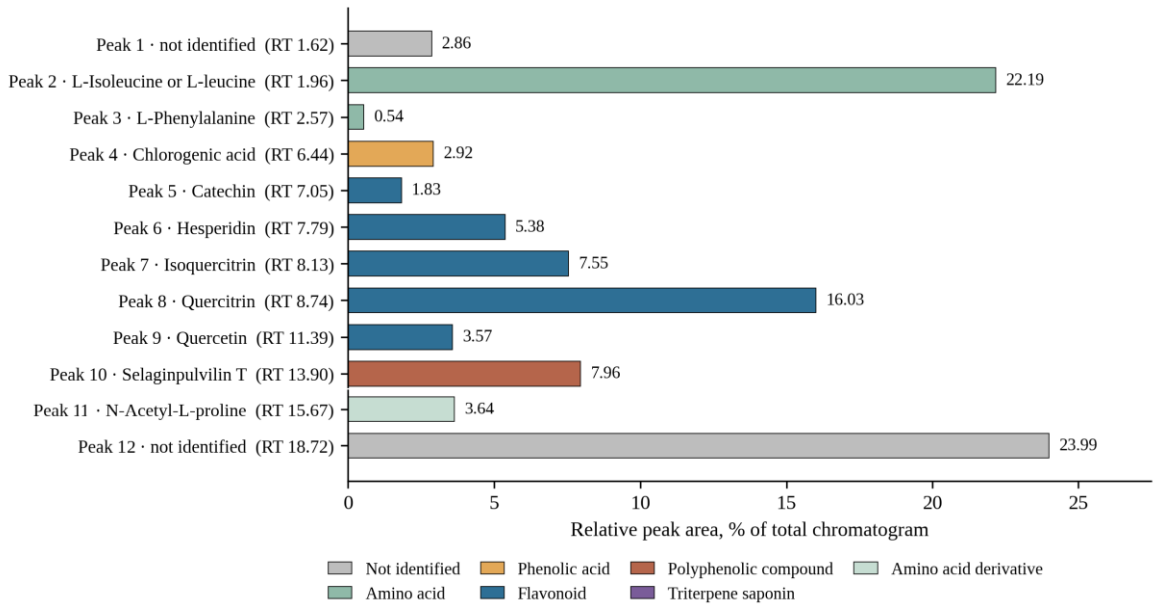

Figure 2. Relative peak areas of the 12 chromatographic peaks registered by LC–MS, coloured by chemical class (data from Table 1). RT — retention time, min.

The analysis showed that the chromatographic profile of the blend is complex and comprises metabolites of different classes. Thirteen chromatographic peaks were registered, of which eleven were assigned to specific chemical compounds on the basis of the available analytical data; two peaks could not be linked to a defined compound under the present analytical conditions. The identified compounds included amino acids, flavonoids, phenolic acids, triterpene saponins and other secondary metabolites.

The largest share among the assigned compounds was the peak attributed to L-isoleucine or L-leucine (22.19%). As these isomers cannot be strictly distinguished on the basis of the present chromatographic result alone, this assignment is provisional; unambiguous differentiation requires authentic standards or additional spectrometric data.

Among the flavonoids, quercetin-3-*O*-α-L-rhamnopyranoside (quercitrin) showed the highest value (16.03%), indicating a considerable accumulation of quercetin-based glycosylated flavonoids in the sample. The detection of isoquercitrin (7.55%) and hesperidin (5.38%) further enriches the flavonoid profile and indicates the presence of both aglycone and glycoside forms. The determination of quercetin itself (3.57%) alongside its glycoside quercitrin points to the co-occurrence of several metabolites derived from the same aglycone, which may reflect the secondary metabolic pathways of the plant material.

Selaginpulvilin T was registered at 7.96%. The relatively high share of this component may be regarded as one of the characteristic features of the chemical profile of the sample. The detection of chlorogenic acid (2.92%) and catechin (1.83%) confirms a substantial content of phenolic metabolites. *N*-Acetyl-L-proline (3.64%) the simultaneous detection of substances belonging to different chemical classes confirms the multi-component nature of the metabolome of the raw material studied.

No defined chemical compound was assigned to peak 1 (2.86%) or peak 12 (23.99%). The relative content of peak 12 accounts for a substantial part of the entire chromatographic profile, and its identification should therefore be a priority direction of further research; the absence of this assignment limits full interpretation of the chemical profile.

Attention should also be paid to the degree of chromatographic separation of the components detected. Low resolution (Rs) values for some peaks may indicate incomplete separation of adjacent components, and caution is required when interpreting identification results for components with close retention times. Optimisation of the chromatographic conditions is advisable for the accurate separation and identification of closely eluting flavonoids.

Overall, the results show that the sample is characterised by a diversity of flavonoids and other polyphenolic metabolites. The co-occurrence of quercitrin, isoquercitrin, hesperidin, quercetin, chlorogenic acid and catechin defines the main groups forming the polyphenolic profile, while the relatively high content of selaginpulvilin T indicates a distinctive secondary metabolite composition. Before the percentages given are interpreted as absolute mass fractions, however, the quantification method applied must be taken into account; in particular, because of the high share of the unidentified peak 12, additional confirmation by LC–MS/MS, HRMS or authentic standards is required for a complete chemical description of the sample.

3.2. Amino acid composition

Determination of the quantity and qualitative composition of amino acids in plant raw material is relevant to pharmaceutical analysis, phytochemistry and drug development, since these data allow the pharmacological value of medicinal plants to be assessed and biologically active additives and phytopreparations to be standardised.

The amino acids of the blend were analysed by HPLC. The results are presented in Figures 3 and 4 and in Table 2.

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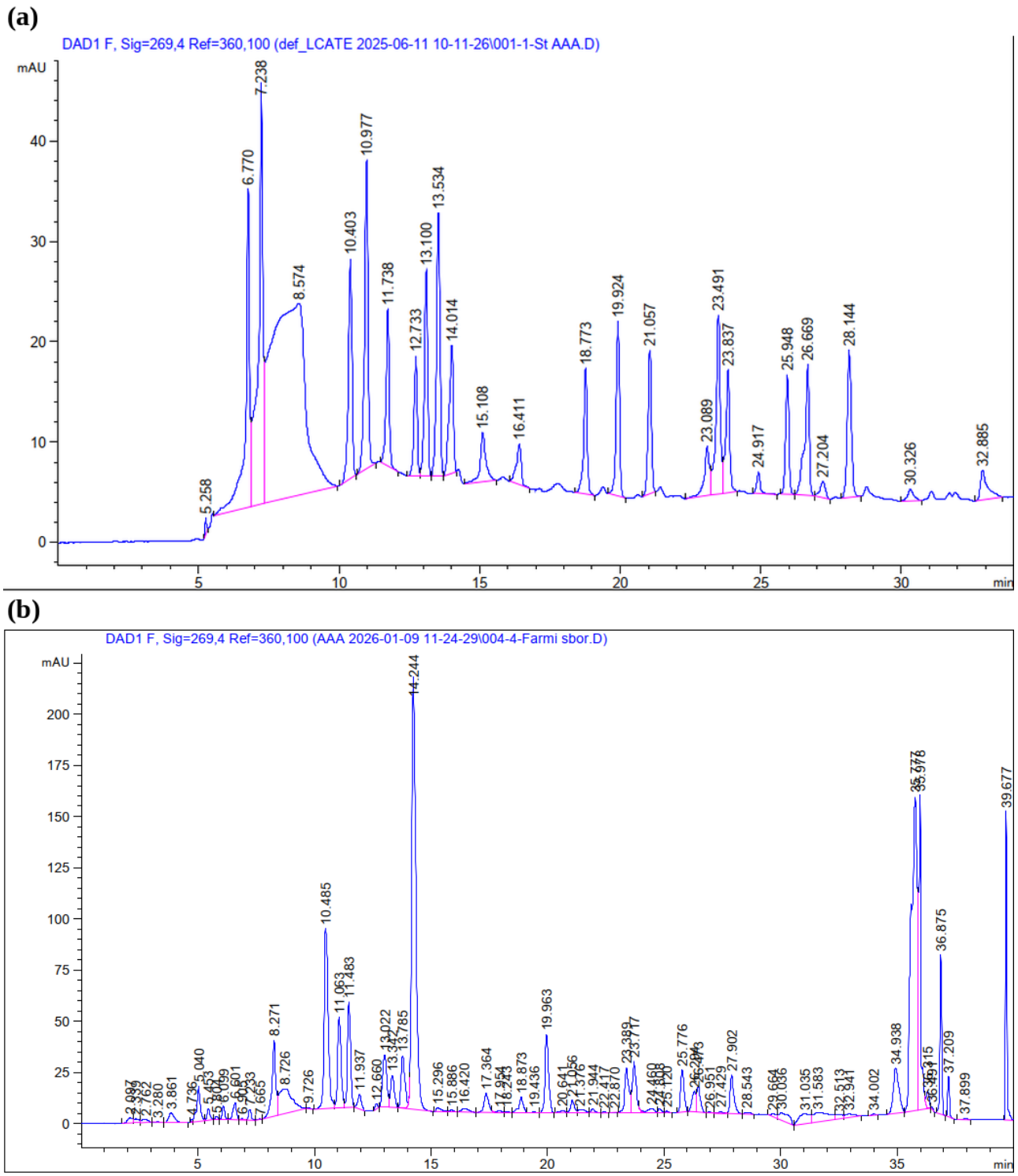

Figure 3. HPLC chromatograms (DAD, 269 nm) of phenylthiocarbamyl amino acid derivatives: (a) working amino acid standard; (b) herbal blend "Lorseptil-ETD".

Table 2: Amino acid composition of the herbal blend "Lorseptil-ETD".

No.	Amino acid	Content, mg/g	Share of total, %
Conditionally essential amino acids			
1	Tyrosine	0.657	2.75
2	Cysteine	0.369	1.55
3	Arginine	0.087	0.36
4	Histidine	2.352	9.86
	Subtotal	**3.465**	**14.52**
Non-essential amino acids			
5	Glutamic acid	0.457	1.91
6	Aspartic acid	1.321	5.54
7	Glycine	0.788	3.3
8	Proline	6.285	26.34
9	Alanine	1.119	4.69
10	Glutamine	0.328	1.38
11	Asparagine	0.765	3.21
12	Serine	1.659	6.96
	Subtotal	**12.722**	**53.33**
Essential amino acids			
13	Threonine	1.741	7.3
14	Valine	0.745	3.12
15	Methionine	0.892	3.74
16	Isoleucine	1.174	4.92
17	Leucine	1.285	5.39
18	Tryptophan	1.457	6.11
19	Phenylalanine	0.205	0.86
20	Lysine	0.169	0.71
	Subtotal	**7.668**	**32.15**
	Total	**23.855**	**100**

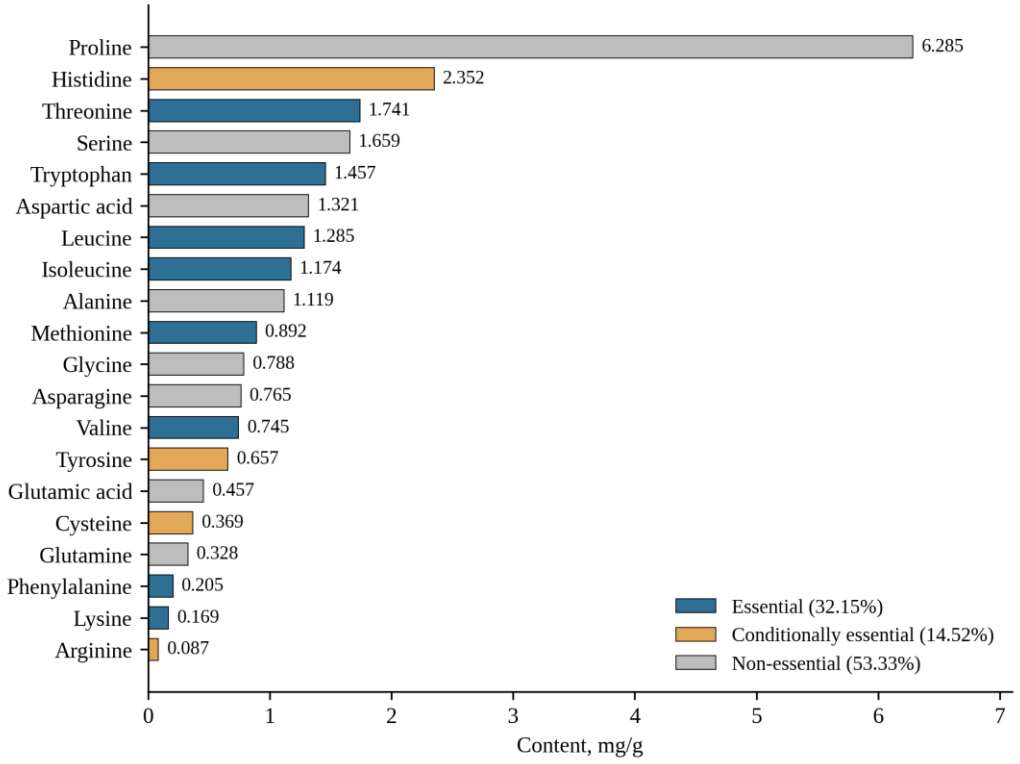

Figure 4. Content of individual amino acids in the herbal blend "Lorseptil-ETD", mg/g, in decreasing order (data from Table 2). Percentages in the legend give the share of each group in the total amino acid content.

Twenty amino acids were identified in the blend. Eight of them are essential and account for 32.15% of the total; conditionally essential amino acids account for 14.52% and non-essential amino acids for 53.33%.

The identified amino acids can be arranged in the following decreasing order:

Proline > histidine > threonine > serine > tryptophan > aspartic acid > leucine > isoleucine > alanine > methionine > glycine > asparagine > valine > tyrosine > glutamic acid > cysteine > glutamine > phenylalanine > lysine > arginine.

Among the conditionally essential amino acids, histidine predominates (2.352 mg/g); among the non-essential amino acids, proline (6.285 mg/g), serine (1.659 mg/g) and aspartic acid (1.321 mg/g); and among the essential amino acids, threonine (1.741 mg/g), tryptophan (1.457 mg/g) and leucine (1.285 mg/g).

By physicochemical classification, acidic amino acids constitute 7.45% (aspartic acid 5.54%, glutamic acid 1.91%), basic amino acids 10.93% (histidine 9.86%, arginine 0.36%, lysine 0.71%), polar uncharged amino acids 20.40% (serine 6.96%, threonine 7.30%, asparagine 3.21%, glutamine 1.38%, cysteine 1.55%), non-polar (hydrophobic) amino acids 51.50% (proline 26.34%, alanine 4.69%, glycine 3.30%, valine 3.12%, leucine 5.39%, isoleucine 4.92%, methionine 3.74%) and aromatic amino acids 9.72% (tryptophan 6.11%, tyrosine 2.75%, phenylalanine 0.86%). Hydrophobic amino acids therefore predominate in the blend.

(Note: the aromatic group overlaps with the hydrophobic and polar groups, so these categories are not mutually exclusive and do not sum to 100%.)

3.3. Amino acid score in comparison with FAO reference values

The amino acid score (AAS) expresses the content of an amino acid in the product as a percentage of the reference value established by the Food and Agriculture Organization (FAO). Values below 100% indicate limiting amino acids. The results of the comparison are presented in Table 3 and Figure 5.

Table 3: Amino acid score of the essential amino acids of the blend in comparison with FAO reference values.

Amino acid	Content, %	FAO reference, %	AAS, %	Assessment
Lysine	0.71	5.5	12.9	Limiting
Tryptophan	6.11	1.1	555.5	Fully satisfies
Threonine	7.30	4.0	182.5	Fully satisfies
Methionine + cysteine	5.29	3.5	151.1	Fully satisfies
Phenylalanine + tyrosine	3.61	6.0	60.2	Partially limiting
Valine	3.12	4.0	78.0	Partially limiting
Isoleucine	4.92	4.0	123.0	Fully satisfies
Leucine	5.39	7.0	77.0	Partially limiting
Histidine	9.86	2.0	493.0	Fully satisfies

Content is expressed as a percentage of total amino acids. AAS — amino acid score (content ÷ FAO reference × 100). AAS > 100% — fully satisfies the FAO reference pattern; AAS < 100% — limiting.

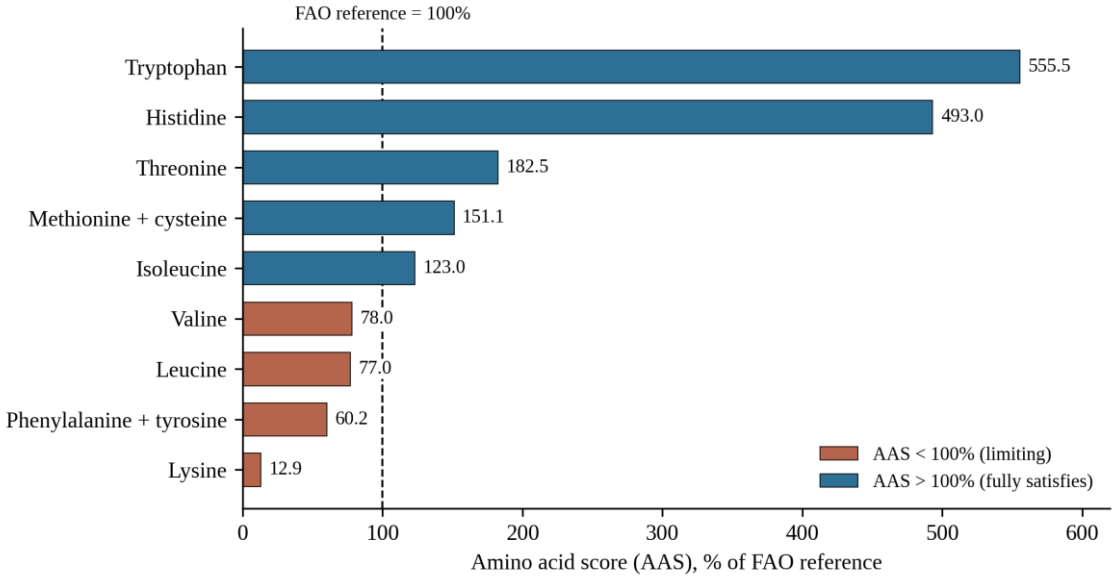

Figure 5. Amino acid score (AAS) of the essential amino acids of the blend relative to the FAO reference pattern; the dashed line marks 100% (data from Table 3).

As Table 3 shows, five of the nine indicators assessed (tryptophan, threonine, methionine + cysteine, isoleucine and histidine) fully satisfy the FAO reference values, three (phenylalanine + tyrosine, valine and leucine) are partially limiting, and lysine is the limiting amino acid with an AAS of 12.9%.

Tryptophan exceeds the FAO reference value more than fivefold and is the precursor of serotonin. Histidine is present at 493% of the reference value and is of importance for tissue repair. Threonine exceeds the reference value twofold and contributes positively to protein balance. The sulphur-containing amino acids methionine and cysteine, which possess antioxidant properties and play a role in stabilising protein structure, together fully satisfy the FAO requirement.

It should be emphasised that the FAO amino acid scoring pattern was developed to evaluate the nutritional quality of dietary protein and not the composition of a topically applied herbal infusion. The comparison presented here is therefore descriptive, and no nutritional claim is made.

3.4. Acute oral toxicity

After administration of the extract at a dose of 20 mL/kg no mortality was observed; all mice tolerated the administration satisfactorily and without signs of acute toxicity. In the second group (body weight 19–22 g), intragastric administration of the higher dose of 25 mL/kg likewise caused no deaths, and all animals remained alive throughout the observation period. On the basis of the complete absence of mortality in both groups, the LD_{50} of the extract on intragastric administration exceeds 25 mL/kg. The results are presented in Table 4.

Table 4: Acute oral toxicity of the purified aqueous extract of the herbal blend "Lorseptil-ETD" in mice.

Animal No.	Group I — 20 mL/kg		Group II — 25 mL/kg	
	Body weight, g	Outcome	Body weight, g	Outcome
1	20	No mortality	19	No mortality
2	19	No mortality	20	No mortality
3	22	No mortality	21	No mortality
4	19	No mortality	20	No mortality
5	20	No mortality	21	No mortality
6	19	No mortality	22	No mortality
LD_{50}	**> 25 mL/kg**			

Route of administration: intragastric, single dose; six animals per group.

A single intragastric administration of the extract at doses of 20 and 25 mL/kg caused no mortality, and all animals in both experimental groups tolerated the administration without signs of acute toxicity, which allows the extract to be classified as a substance of low acute enteral toxicity on single administration.

3.5. Wound-healing activity

The wound process proceeded in phases in all animals, but the rate and intensity of the reparative processes differed appreciably between groups.

In the control group, pronounced exudation and an inflammatory reaction were observed during the first 24 h. Active granulation and signs of inflammation persisted up to days 4–7. A reduction of the wound area of approximately 50% was observed by day 13 and of approximately 85% by day 19, with complete healing occurring on day 24.

In Group II (Lorseptil-ETD), the wound surface was already clean on day 4, exudation was minimal and the inflammatory reaction considerably reduced. The wound area decreased by approximately 35–40% by day 10, by 65–70% by day 13 and by 80–85% by day 16; complete healing was observed on day 21.

In Group III (Rotokan®), acceleration of the reparative processes was likewise observed compared with the control. The wound area decreased by approximately 60–65% by day 13 and by 80–85% by day 16, and complete healing occurred on day 20. The results are summarised in Table 5 and Figure 6.

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Table 5: Comparative macroscopic characteristics of wound healing in rats treated with the herbal blend "Lorseptil-ETD" and with Rotokan® (JSC Tverskaya Pharmaceutical Factory, Russia); six animals per group.

Day	Group I (control)	Group II (Lorseptil-ETD)	Group III (Rotokan®)
1	Abundant exudate, moderate inflammation, wound edges moist	Abundant exudate, moderate inflammation, wound edges moist	Abundant exudate, moderate inflammation, wound edges moist
4	Pronounced granulation, suppuration	Wound surface clean, granulation less pronounced	Moderate exudate, wound surface clean, granulation less pronounced
7	Active granulation, inflammation persists	Moderate granulation, wound edges dry	Moderate granulation, wound edges dry
10	Active formation of granulation tissue	Area ↓ ~35–40%, epithelialisation less pronounced	Area ↓ ~35–40%, epithelialisation less pronounced
13	Area ↓ ~50%, active epithelialisation	Area ↓ ~65–70%, marked granulation, partial epithelialisation	Area ↓ ~60–65%, marked granulation, partial epithelialisation
16	Area ↓ ~70%, pronounced epithelialisation	Area ↓ ~80–85%, epithelialisation continuing	Area ↓ ~80–85%, epithelialisation continuing
19	Area ↓ ~85%, almost closed, scarring beginning	Area ↓ ~90–95%, close to complete healing	Area ↓ ~95–97%, close to complete healing
20	—	Area ↓ ~97–98%, close to complete healing	Complete healing
21	Area ↓ ~95%, virtually complete epithelialisation	Complete healing	—
24	Complete healing	—	—

Wound area reduction was estimated visually and is given as an approximate percentage of the initial defect; no planimetric measurement or statistical comparison was performed. — healing already complete.

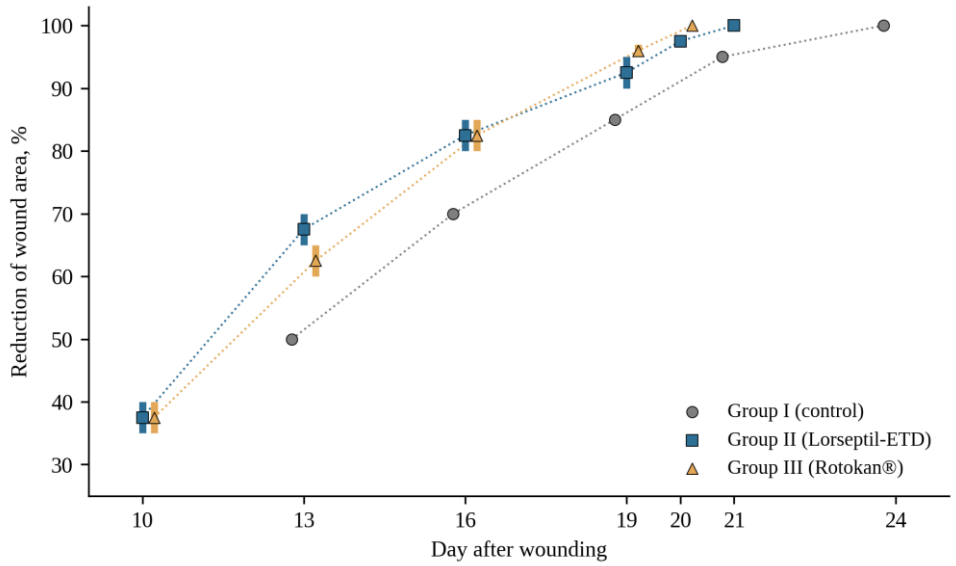

Figure 6. Reduction of wound area in rats with a full-thickness excision wound (data from Table 5). Values are visual estimates as reported; vertical bars show the reported ranges and markers their midpoints. Points are offset slightly along the horizontal axis for clarity; no measurement was reported for the control group on day 10.

The preclinical study showed that the herbal blend possesses wound-healing, anti-inflammatory and antiseptic properties. Its application promoted faster cleansing of the wound surface, reduced the intensity of exudation and inflammatory reactions and accelerated granulation and epithelialisation compared with the control group. In terms of the macroscopic dynamics of the reparative processes, the preparation showed a course comparable with that of the reference preparation Rotokan®.

4. DISCUSSION

The results obtained show that the herbal blend "Lorseptil-ETD" is a chemically complex material in which polyphenolic metabolites and free amino acids occur together, and that its aqueous extract combines low acute enteral toxicity with a favourable macroscopic course of healing in a full-thickness excision wound model.

The polyphenolic fraction of the blend is dominated by quercetin and its glycosides. The co-occurrence of quercitrin, isoquercitrin and free quercetin, together with catechin and chlorogenic acid, is consistent with the composition expected from the three species combined: catechins and condensed tannins are characteristic of oak bark, where they are responsible for the astringent and antibacterial properties for which the raw material is recommended in inflammation of the oral mucosa [2, 3], whereas quercetin glycosides are a well-documented constituent of both *H. perforatum* and *C. officinalis* [5, 7]. Flavonoids of this type are widely reported to modulate the inflammatory phase of wound healing and to reduce oxidative damage in granulation tissue, which provides a plausible mechanistic basis for the effects observed in the present model [5, 8].

The detection of glycyrrhizic acid, a triterpene saponin, indicates that the blend also contains surface-active saponin components; these contribute to the wetting and cleansing of the wound surface and may facilitate the penetration of the polyphenolic constituents. This is consistent with the mode of application used here, in which the extract was delivered by irrigation directly into the wound cavity.

The amino acid profile is dominated by proline (26.34% of total amino acids), which is of interest in the context of the biological effect observed: proline and hydroxyproline are the principal constituents of collagen, and the availability of free proline is one of the factors that determines the rate of collagen deposition in granulation tissue. Histidine, the second most abundant amino acid detected, is likewise associated with tissue repair. It should be emphasised, however, that these correlations are hypothesis-generating: the present study did not measure hydroxyproline content, collagen deposition or any biochemical marker of repair, and the contribution of the free amino acid fraction of a topically applied infusion to healing cannot be quantified from the present data.

The absence of mortality at both doses indicates that the extract belongs to substances of low acute enteral toxicity on single administration. This finding must nevertheless be interpreted with caution. The doses were expressed as volumes of a 1:10 aqueous extract, and 25 mL/kg approaches the maximum volume that can be administered by gavage to a mouse; the result therefore establishes that no toxicity occurs up to the maximum administrable volume, rather than defining a toxicological ceiling. Expressing the dose in mg/kg of dry extract and extending the study to the OECD limit dose would allow direct comparison with the literature.

In the excision model, both treated groups reached complete healing 3–4 days earlier than the untreated control, and the macroscopic dynamics were comparable with those of the reference preparation Rotokan®, itself a combined extract of camomile, calendula and yarrow. This comparability is the practically relevant observation: it suggests that a blend composed of locally available raw material may reproduce the effect of an imported combined preparation. The comparison remains preliminary, however: the assessment was macroscopic and semi-quantitative, without planimetry, statistical analysis or histological confirmation, and the study included no single-species comparator arms that would show what the blend adds over its individual components.

4.1. Limitations

The present study has several limitations. First, two chromatographic peaks, one of them accounting for almost a quarter of the total peak area, remain unidentified, so the chemical characterisation of the blend is incomplete; confirmation by HRMS or authentic standards is required. Second, the quantitative LC–MS data are expressed as relative peak areas and not as absolute contents. Third, the acute toxicity assessment was limited to two volumes in a small number of animals and does not constitute a full OECD 423 study. Fourth, and most importantly, wound healing was assessed macroscopically without planimetric measurement of wound area, so no statistical comparison between groups was possible and the differences described are descriptive rather than demonstrated; histological confirmation and single-species comparator groups were likewise absent.

Fifth, no antimicrobial testing against the oral pathogens relevant to the proposed indication was performed, although the intended application is in inflammatory diseases of the oral cavity.

5. CONCLUSIONS

1 LC–MS analysis of the herbal blend "Lorseptil-ETD" (*Quercus robur* L., *Hypericum perforatum* L., *Calendula officinalis* L., 1:1:1) revealed a multi-component metabolite profile including the flavonoids catechin, hesperidin, isoquercitrin, quercetin-3-*O*-α-L-rhamnopyranoside and quercetin, of which quercetin-3-*O*-α-L-rhamnopyranoside predominated (16.03% of total peak area). The detection of glycyrrhizic acid, selaginpulvilin T and chlorogenic acid showed that the blend also contains components of polyphenolic and saponin nature.

2 Twenty amino acids were identified with a total content of 23.855 mg/g, essential amino acids accounting for 32.15%. Proline and histidine predominated quantitatively, and hydrophobic amino acids made up 51.50% of the total. Comparison with FAO reference values showed that five of the nine essential amino acid indicators fully satisfy the reference pattern, while lysine is the limiting amino acid.

3 A single intragastric administration of the purified aqueous extract at 20 and 25 mL/kg caused no mortality, indicating an LD_{50} above 25 mL/kg and allowing the extract to be classified as a substance of low acute enteral toxicity on single administration.

4 In a full-thickness excision wound model the extract promoted faster cleansing of the wound surface, reduced exudation and accelerated granulation and epithelialisation compared with untreated controls, with complete healing on day 21 versus day 24 in the control group.

5 In the macroscopic course of wound healing the extract showed a dynamic comparable with that of the reference preparation Rotokan®. Confirmation of this observation requires a quantitative study with planimetric measurement, statistical analysis and histological assessment.

DECLARATIONS

Ethical approval. The preclinical experiments were carried out at the testing laboratory of the Scientific Centre for Standardisation of Medicines (Tashkent), which holds a Good Laboratory Practice certificate (No. GLP-03:2025, O'zDSt 2762:2018) and ISO/IEC 17025:2019 accreditation (No. O'ZAK.SL.0314), in accordance with the national requirements for preclinical studies of medicinal products in force in the Republic of Uzbekistan.

Conflict of interest. The authors declare that they have no conflict of interest.

Data availability. The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Use of AI tools. Generative AI was used to assist with translation of the manuscript into English and with language editing. The authors reviewed and edited all content and take full responsibility for the content of the publication.

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