

Article

Integrated Pharmacognostic, LC-MS Metabolomic Profiling and Biological Evaluation of *Elymus repens* (L.) Gould

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Abstract

Elymus repens (L.) Gould has a long history of use in traditional medicine across the British Isles, particularly among Gaelic and Anglo-Saxon communities, where it has been employed as a diuretic and anti-inflammatory agent. Despite its ethnopharmacological significance, comprehensive insights into its phytochemical composition and biological activities remain limited. The present study aimed to provide an integrated characterization of *E. repens* through macro- and microscopic analyses, advanced phytochemical profiling, and evaluation of the biological activities of the rhizome part. Microscopic examination revealed distinct anatomical features differentiating rhizome and stem, leaf tissues, supporting accurate identification and pharmacognostic standardization. Chemical profiling using Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-QToF-MS) enabled the tentative identification of 93 metabolites, including amino acids, in aerial and rhizome extracts. These compounds were primarily classified into polyamines (e.g., feruloylputrescine, hydroxycoumaroylagmatine), phenolic acids (gallic, vanillic, and ferulic acids), flavonoids (apigenin, tricetin, saponarin analogues), amino acids (arginine, tyrosine, tryptophan), organic acids (malic, succinic acids), nucleosides (adenosine, thymidine), and phospholipids. Biological evaluation demonstrated that the hydroethanolic rhizome extract exhibited notable antifungal activity against *Aspergillus fumigatus* (IC₅₀ = 34.9 µg/mL). Additionally, hydroethanolic extracts of both the aerial and rhizome parts showed no cytotoxicity in the *Artemia salina* lethality assay at concentrations up to 10 mg/mL, indicating a favorable preliminary safety profile. Of particular interest is tricetin, which possesses anti-inflammatory, antioxidant, antimicrobial, and potential nephroprotective therapeutic effects; its mechanism of action is associated with the suppression of oxidative stress, the inhibition of pro-inflammatory mediators, and the disruption of metabolic processes in microbial cells. Overall, this study provides a comprehensive phytochemical and pharmacognostic characterization of *E. repens*, highlighting its potential as a source of bioactive compounds. The metabolite profile obtained directly from the biologically active extract,



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combined with its proven antifungal activity, serves as direct confirmation of the antimicrobial aspect of this plant's traditional use, while its broader applications in ethnomedicine require targeted pharmacological testing. These findings contribute valuable data for chemotaxonomic classification and future pharmacological investigations.

Keywords: natural products; microscopy; phytochemical profiling; LC-QToF-MS; antimicrobial activity; cytotoxic activity

1. Introduction

Elymus repens (L.) Gould, commonly known as quack grass, couch grass, or dog grass, is a perennial species belonging to the family Poaceae (grass family), one of the most ecologically and economically significant plant families worldwide. Native to Eurasia, it has spread widely across North and South America, Asia, and Australia due to its strong adaptability and invasive growth habit [1–3]. Traditionally, this plant has been valued for its medicinal uses, particularly in folk medicine in the British Isles (Gaels: Irish Celts and Anglo-Saxons), where both the aerial parts and rhizomes are prepared as teas, ointments, or infusions to treat digestive disorders, urinary tract conditions, skin diseases, and joint pain [3–8]. For example, in Serbian regions such as Timok and Svrlijig, *E. repens* is used to alleviate stomach pain and kidney stones [4]. In South Kosovo, it is used for hemorrhoids and urinary and respiratory ailments, while in Bosnia and Herzegovina, it is consumed as a tea to relieve renal stones [4]. In Poland, the plant is traditionally used as a sedative and diuretic and to relieve pain and spasms in the urinary tract [9,10].

Ethnobotanical records from North American Indigenous communities: Apache (A), White Mountain (WM), Cherokee (CHE), Gosiute, Iroquois, and Okanagan-Colville (OC) further highlight the plant's versatile applications in both food and traditional medicine. The rhizomes have been roasted as a coffee substitute, boiled into syrups, or ground into flour, while decoctions and infusions are used to treat kidney, liver, and urinary disorders, incontinence, fever, jaundice, and rheumatism [2]. In modern herbal practice, *E. repens* is recognized as a soothing diuretic, demulcent, and tonic that alleviates urinary tract irritation and spasm and is widely recommended in pediatric and mild urinary conditions due to its perceived safety and gentle pharmacological action [3]. Despite its extensive traditional use, especially for urinary and renal conditions, scientific validation of its therapeutic efficacy remains limited and fragmented [5].

Pharmacological studies, however, suggest promising biological activities. Extracts from *E. repens* rhizomes have demonstrated antiurolithiatic effects in animal models [11], hypoglycemic activity [12], and anti-inflammatory effects in topical formulations [13]. Phytochemical investigations have identified a diverse range of bioactive compounds, including saponins, tannins, flavonoids, iridoids, anthraquinones, and coumarins [5,13], indicating a chemically complex profile that may underpin its multi-target pharmacological effects. Additionally, mass spectrometric analyses have revealed a rich composition of phenolic acids, saccharides, amino acids, and flavonoids, including hesperidin [14], a compound known for its antioxidant and vascular-protective properties.

Nevertheless, most existing studies have focused primarily on rhizomes and their aqueous extracts, whereas the phytochemical composition and biological potential of the aerial parts remain largely unexplored. Therefore, the present study aims to investigate the macro- and microscopic characteristics, chemical composition, and biological activity of both the aerial parts and rhizomes of *E. repens* using LC-QToF-MS, a high-resolution

analytical technique that enables comprehensive metabolite profiling, to provide a more complete understanding of the plant's therapeutic potential and applications.

2. Materials and Methods

2.1. Plant Collection and Identification

The aerial and underground parts of *E. repens* were collected at 4 km from Koktyube village, Talgar district, Almaty region (N 43°12'06.2"; E 076°41'14.7"). Plant material was collected in June 2024. The aerial parts were separated from the rhizomes and dried in accordance with Good Agricultural and Collection Practice (GACP) standards. After drying, the total dry weight of the raw materials was 1000 g.

Identification of plant material was carried out by Veselova P.V., head of the Higher Plant Flora laboratories of the Republican State Enterprise "Institute of Botany and Phytointroduction". Confirmation of the authenticity of the specimens was issued by official certificate No. 01-05/331 dated 21 June 2024 (index №. 411).

The collected plant material was stored in the botanical repository of the National Center for Natural Products Research (NCNPR) at the University of Mississippi, USA, under accession #26550.

2.2. Macro- and Microscopic Description of Raw Materials of *E. repens*

2.2.1. Preparation of Samples for Macroscopy and Microscopy

Freshly collected stems, roots, and leaf were subjected to pre fixative with FAA (10% Formalin/5% Glacial Acetic Acid/50% ethanol (using absolute EtOH)/35% de-ionized (DI) water and processed for detailed anatomical studies with several hand sections (~20 µm thick) stained with toluidine blue O (TBO) for basic histology observations [15,16]. All mounts were prepared on glass slides with water. Photomicrographs were obtained using an Olympus BX53 compound microscope (Olympus Corp., Tokyo, Japan) equipped with an Olympus DP74 camera. Images were processed using Cell Sens Standard Imaging Software version 3.1, build 21199.

2.2.2. Preparation of Samples for Scanning Electron Microscopy (SEM) and EDS Analysis

The specimens were fixed in FAA (formalin/glacial acetic acid/ethanol/DI water) in the following percentages: 10% formalin/5% glacial acetic acid/50% ethanol (absolute)/35% deionized water [17]. The samples were then washed under running water and passed through a series of 30%, 50%, 70%, 90%, and 100% ethanol for 30 min per step. The processed samples were dried using a Leica CPD300 critical point dryer (Leica Microsystems, Wetzlar, Germany) supplied with liquid CO₂ [18]. Dried samples were mounted on aluminum stubs with double-sided adhesive carbon tape and then coated with 10 nm gold–palladium (60:40) using a Desk V HP sputter coater (Denton Vacuum, Moorestown, NJ, USA) supplied with argon gas. The samples were imaged using a JSM-7200FLV field-emission SEM (JEOL Ltd., Tokyo, Japan).

2.3. Chemicals and Reagents

All chemicals and reagents used in the present study were of analytical purity. Ethanol (96% v/v, LOT 252567, Fisher Scientific, Waltham, MA, USA), at 70% was used for the hydroalcoholic extraction. Acetonitrile (for HPLC ≥ 99.9%, LOT 256446, Fisher Chemicals, Waltham, MA, USA), methanol (100%, HPLC grade, LOT 232035, Fisher Chemicals, Waltham, MA, USA), and formic acid (for HPLC ≥ 99+%, LOT B0549300A, Thermo Fisher Scientific, Waltham, MA, USA) are of HPLC-certified grade, and water (for HPLC, LOT 255436, Fisher Chemicals, USA) was purified using a Milli-Q system (Millipore, Bedford, MA, USA). Dimethyl sulfoxide (DMSO, ≥99.9%, LOT 218764, Fisher Chemicals, Waltham,

MA, USA) was used to dissolve the dry extracts prior to antimicrobial testing. The samples were dissolved in DMSO as 20 mg/mL stocks.

2.4. Extraction Process

The aerial and rhizome parts of the plant were dried in the shade at room temperature for 10 days and then ground using a cutting mill (SM 300, RETSCH GmbH, Haan, Germany). Ultrasound-assisted extraction was performed from aerial and rhizome parts separately, using an ultrasonic bath (Stegler, 5DT, Moscow, Russia) with 5 L of ethanol (70%), 120 W power, and an ultrasonic frequency of 40 kHz at 45 °C. For each variant, 500 g of aerial and rhizome parts were used for extraction. The extraction time was 30 min. The obtained extracts were filtered through filter paper (Whatman №1, Little Chalfont, UK) and evaporated under reduced pressure at a temperature not exceeding 40 °C using a rotary evaporator (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany), until dryness. The concentrated samples were stored in dark glass vials at 4 °C until further studies.

Ultrasonic extraction using 70% ethanol at a bath volume of 5 L, 120 W power, 40 kHz frequency, 45 °C temperature, and 30 min duration yielded high yields (46.22 g and 19.02 g for aerial and rhizome parts, respectively).

2.5. Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-QToF-MS)

2.5.1. Sample Preparation for LC-QToF-MS

Twenty-five milligrams of the dried 70% hydroethanolic extract was reconstituted in 1 mL of methanol, vortexed, sonicated for 30 min, vortexed again, and filtered into an LC vial prior to analysis.

2.5.2. Instrumentation Setup for Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-QToF-MS)

Phytochemical profiling in aerial and rhizome parts of *E. repens* was conducted by coupling the LC system to the quadrupole time-of-flight mass spectrometer (Q-ToF) (Model #G6575A, Agilent Technologies, Santa Clara, CA, USA) equipped with an electrospray ionization interface (ESI) in the positive and negative ionization modes. Chromatographic separation was performed on an Agilent Poroshell 120 EC-C18 column (150 mm × 2.1 mm, 2.7 µm) using an Agilent 1290 system at 40 °C. A binary gradient of water (A) and acetonitrile (B), both containing 0.1% formic acid, at a flow rate of 0.21 mL/min. The gradient program was: 0–2 min (97% A/3% B), 25 min (70% A/30% B), 35 min (30% A/70% B), 40 min (100% B), hold for 5 min, then re-equilibrate for 5 min (97% A/3% B). Injection volume was 1 µL. Nitrogen was used as the desolvation gas and set to 325 °C with a flow rate of 11 L/min. Other parameters used were nebulizer pressure, 30 psi; sheath gas temperature, 300 °C; sheath gas flow, 11 L/min; capillary voltage, 3000 V; and fragmentor voltage, 150 V. The mass analysis range was 50–1100 *m/z*. Accurate mass measurements were obtained using reference ion correction using reference masses at *m/z* 121.0509 (protonated purine) and 922.0098 [protonated hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine or HP-921] in the positive ion mode, while *m/z* 112.9856 (deprotonated trifluoroacetic acid-TFA) and 1033.9881 (TFA adducted HP-921) were employed in the negative ion mode. Samples were analyzed in all-ion MS-MS mode, where experiment 1 was carried out with a collision energy of zero and experiment 2 with a fixed collision energy of 45 eV. The precise molecular mass and molecular formula were processed with MassHunter Qualitative Analysis software, Ver. B.7.00 [19,20].

2.6. Antimicrobial Activity

The antimicrobial activity of hydroethanolic extracts of aerial and rhizome parts of *E. repens* was evaluated against eight clinically relevant microorganisms, including bacteria

and fungi. In primary antimicrobial screening, the samples were tested for their ability to inhibit the growth of five pathogenic bacterial strains: Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC1708), *Escherichia coli* (ATCC2452), *Pseudomonas aeruginosa* (ATCC BAA-2108), *Klebsiella pneumoniae* (ATCC2146), and Vancomycin-resistant *Enterococcus faecium* (VRE) (ATCC700221); as well as three fungal strains: *Candida albicans* (ATCC90028), *Cryptococcus neoformans* (ATCC90113), and *Aspergillus fumigatus* (ATCC204305).

The antifungal drug control was amphotericin B. The IC₅₀s were calculated using the XLfit 4.2 software (IDBS, Alameda, CA, USA) with the fit model 201. The antimicrobial activity of hydroethanolic extracts from the aerial and rhizome parts of *E. repens* was evaluated using a method based on CLSI recommendations and adapted for plant extracts [21].

2.7. Cytotoxic Activity

Artemia salina lethality assay was selected as a rapid, reproducible and cost-effective preliminary bioassay for the general cytotoxicity screening of the extracts. Since its introduction by Meyer et al., this assay has been widely used in natural-product research because larval lethality correlates well with cytotoxicity in mammalian cell lines and with acute oral toxicity in rodents, making it a validated predictive model for the toxic potential of botanical extracts [22–24]. According to the toxicity criteria of Meyer et al., extracts are classified as highly toxic (LC₅₀ < 30 µg/mL), toxic (30–1000 µg/mL) or non-toxic (LC₅₀ > 1000 µg/mL).

The method is based on comparing the number of dead larvae in the test sample and in the control water without toxic substances. Acute toxicity was defined as the death of ≥50% of larvae compared to the control.

A. salina eggs were incubated in artificial seawater (salinity 8.0–8.5; stable pH) with aeration for 72 h. One-day-old larvae were used in the experiment, with 20–40 individuals per test tube. Actinomycin D was used as a control. *E. repens* extracts (70% ethanol) were tested at concentrations of 1, 5, and 10 mg/mL. Each sample was examined in three parallel replicates at 20 ± 5 °C and under natural lighting. Mortality percentage was calculated as the ratio of the number of larvae killed by the plant extract to the total number of larvae [25,26].

3. Results

3.1. Macro- and Microscopic Description of *E. repens*

3.1.1. External Morphology of *E. repens*

This grass is a tufted, perennial, rhizomatous plant that ranges in color from white to yellow. It has a creeping, branched underground stem (Figure 1A,B). The stem is green, erect, rounded, and hollow, with internodes that are glabrous and slightly rough near the nodes. The leaf blades are typically flat, measuring between 6 and 30 cm in length and 3 and 10 mm in width. They may be glabrous or have loose hairiness on the upper side. The spike is either lax or dense, ranging from 5 to 15 (up to 20) cm long, and is erect and straight. The rachis features scabrid joints along the margins.

The spikelets are 5 to 7-flowered, measuring 8 to 17 mm long. The glumes are subequal, lanceolate to lanceolate-oblong, and range from 5 to 15 mm long, with an acute, mucronate, or shortly awned tip. They are scabrid on the nerves above. The lemma is lanceolate-oblong, measuring 6 to 11 (up to 13) mm long, glabrous, smooth, and acute with a subulate tip. The palea is nearly as long as the lemma, and the anthers range from 3.5 to 6 mm long.

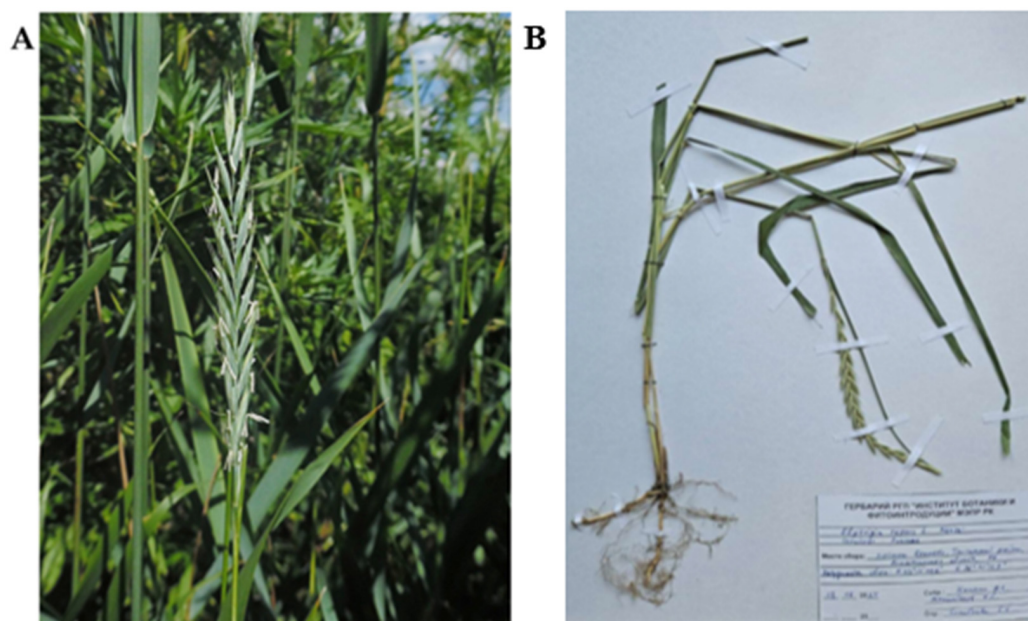


Figure 1. (A) Habit of *E. repens*. (B) Herbarium specimen preserved for the repository.

3.1.2. Microscopic Description

Leaf

The morpho-anatomical characteristics of the leaf indicate that the leaf blade is linear and slightly folded at the margins, a trait typical of monocots. The venation is parallel. The stomata are amphistomatic and dumbbell-shaped, as in graminaceous plants (Figure 2A,B,E,F). Additionally, the leaf surface has a rough texture, with sharp, pointed, prickly trichomes that are outgrowths of both the upper and lower epidermis (Figure 2A–D). The transverse section of the leaf consists of a single layer of rectangular-shaped epidermal cells covered by a thin cuticle. The outer epidermis has a significant waxy coating. The presence of stomata is less frequent on the abaxial (upper) surface compared to the adaxial (lower) surface. This difference is primarily due to the presence of bulliform epidermal cells, and there is also a thinner waxy layer on the lower side of the leaf. These two characteristics significantly influence leaf adaptation, helping balance water loss during respiration. The lower layers of the epidermis contain a few elongated cells located between the vascular strands in the veins. The collateral vascular bundles are located in the leaf mesophyll region and are surrounded by a sclerenchymatous cap, which is more lignified, as indicated by the lumen under polarized light (Figure 2L). These bundles consist of spongy parenchyma cells that are filled with chloroplasts (Figure 2H–L).

Inflorescence

The inflorescence of *E. repens* is a spike, a type of racemose inflorescence. In this arrangement, the central axis is elongated, and sessile spikelets are alternately positioned on either side in a distichous pattern. Scanning electron microscopy reveals two types of trichomes on the glume surface covering the floral parts (Figure 3A–D). The margins feature pointed, angled trichomes, while the surface has short, pointed trichomes, with occasional type 2 trichomes as well.

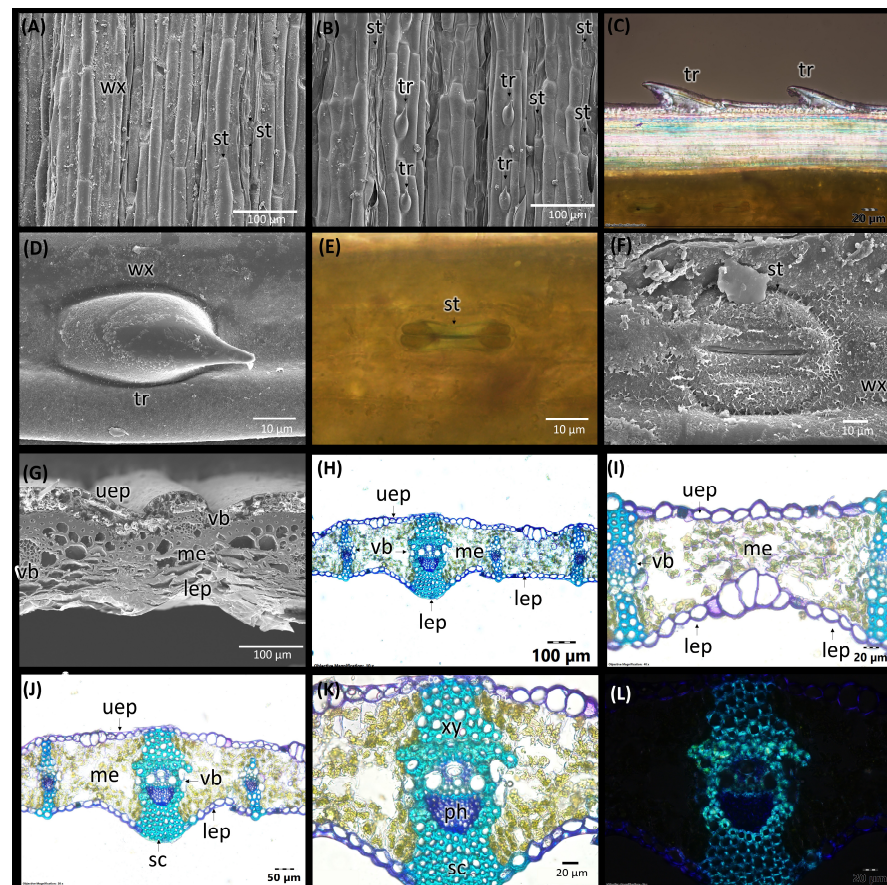


Figure 2. Micro-morphology of the leaf of *E. repens* observed under Scanning Electron Microscope (SEM), Brightfield Microscope (BM), and Polarizer (P). (A) Upper surface of leaf. (B) Lower surface of leaf. (C,D) Trichome structure present in the margin and on surface of the leaf. (E,F) Stomata; (G–L) Transverse section of leaf viewed under the SEM, BM, and P. st—stomata, tr—tr-trichome, wx—wax, uep—upper epidermis, lep—lower epidermis, vb—vascular bundle, me—mesophyll, sc—sclerenchyma.

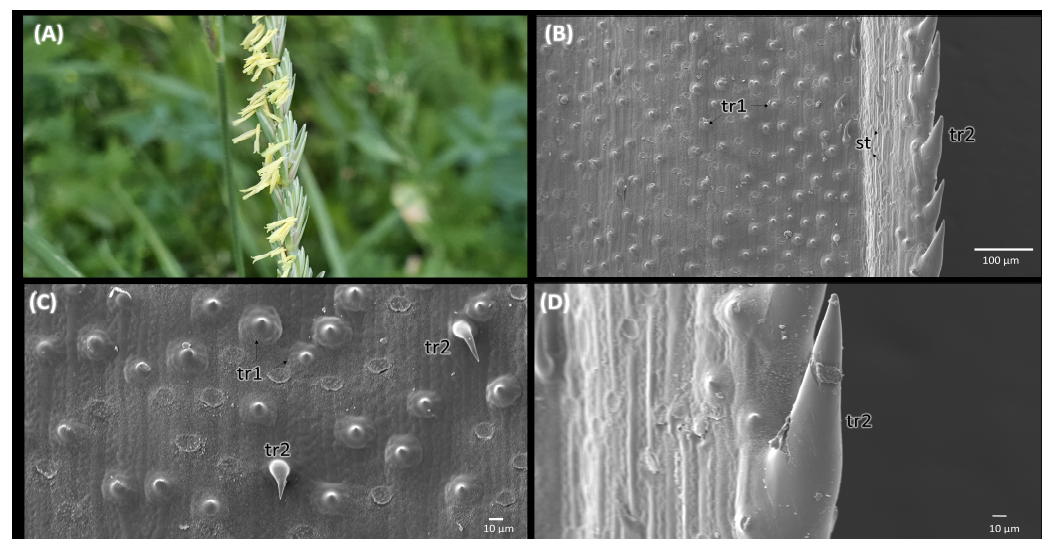


Figure 3. Micro-morphology of inflorescence of *E. repens*. (A) External morphology of Spikelet, (B–D) SEM image of glume surface showing the two types of trichome presence, single-cell (tr1) and two-cell thick (tr2). There is noticeable stomata (st) presence as well. Scale Bar: (B) = 100 µm and (C,D) = 10 µm.

Micromorphology of Stem and Root

The stem anatomy of *E. repens* displays typical monocot characteristics. The outermost layer is the epidermis, a single layer of compactly arranged cells covered with a thick cuticle. Below the epidermis is a narrow zone of hypodermis, consisting mostly of 2–3 layers of sclerenchymatous cells that lignify to provide mechanical support. The ground tissue is the cortex and is composed of two rings of vascular bundles, which are collateral and covered by sclerenchymatous caps. These bundles are scattered throughout the ground tissue, numerous and closed. Each vascular bundle has xylem towards the inner side and phloem towards the outer side. Secondary growth is absent, as is typical in monocot stems (Figure 4A–F).

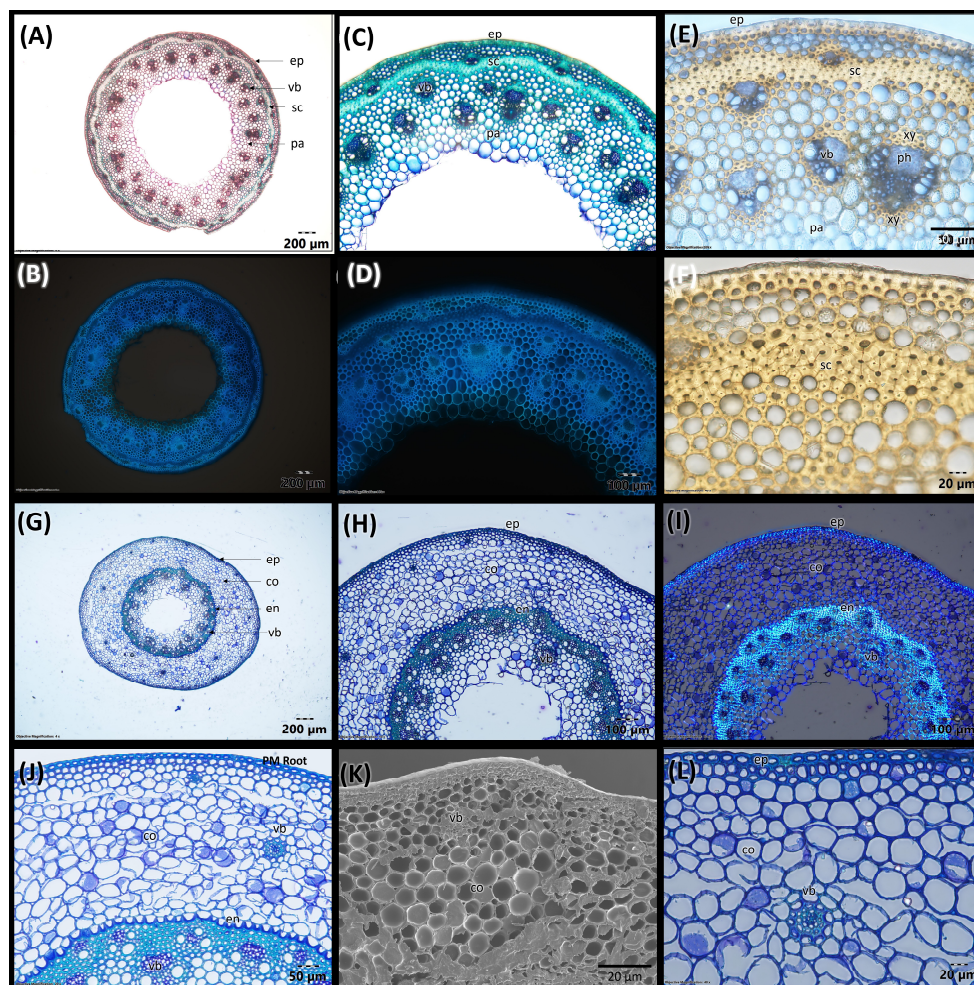


Figure 4. Micro-morphology of stem (A–F) and root (G–L) of *E. repens* observed under Scanning Electron Microscope (SEM), Brightfield Microscope (BM) and Polarizer (P). ep: epidermis, vb: vascular bundle, hp: hollow pith, pa: parenchyma cells, xy: xylem, ph: phloem, sc: sclerenchyma, en: endodermis, pe: phenolic content.

The anatomy of the root of *E. repens* is typical of a monocotyledonous rhizome. The outermost layer is the epidermis, which bears root hairs that aid in the absorption of water and minerals. Beneath it lies the broad cortex, composed mainly of 7–10 layers of parenchymatous cells, some of which show the presence of phenolic compounds, confirmed with the color reaction to a bluish-slight pink with TBO. The single-layered endodermis forms the innermost layer of the cortex and is characterized by the presence of Casparian strips (Figure 4J). The vascular bundles are arranged in a radial pattern, with xylem and

phloem alternating around a central pith. In *E. repens*, like other monocots, the root typically has polyarch xylem (many xylem strands) and a well-developed central pith (Figure 4G–L).

3.2. Identification and Characterization by LC-QToF-MS

The phytochemical profiles of *E. repens* aerial parts (A) and rhizomes (R) were investigated using Liquid Chromatography-Tandem Mass Spectrometry with Electrospray Ionization in both positive and negative ionization modes. This comprehensive analysis, summarized in Table 1, revealed a diverse array of compounds (93 compounds) across several major chemical classes, including polyamines, phenolic compounds, amino acids and derivatives, flavonoids, organic acids, nucleosides, phospholipids, and other compounds. The comparative analysis revealed both qualitative similarities and differences between tissues, and fragmentation data provided tentative compounds, compound identifications, and insights into metabolic differences.

Table 1. Overview of metabolite classes preliminarily identified in the aerial (A) and rhizome (R) parts of *E. repens* using LC-QToF-MS.

Compound Class	#	Representative/Notable Compounds	A/R	References
Polyamines (ornithine alkaloids/HCAAs)	6	feruloyl- and diferuloylputrescine, coumaroyl-/hydroxycoumaroylagmatine	A, R	[27–29]
Phenolic acids	26	Chlorogenic acid isomers (3-/4-/5-CQA), gallic, vanillic, caffeic, ferulic, <i>p</i> -coumaric acids	A > R	[30]
Flavonoids	22	Luteolin di-/mono-glycosides, tricin derivatives, apigenin C-glycosides, salcolin A/B (flavonolignans)	A > R	[19,31]
Amino acids & derivatives	22	Tryptophan, tyrosine, arginine, fructosylvaline, fructosyl-(iso)leucine	A, R (Trp: R > A)	[32,33]
Organic acids	5	Malic, citric, furoic, succinic, fumaric acids	A, R	[33]
Nucleosides	2	Adenosine, thymidine	R > A	[34]
Phospholipids	5	Phosphatidylcholines (diagnostic <i>m/z</i> 184.0735)	A, R	[35]
Other compounds	5	Sucrose, choline, hydroxymethylcoumarin, furandione, azelaic acid	A, R	[36]
Total	93			

A complete summary, including exact masses, molecular formulas, fragment ions, and references, is provided in Supplementary Tables S1 and S2.

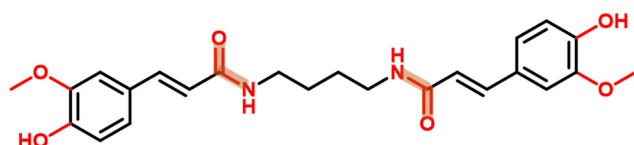
The results of the study can be summarized as follows: analysis by LC-QToF-MS identified 93 metabolites from the aerial (A) and underground (R, rhizome) parts of *E. repens*, belonging to eight major chemical classes (Table 1). The aerial and rhizome parts of *E. repens* shared the majority of the identified metabolites, but several organ-related differences were evident, particularly among phenolic compounds and flavonoids. Chlorogenic acid isomers (19–21), coumaroylquinic acid isomers (28–29), sinapic acid (32), luteolin diglucoside (33), vicenin-2 (35), luteolin glucosides (37–38), luteolin/kaempferol rutinosides (40–41), and tricin (52) were clearly detected in the aerial part but occurred only at trace levels in the rhizome. Isoquercetin (42) was detected in the aerial part but was not detected in the rhizome, whereas ferulic acid (31) showed the opposite tendency, being present only at a trace level in the aerial part but clearly detected in the rhizome. Thymidine (64) was also detected more distinctly in the rhizome than in the aerial tissue. In contrast,

the polyamines, organic acids, phospholipids, and most other constituents showed broadly similar qualitative occurrence in both organs. These findings suggest that the most apparent tissue-dependent variation in *E. repens* is associated with phenolic and flavonoid composition; however, because the present data are based on detection status rather than quantitative abundance, the differences should be interpreted as qualitative rather than concentration-dependent (Tables S1 and S2).

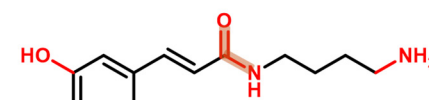
A complete annotated list of all 93 identified compounds, including individual numbers, exact m/z values, and relative abundances in each plant part, is provided in Supplementary Tables S1 and S2. Table S1 summarizes the tentative identification of metabolites in the aerial and rhizome extracts of *E. repens* based on accurate-mass precursor ions and diagnostic MS/MS fragmentation, whereas Table S2 specifically presents the AQC-derivatized amino acid profiles. Unlike the tentative assignments in Table S1, the amino acids in Table S2 were identified by comparison with authentic reference compounds.

Given that polyamines (ornithine alkaloids/HCAAs) constitute a class first identified in *E. repens*, the structural formulas and mass spectrometric fragmentation patterns of four representative compounds are presented below, including two ornithine alkaloids—diferuloylputrescine and feruloylputrescine isomer—as well as tricin-*O*-rutinoside (flavonoids) and chlorogenic acid (5-CQA), illustrating the approach to structural annotation of the main identified classes of metabolites (Figure 5).

Hydroxycinnamoyl polyamines (Phenolamides)

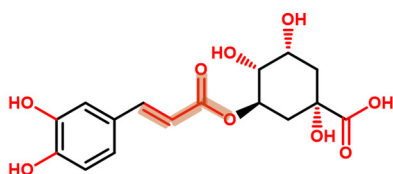


Diferuloylputrescine (6) m/z 441.2034 $[M+H]^+$
 $\downarrow$ $-C_{10}H_8O_3$ (176.0473 Da) $\rightarrow m/z$ 265.1550 $[M+H-C_{10}H_8O_3]^+$
 $\downarrow$ m/z 177.0548 $[C_{10}H_8O_3+H]^+$, feruloyl-derived ion
 $\rightarrow -CH_4O$ (32.0262 Da) $\rightarrow m/z$ 145.0287
 $\rightarrow -CO$ (27.9949 Da) $\rightarrow m/z$ 117.0336



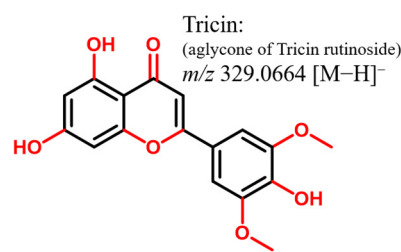
Feruloylputrescine isomer (3) m/z 265.1548 $[M+H]^+$
 $\downarrow$ 177.0547 $[C_{10}H_8O_3+H]^+$, feruloyl-derived ion
 $\rightarrow -CO$ (27.9949 Da) $\rightarrow m/z$ 149.0598
 $\rightarrow -CH_4O$ (32.0262 Da) $\rightarrow m/z$ 117.0335
 $\rightarrow -CO$ (27.9949 Da) $\rightarrow m/z$ 89.0385

Phenolic acids



5-*O*-Caffeoylquinic acid (5-CQA, 19) m/z 353.0879 $[M-H]^-$
 $\downarrow$ m/z 191.0561 [quinic acid- H] $^-$ ($-C_9H_6O_3$, 162.0317 Da)
 $\downarrow$ m/z 173.0459 ($-H_2O$)
 $\downarrow$ m/z 179.0353 [caffeic acid- H] $^-$
 $\downarrow$ m/z 135.0458 ($-CO_2$)

Flavonoids



Tricin-*O*-rutinoside (45) m/z 637.1775 $[M-H]^-$
 $\rightarrow -C_6H_{10}O_4$ (-146.0579 Da, rhamnosyl) $\rightarrow m/z$ 491.1205
 $\rightarrow -C_6H_{10}O_5$ (-162.0528 Da, glucosyl) $\rightarrow m/z$ 329.0664 [tricin- H] $^-$
 $\rightarrow -CH_3$ (15.0235 Da) $\rightarrow m/z$ 314.0438
 $\downarrow$ m/z 177.0555 (RDA)
 $\downarrow$ m/z 151.0045 (RDA)

Figure 5. Representative structures and proposed ESI(+)/(−)-LC–QToF–MS/MS fragmentation pathways of selected *E. repens* constituents. Structural moieties involved in fragmentation and corresponding cleavage sites are highlighted in red.

Figure 5 represents chemical structures and proposed fragmentation pathways of selected constituents of *E. repens* obtained by ESI(+)/(−)-LC–QToF–MS/MS. Structural moieties involved in the proposed fragmentation pathways and the corresponding cleavage sites are highlighted in red to facilitate visualization of the formation of diagnostic product ions. Diferuloylputrescine (6) showed a protonated precursor ion at m/z 441.2034 $[M+H]^+$, yielding m/z 265.1550 $[M+H-C_{10}H_8O_3]^+$ following loss of a hydroxycinnamoyl-related

neutral moiety (176.0473 Da). A complementary feruloyl-derived ion was observed at m/z 177.0548 $[\text{C}_{10}\text{H}_8\text{O}_3+\text{H}]^+$, followed by sequential losses of CH_4O (32.0262 Da) and CO (27.9949 Da) to give m/z 145.0287 and 117.0336, respectively. Feruloylputrescine isomer (3) produced $[\text{M}+\text{H}]^+$ at m/z 265.1548 and the characteristic feruloyl-derived ion at m/z 177.0547, which further fragmented to m/z 149.0598 by loss of CO , m/z 117.0335 by subsequent loss of CH_4O , and m/z 89.0385 by an additional CO loss. 5-O-Caffeoylquinic acid (5-CQA, 19) generated $[\text{M}-\text{H}]^-$ at m/z 353.0879 and the diagnostic quinic acid ion at m/z 191.0561 $[\text{quinic acid}-\text{H}]^-$ following loss of the caffeoyl residue ($\text{C}_9\text{H}_6\text{O}_3$, 162.0317 Da), with further dehydration to m/z 173.0459. A complementary caffeic acid-derived ion at m/z 179.0353 $[\text{caffeic acid}-\text{H}]^-$ subsequently yielded m/z 135.0458 through CO_2 loss. Tricin-O-rutinoside (45) showed $[\text{M}-\text{H}]^-$ at m/z 637.1775 and underwent sequential losses of a rhamnosyl residue ($\text{C}_6\text{H}_{10}\text{O}_4$, 146.0579 Da) and a glucosyl residue ($\text{C}_6\text{H}_{10}\text{O}_5$, 162.0528 Da), producing ions at m/z 491.1205 and the tricin aglycone at m/z 329.0664 $[\text{tricin}-\text{H}]^-$, respectively. Further fragmentation of the tricin aglycone produced m/z 314.0438 through CH_3 loss, together with retro-Diels–Alder (RDA) product ions at m/z 177.0555 and 151.0045. These diagnostic products illustrate the representative fragmentation behavior of hydroxycinnamoyl polyamines (phenolamides), caffeoylquinic acids, and tricin glycosides detected in *E. repens*.

3.3. Antimicrobial Activity of *E. repens* Extracts

Samples were tested for their ability to inhibit the growth of five human pathogenic bacterial strains (Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC1708), *Escherichia coli* (ATCC2452), *Pseudomonas aeruginosa* (ATCC BAA-2108), *Klebsiella pneumoniae* (ATCC2146), *Vancomycin-resistant Enterococcus faecium* (VRE) (ATCC700221)) and three fungi (*Candida albicans* (ATCC90028), *Cryptococcus neoformans* (ATCC90113), and *Aspergillus fumigatus* (ATCC204305)).

However, pharmacological studies revealed that activity against *Aspergillus fumigatus* was observed exclusively in the hydroethanolic extract of the rhizome part (*A. fumigatus* IC_{50} = 34.9, Test Conc. = 200–8 $\mu\text{g/mL}$). The tested extracts did not inhibit the growth of five strains of human-pathogenic bacteria and two strains of fungi. The antimicrobial activity of the aerial part and rhizome extracts of *E. repens* against the tested microbial strains is presented in Table 2.

Table 2. IC_{50} ($\mu\text{g/mL}$) of extracts against microbial strains.

Microorganism	Extracts from the Aerial Parts of <i>E. repens</i>	Extracts from the Rhizome Parts of <i>E. repens</i>	Amphotericin B (5 $\mu\text{g/mL}$)
<i>A. fumigatus</i>	NA	34.9	1.934

The results in IC_{50} ($\mu\text{g/mL}$). NA—not active at 200 $\mu\text{g/mL}$.

3.4. Cytotoxic Activity of *E. repens* Extracts

The results of the study are presented in Table 3. The reference drug, actinomycin D, exhibited pronounced cytotoxic activity across all tested concentrations, resulting in high larval mortality ranging from 74% to 96%, thereby confirming the sensitivity and validity of the *Artemia salina* bioassay system. In contrast, the hydroethanolic extracts of both the aerial and rhizome parts of *Elymus repens* showed no cytotoxicity. Notably, no larval mortality was observed even at the highest concentrations tested, indicating a lack of acute toxicity. Both extracts produced highly consistent and comparable results, with no statistically significant differences in their effects on *A. salina* viability. These findings strongly suggest that *E. repens* extracts possess a favorable safety profile and are biologically

non-toxic under the experimental conditions employed. The absence of cytotoxicity further supports their traditional use in herbal medicine and indicates their potential suitability for therapeutic applications requiring low toxicity.

Table 3. Results of the cytotoxic activity study against *A. salina*.

Sample	Concentration mg/mL	% of Surviving Larvae in the Sample	% Mortality
Actinomycin D	10	0	96 *
	5	2	94 *
	1	22	74 *
Hydroethanolic extracts of <i>E. repens</i> .	10	96	0
	5	96	0
	1	96	0

* $p < 0.05$ compared to negative control.

Table 3 presents the data on cytotoxic activity of extracts of the aerial and rhizome parts of *E. repens*. The results obtained were similar; so, they were combined.

4. Discussion

This study provides a comprehensive investigation of the morphological characteristics, chemical composition, and biological activity of the aerial and rhizome parts of *Elymus repens* collected in Kazakhstan, establishing a clear link between structural features, metabolite diversity, and functional bioactivity.

Microscopic examination revealed distinct anatomical traits, including two concentric rings of vascular bundles in the stem, well-developed sclerenchyma tissue in the roots, and a high density of trichomes on the leaf surfaces. These features are consistent with adaptive xeromorphic characteristics that enhance environmental resilience and can serve as reliable taxonomic markers. Furthermore, diagnostic micromorphological attributes were identified, including the absence of epidermal papillae, non-penetration of bulliform cells into the mesophyll, ribbed leaf architecture, deeply sunken stomata, and thickened cuticular structures. These markers enable accurate taxonomic differentiation and facilitate the assessment of phylogenetic relationships with related species; consistent with previous reports and pharmacopoeial standards [37–40], they further confirm the authenticity of the study material.

High-resolution chemical profiling demonstrated that *E. repens* is a metabolically diverse species, containing a wide spectrum of bioactive compounds, including polyamines, phenolic acids, amino acids, flavonoids, organic acids, nucleosides, phospholipids, and other secondary metabolites. Key phenolic acids—chlorogenic, caffeic, and ferulic acids—were detected alongside flavonoid derivatives of apigenin and luteolin, corroborating earlier findings and confirming their conserved presence within this species [14]. These metabolites are well known for their antioxidant, anti-inflammatory, and antimicrobial properties, suggesting a mechanistic basis for the observed biological activities [30]. In addition, simple sugars (sucrose/maltose) and a conserved amino acid profile, comparable to that of other cereals, point to the preservation of essential primary metabolic pathways within the Poaceae family [32,41]. Notably, we also identified flavonolignans such as salcolin A/B and C/D, which further strengthens the pharmacological relevance of *E. repens*, as these compounds are associated with potent antioxidant, antibacterial, and anti-inflammatory activities. Collectively, phenolic acids and flavonolignans likely coexist as a synergistic phytochemical network that drives the plant's bioactivity [42].

A comparison with the existing literature data shows that a significant portion of the obtained profile confirms previously published phytochemical data on *E. repens*: isomers of caffeoyl-/feruloylquinic (chlorogenic) acid, free phenolic acids (gallic, vanillic, caffeic, ferulic, p-coumaric), C-glycosides of luteolin and apigenin, tricin derivatives, tryptophan and related amino acids and low-molecular-weight organic acids (malic, citric, succinic)—all consistent with previous HPLC-MS and pharmacopoeial data [14,43]. On the other hand, to the best of our knowledge, several components detected in *E. repens* are reported here for the first time, primarily a series of hydroxycinnamic acid amide conjugates with polyamines (ornithine)—hydroxycinnamoylputrescine, feruloyl- and diferuloylputrescine, coumaroyl- and hydroxycoumaroyl-argmatine—along with flavonolignans (salcolin A/B) and several nucleosides and phospholipids. Although hydroxycinnamic acid amides have been well studied in the Poaceae family (e.g., in wheat, maize, and *Brachypodium*) [28,29], their systematic characterization in *E. repens* has not been previously described. Moreover, while early studies focused primarily on the rhizome, the current study provides a comparative profile of both aerial and rhizome tissues. The differences between the present profile and data from earlier studies are most likely due to the geographical origin of the material (Kazakhstan), growth conditions, the plant organ analyzed, the extraction procedure, and the analytical platform used (LC-QToF-MS versus HPLC-PDA-ESI-MS/MS) [43].

To directly link the chemical composition to the tested biological activity, the dried 70% hydroethanolic extract used in the bioassays was itself reconstituted in methanol and subjected to LC-QToF-MS analysis (Section 2.5.1). This analysis identified 93 metabolites directly in the extract used for biological evaluation (Table 1, Supplementary Table S1), including all six polyamines and the flavonolignans salcolin A/B highlighted above, thereby providing extract-matched chemical characterization rather than characterization of a separate preparation.

The selective antifungal activity of the hydroethanolic rhizome extract against *A. fumigatus* is consistent with existing data on the presence of antimicrobial substances in *E. repens* rhizomes, including hevein-like antimicrobial peptides [44] and antifungal phenolic/flavonoid components. This activity is directly supported by the LC-QToF-MS profile of the same hydroethanolic extract (Table 1), which confirmed a phenolic- and flavonoid-rich composition in the rhizome fraction, providing extract-matched chemical evidence for the observed antifungal effect. This is consistent with a growing body of literature indicating that phenolic- and flavonoid-rich plant extracts represent a promising alternative source of antifungal agents against *Aspergillus* spp., including *A. fumigatus*, particularly in light of rising resistance to conventional antifungal drug classes such as polyenes, echinocandins, and azoles [45]. The lack of activity against the tested bacteria and two other fungi indicates a selective rather than broad-spectrum effect, consistent with previous data showing that *E. repens* does not act as a potent direct antibacterial agent but rather through anti-adhesive and physicochemical mechanisms [46]. Regarding antioxidant capacity, it should be noted that this study did not directly measure antioxidant activity; its potential is therefore discussed based on the identified phenolic and flavonoid composition (e.g., chlorogenic acids, luteolin derivatives, and tricin—all well-documented free-radical scavengers) and in light of earlier data on this and related Poaceae species [13,14].

These findings are further supported by the existing literature reporting antiurolithiatic effects of *E. repens*, including reduced calcium oxalate stone formation, likely mediated by its polyphenolic and saponin content [47]. The moderate bioactivities observed may also be linked to phenolic acids and flavonoids capable of modulating oxidative stress pathways and inducing apoptosis in pathological cells, as demonstrated in related plant systems with urological relevance; such mechanisms may further contribute to antiadhesive and protective effects against uropathogenic bacteria [46].

Cytotoxicity assessment using *Artemia salina* demonstrated a complete absence of toxicity for both aerial and rhizome extracts, even at elevated concentrations, supporting a favorable safety profile consistent with the plant's long-standing traditional use, including in treatments involving prolonged or repeated administration.

Overall, this is the first study to systematically correlate anatomical features, comprehensive metabolite profiling, and biological activity in both the aerial and rhizome parts of *E. repens*. These findings expand current knowledge of the species by showing that its biological efficacy arises from a complex interplay of structurally diverse metabolites, and underscore its potential as a safe, valuable source of natural antifungal and bioactive compounds for further development for pharmaceutical, nutraceutical, and cosmetic applications.

This study has certain limitations. The biological evaluation was limited to screening for in vitro antimicrobial activity and cytotoxicity against *Artemia*; pharmacological models that directly reflect the traditional indications of *E. repens*—such as diuretic, anti-adhesive (against uropathogenic bacteria), antiurolithiatic, and anti-inflammatory models—were not investigated, and no direct antibacterial activity against the uropathogens under study was detected. Furthermore, antioxidant activity was not measured experimentally and is discussed only based on identified components and literature data. Accordingly, the traditional uses of this species are considered here from the perspective of phytochemical rationale rather than direct pharmacological evidence. Future studies should include the isolation of active compounds guided by bioassay results, combined with target-specific pharmacological studies, to validate the ethnomedical applications.

5. Conclusions

In summary, this study provides a robust, integrative evaluation of *Elymus repens*, establishing a clear link between its morphological characteristics, chemical composition, and biological activity across both aerial and rhizome tissues. The combined anatomical, phytochemical, and bioactivity data not only confirm the botanical authenticity and taxonomic reliability of the material but also highlight the species as a chemically rich and biologically relevant member of the Poaceae family. The chemical diversity revealed by direct LC-QToF-MS profiling of the 70% hydroethanolic extract—the same extract used in the biological assays—including phenolic acids, flavonoids, and flavonolignans, together with its demonstrated selective antifungal activity and absence of cytotoxicity, provides direct phytochemical support for the antimicrobial dimension of *E. repens*' traditional use, particularly for urinary tract and skin-related indications where an infectious component is well documented. Notably, no direct antibacterial activity was detected against the uropathogenic strains studied, consistent with the prevailing view that the beneficial effects of *E. repens* on the urinary system are primarily attributable to its diuretic, demulcent, anti-adhesive, and antiurolithiatic actions rather than a direct antimicrobial effect; direct pharmacological validation of these specific mechanisms using appropriate models was beyond the scope of this study and is identified as a priority for future research. Importantly, the observed tissue-specific bioactivity highlights the need for targeted selection of plant parts in pharmacological formulations.

From a pharmaceutical and pharmacognostic perspective, these findings position *E. repens* as a promising source of safe, multi-functional natural compounds with potential applications in antifungal therapies, urological formulations, and functional herbal products. The work also underscores the importance of comprehensive metabolite profiling and anatomical standardization in the quality control and authentication of medicinal plants. Future research should focus on bioactivity-guided isolation of key active compounds, detailed mechanism-of-action studies, and in vivo validation of pharmacological

effects. Additionally, advanced omics-based approaches (metabolomics, transcriptomics) and formulation development studies could further elucidate synergistic interactions and enhance therapeutic efficacy. Such efforts would strengthen the scientific basis of *E. repens* in modern medicine and support its integration into evidence-based phytopharmaceutical development and standardized pharmacognostic practice.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/scipharm94030075/s1>.

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Abbreviations

The following abbreviations are used in this manuscript:

LC-QToF-MS Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry

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