Review

Chemical Diversity in *Leuenbergeria bleo*: From Small-Molecule Phytochemicals to Bioactive Microproteins

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Chemical Diversity in *Leuenbergeria bleo*: From Small-Molecule Phytochemicals to Bioactive Microproteins

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Abstract

Leuenbergeria bleo (Kunth) DC. (Cactaceae), previously classified as *Pereskia bleo*, represents a phylogenetically basal cactus species with a disjunct distribution across Central America, Southeast Asia, and southern China. Phytochemical investigations have traditionally emphasized small-molecule secondary metabolites, including phenolics, alkaloids, and terpenoids, which contribute to antioxidant and anti-inflammatory activities. However, recent peptidomic analyses have expanded this chemical space through the discovery of bleogens, a family of hyper-stable, cysteine-rich microproteins with specific antifungal and wound-healing properties. This review systematically integrates botanical characteristics, ethnomedicinal applications, and pharmacological profiles, providing a comparative analysis of the plant's small-molecule constituents versus its peptidyl biologics. It identifies the co-existence of these distinct chemical classes as a defining feature of the plant's efficacy while highlighting the need for future research into their potential interactions.

Keywords: *Leuenbergeria bleo*; *Pereska bleo*; phytochemistry; bleogens; cysteine-rich peptides; traditional medicine; pharmacological activities; wound healing

1. Introduction

Leuenbergeria bleo (Kunth) DC. holds a distinctive place in plant biology as a member of the phylogenetically basal lineages within the Cactaceae family [1–3]. Recently reclassified from the genus *Pereskia* based on molecular phylogenetic evidence, *L. bleo* exhibits ancestral traits such as persistent leaves and non-succulent stems, distinguishing it from more derived cacti [1]. Native to the humid lowlands of Central America and widely naturalized across Southeast Asia and southern China, this species has been documented in ethnomedicinal practices for treating metabolic disorders, inflammatory conditions, wounds, and malignancies [4]. These traditional applications have spurred scientific interest in its phytochemical profile, prompting systematic investigations into its bioactive constituents.

Historically, phytochemical studies of *L. bleo* have focused primarily on small-molecule secondary metabolites [4,5]. These efforts have led to the isolation of various phenolic compounds, alkaloids, and terpenoids, which were generally associated with the plant's observed antioxidant and cytotoxic activities [4–7]. However, the traditional use of *L. bleo* for tissue repair has long suggested underlying mechanisms that extend beyond simple antioxidant effects. Recent investigations have addressed this gap by identifying bleogens, a novel family of cysteine-rich peptides (CRPs), as the primary bioactive constituents responsible for its wound-healing properties [8–10].

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This review synthesizes current knowledge of the botanical features, ethnomedicinal uses, and chemical diversity of *L. bleo*, critically examining both the well-characterized small-molecule landscape and emerging peptidomic discoveries.

2. Botanical Characteristics

L. bleo, commonly known as rose cactus or leaf cactus, represents a morphological bridge between the leafy ancestors of cacti and their more derived, succulent descendants. This perennial shrub or small tree reaches 0.6–8 m in height and features extensively branched woody stems adorned with areoles bearing fascicles of spines (typically 1–5 per areole, up to 5 cm long), characteristics that distinguish it from the stem-succulent members of the Cactaceae family [11–13]. The root system is fibrous and relatively shallow, facilitating adaptation to rocky or disturbed soils.

The leaves are simple, ovate-lanceolate to oblanceolate, measuring 6–21 cm in length, with prominent venation that supports efficient photosynthesis in shaded, humid environments [11–13]. Unlike more derived cacti, *L. bleo* retains persistent leaves, which reduce water loss while maintaining photosynthetic capacity in its mesic habitats. Reproductive structures consist of hermaphroditic flowers (3–5 cm in diameter) with vivid orange-red perianth segments and numerous stamens [14,15]. Flowering occurs seasonally and is typically diurnal, attracting pollinators such as bees and other insects. The resulting fruits are turbinate or globose berries (2–4 cm wide) containing numerous black seeds embedded in mucilaginous pulp, dispersed by birds and small mammals.

Exhibiting notable phenotypic plasticity, *L. bleo* thrives in diverse conditions, from the well-drained soils of its native Central American lowlands to disturbed habitats in its naturalized ranges across Southeast Asia and southern China [16,17]. This adaptability underscores its evolutionary significance as a model for studying the early diversification of the Cactaceae [18,19].

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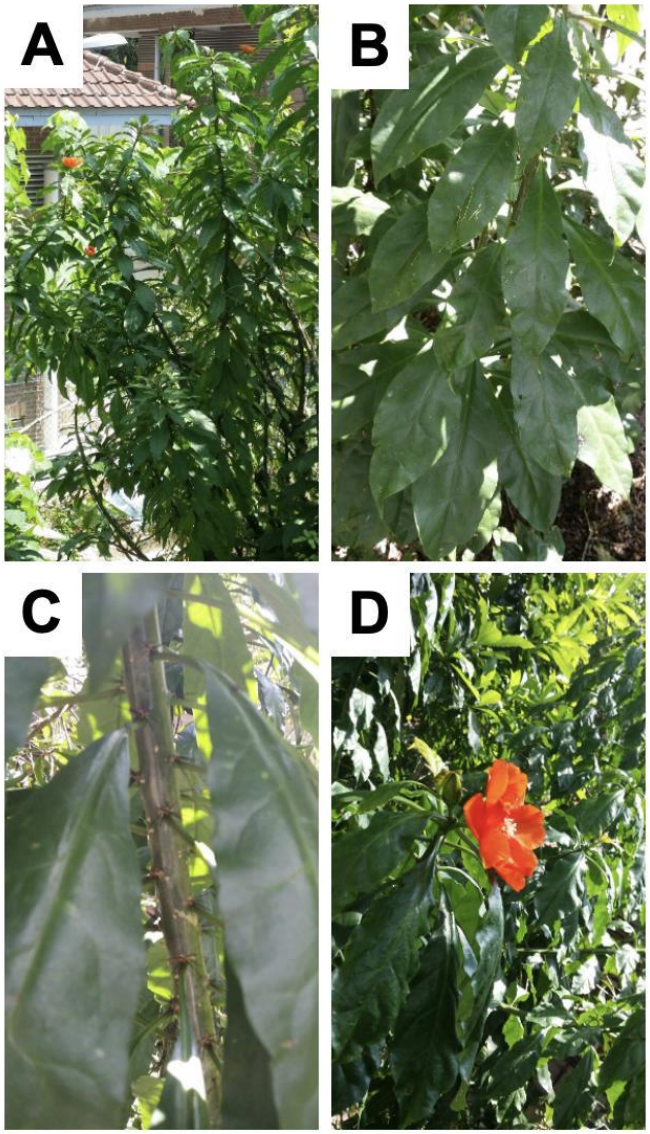

Figure 1. Photographs of (A) *Leuenbergeria bleo* (Kunth), (B) its leaves, (C) thorns, and (D) flowers.

3. Traditional Usage

Ethnomedicinal records of *L. bleo* reveal a convergence of applications across geographically distant cultures, influenced by its naturalization from Central America to Southeast Asia. This transoceanic distribution has fostered shared emphases on detoxification, anti-inflammatory effects, and tissue repair, often attributed to the plant's purported cooling and purifying properties in traditional medicine systems [4,5]. Such uses reflect its role as a versatile folk remedy, bridging indigenous knowledge with contemporary herbal practices.

3.1. Southeast Asian Traditional Medicine

In Malaysia and Singapore, *L. bleo* is commonly known as "jarum tujuh bilah" (seven needles), referring to its spiny areoles [4,5]. Fresh or dried leaves are consumed raw as a vegetable (*ulam*) or prepared as decoctions, primarily for metabolic conditions such as diabetes mellitus, hypertension, and rheumatism [5,20]. Topically, mashed leaf poultices are applied to boils, wounds, and skin inflammations to promote healing and reduce infection [5,20]. Ethnobotanical surveys in Singapore indicate its prominence in complementary therapies, with 36% of respondents using it for cancer support and 32% for general wellness, often in combination with other herbs [21].

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3.2. Central American Indigenous Medicine

In its native regions of Panama and Costa Rica, *L. bleo* features in indigenous pharmacopeias, where crushed leaves are applied as cataplasms for wound cleansing, infection prevention, and tissue repair [22,23]. Internal decoctions, sometimes combined with other plants, are used to address gastrointestinal disorders and to manage snakebite sequelae, including muscle spasms, swelling, and systemic inflammation [24–28]. These practices reflect traditional uses documented among various indigenous communities in the region.

3.3. Contemporary Challenges

Despite widespread use, contemporary applications of *L. bleo* face challenges related to standardization, dosage variability, and potential safety concerns. The absence of regulated preparations emphasizes the need for rigorous scientific validation to bridge traditional knowledge with evidence-based medicine. The following sections critically evaluate the pharmacological data supporting these ethnomedicinal claims, examining the distinct contributions of small-molecule metabolites and emerging peptidomic compounds.

4. Extract-Level Pharmacology

Pharmacological investigations using various solvent extracts have provided validation for several ethnomedicinal claims of *L. bleo*, though most evidence remains confined to in vitro studies. This section synthesizes key bioactivities observed at the crude extract level, contextualizing them within traditional uses while noting limitations in mechanistic understanding and clinical translation.

4.1. Antioxidant Activity

Methanolic and aqueous leaf extracts demonstrate potent free radical scavenging in DPPH assays, correlating with phenolic content [29–32]. Complementary assays, including ABTS, ferric reducing antioxidant potential (FRAP), and total antioxidant capacity evaluations, corroborate these effects [33,34]. These findings support traditional uses for conditions involving oxidative stress, such as chronic inflammation and cancer prevention, though in vivo validation remains limited.

4.2. Anti-Inflammatory Effects

Extracts inhibit key inflammatory mediators in vitro, consistent with ethnomedicinal applications for rheumatism and skin inflammation [29,35,36]. Preliminary in vivo studies suggest potential mechanisms involving modulation of bradykinin, histamine, or interleukin pathways [35,36]. However, direct mechanistic characterization of *L. bleo* extracts remains limited, warranting further investigation to establish definitive anti-inflammatory pathways and dose-response relationships.

4.3. Cytotoxic and Anticancer Potential

Extracts exhibit dose-dependent cytotoxicity against human cancer cell lines, including HeLa (cervical), MCF-7 (breast), and HCT-116 (colon) [6,27,37–40]. Proposed mechanisms include apoptosis induction via caspase-3 activation and c-myc downregulation [37,39]. While these results align with traditional anticancer uses, they remain preliminary, lacking robust in vivo tumour suppression data and selectivity assessments against non-cancerous cells.

4.4. Antimicrobial Activity

L. bleo exhibits broad-spectrum antimicrobial effects against bacteria and fungi, validating its traditional role in wound care [29,30,41]. Methanolic and ethanolic extracts demonstrate inhibitory activity against Gram-positive bacteria, Gram-negative bacteria, and fungi, with MICs generally ranging from 100 to 1800 µg/mL depending on the microorganism and extract preparation [29,30].

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Potential mechanisms include membrane disruption and biofilm inhibition, offering a foundation for developing novel antimicrobial agents, particularly antifungals targeting drug-resistant strains [41,42].

4.5. Metabolic and Cardiovascular Effects

L. bleo demonstrates metabolic regulatory activities that align with traditional applications for diabetes mellitus and hypertension [43–45]. Aqueous extracts significantly reduced blood glucose levels in streptozotocin-induced diabetic rats, restored serum insulin, and improved lipid profiles (reduced total cholesterol, triglycerides, and low-density lipoprotein; elevated high-density lipoprotein), though no hypoglycemic effect was observed in normoglycemic animals [43]. Ethanolic extracts produced dose-dependent antihypertensive effects in NaCl-induced hypertensive rats, with the highest dose achieving efficacy comparable to captopril, likely through increased urinary sodium and potassium excretion [44]. While these findings support ethnomedicinal use for metabolic disorders, mechanistic details remain incomplete, and clinical translation requires comprehensive safety assessments and identification of active constituents responsible for these effects.

4.6. Analgesic and Larvicidal Activities

L. bleo leaf extracts demonstrate central and peripheral antinociceptive effects in rodent pain models [35,36]. Butanol fractions (p.o.) produced effects superior to morphine in hot plate tests, while various fractions reduced pain responses in capsaicin-, glutamate-, and formalin-induced nociception models [35,36]. The antinociceptive mechanism involves opioid receptor activation and nitrergic pathways, as demonstrated by naloxone and L-NAME antagonism, with isolated compounds β-sitosterol and vitexin contributing to the observed effects [35]. These findings support traditional applications for rheumatism and gastric pain, though the moderate activity observed and the lack of identification of primary active constituents warrant further investigation [36].

Ethanolic fruit endocarp extracts exhibit larvicidal activity against *Aedes aegypti* third-instar larvae, with fractionated extracts achieving LC_{50} values of 223.12–707.94 ppm at 24–48 hours exposure [46]. While this supports potential applications in dengue vector control, safety assessments for non-target organisms and efficacy against other mosquito vectors remain unexplored [46].

Collectively, these pharmacological activities suggest a diverse chemical repertoire underlying *L. bleo*'s therapeutic potential. However, the lack of standardized extracts, incomplete mechanistic characterization, and absence of clinical data limit translational application. The subsequent sections dissect the underlying phytochemistry, distinguishing contributions from small-molecule metabolites and cysteine-rich peptides.

5. Small-Molecule Constituents and Their Bioactivities

The small-molecule profile of *L. bleo*, characterized primarily from leaf and fruit extracts, encompasses a diverse array of alkaloids, phenolics, sterols, terpenoids, carotenoids, and fatty acids [4,5]. These classes of secondary metabolites provide the chemical basis for many of the plant's observed bioactivities, though the distinction between individual compound effects and synergistic extract activities remains an active area of investigation. This section highlights representative constituents and their pharmacological relevance.

5.1. Alkaloids

L. bleo leaves contain alkaloids, notably β-phenethylamine derivatives including 3,4-dimethoxy-β-phenethylamine, 3-methoxytyramine, and tyramine [4,47]. While 3,4-dimethoxy-β-phenethylamine shares structural similarities with mescaline, it lacks the 3,4,5-trimethoxy substitution pattern required for the potent hallucinogenic effects seen in other cacti like *Lophophora williamsii* [48,49]. Functionally, 3-methoxytyramine and tyramine may influence vascular and neurological systems, with tyramine known for its vasopressor activity [50,51].

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5.2. Phenolics and Flavonoids

Phenolic compounds represent the most abundant bioactive in *L. bleo* leaves, correlating with the plant's antioxidant capacity [4,30]. Total phenolic content varies by extraction solvent, with ethyl acetate fractions typically yielding the highest concentrations [4,30]. Key isolates include the flavonoid aglycones quercetin and myricetin, the flavan-3-ols catechin and epicatechin, and α-tocopherol (Vitamin E) [52]. The lipophilic phenol 2,4-di-tert-butylphenol has emerged as a potent cytotoxic agent, demonstrating significant activity against KB and MCF-7 cancer cell lines [27]. Quercetin and its glycosides (e.g., vitexin) are implicated in the plant's antinociceptive effects via opioid receptor modulation and contribute to broad-spectrum antioxidant defense [35,36].

5.3. Sterols

The sterol profile of *L. bleo* leaves is dominated by β-sitosterol and its glucoside, alongside minor constituents such as campesterol and stigmasterol [4,27]. β-sitosterol exhibits moderate cytotoxicity against breast cancer cell lines and contributes to the plant's antimicrobial defense [27]. In complex mixtures, these sterols often show lower individual cytotoxicity (IC_{50} >100 μg/mL), suggesting that their pharmacological impact, particularly in anti-inflammatory and antirheumatic applications, may rely on synergistic interactions with other lipid-soluble constituents rather than potent single-agent activity [27].

5.4. Terpenoids and Lactones

Essential oil and organic solvent extracts yield bioactive terpenoids, including phytol (a diterpene alcohol) and dihydroactinidiolide (a terpene lactone) [27,29]. Phytol contributes to the plant's moderate cytotoxic activity [27]. Dihydroactinidiolide complements these effects with additional cytotoxic and antioxidant properties [27].

6. Microproteins and Cysteine-Rich Peptides (CRPS)

While small-molecule metabolites have historically dominated the pharmacological characterization of *L. bleo*, recent investigations have unveiled a significant proteomic dimension to its therapeutic profile. Investigations by Loo et al. (2017) identified a novel suite of ribosomally synthesized microproteins, marking a paradigm shift in understanding the plant's bioactivity [8].

6.1. Discovery and Identification

The discovery of "bleogens" represents a pivotal advancement in Cactaceae phytochemistry, moving beyond traditional alkaloid and phenolic profiling to plant peptidomics. High-resolution mass spectrometric analysis of aqueous *L. bleo* leaf extracts revealed a distinct cluster of peptide signals in the 3000–5000 Da mass range [8]. By integrating liquid chromatography-tandem mass spectrometry (LC-MS/MS) with transcriptomic mining, researchers successfully matched these peptide masses to their encoding precursor genes, leading to the characterization of the bleogen family (pB1–pB15) [8]. Bleogen pB1 (pB1) was identified as the archetypal member due to its high abundance, with extraction yields approximating 100 mg/kg wet leaf weight, and consistent detection in untargeted analyses. These mature peptides are typically 30–40 amino acid residues in length, distinguishing them from larger proteinaceous toxins or enzymes [8].

6.2. Structural Architecture and Stability

Bleogens are compact CRPs characterized by the six-cysteine hevein-like peptide (6C-HLP) signature motif ($CX_nCX_nCCX_nCX_nC$) and adopt highly stable, disulfide-constrained tertiary structures [8]. Nuclear magnetic resonance (NMR) analysis of bleogen pB1 revealed a canonical cystine-knot connectivity (Cys I–IV, II–V, III–VI) that stabilizes two antiparallel β-sheets and four interconnecting loops [8]. This framework imparts exceptional physicochemical stability, including thermal tolerance

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up to 100 °C, acid resistance down to pH 2, and robust protease resistance, attributes that align with potential defensive functions in plants and hold promise for therapeutic development [8].

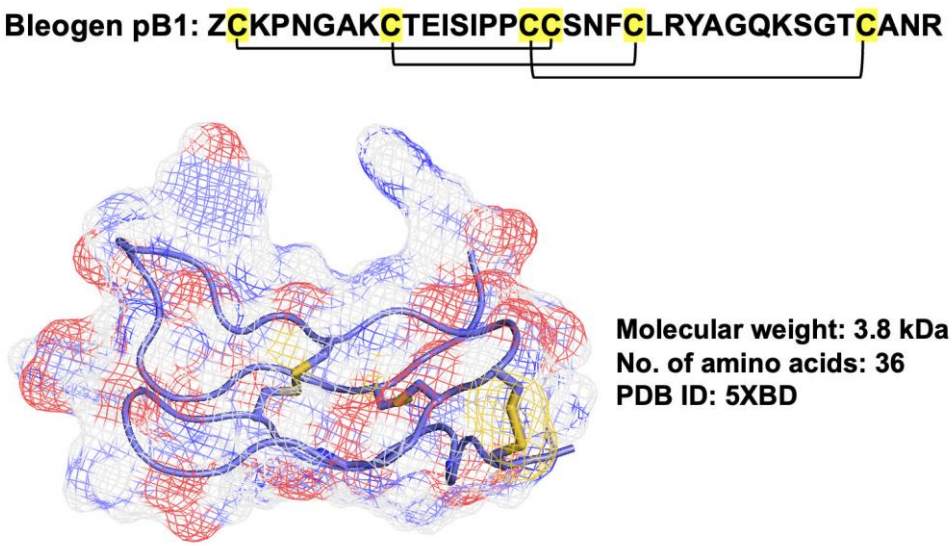

Figure 2. Primary, secondary and tertiary structure of bleogen pB1 microprotein.

6.3. Genomic Organization and Biosynthesis

Unlike non-ribosomal peptides, bleogens are gene-encoded products processed from secretory precursor proteins. Transcriptomic analysis indicates diverse precursor architectures. Bleogen pB1 arises from a Type I precursor, containing an N-terminal endoplasmic reticulum (ER) signal peptide (29–31 residues) immediately followed by the mature peptide domain [8]. Conversely, pB2–pB11 are derived from Type II precursors, which feature an intervening C-terminal propeptide region likely involved in vacuolar targeting and regulating proteolytic maturation [8]. A subset of these, pB12–pB15 (Type IIb), display complex architectures with tandem repeats of the mature domain, suggesting evolutionary gene duplication events that enable the multimeric release of active peptides from a single transcript [8].

6.4. Classification Within Plant Cysteine-Rich Peptides

The characterization of bleogens positions *L. bleo* as a significant source of plant CRPs, comparable to species producing cyclotides, defensins, thionins, and knottins [53]. However, structurally, bleogens are distinct. They strictly adhere to the hevein-like fold and 6C-HLP disulfide pattern, differentiating them from the cyclic backbones of cyclotides or the distinct γ-core motifs found in plant defensins [8]. This classification expands the known structural diversity of the Cactaceae peptidome and suggests that *L. bleo* has evolved specific peptide scaffolds distinct from those found in other medicinal plant families (e.g., Violaceae or Rubiaceae).

6.5. Pharmacological Activities and Translational Potential

The bioactivity of bleogens provides a molecular rationale for the traditional use of *L. bleo* in wound care and infection management. Bleogen pB1 exhibits potent, selective antifungal activity against *Candida albicans* and *Candida tropicalis*, achieving low-micromolar minimum inhibitory concentrations (MICs) without inducing cytotoxicity in mammalian cells at concentrations up to ~100 μM [8].

Beyond antimicrobial effects, pB1 functions as a wound-healing accelerator. In vitro studies demonstrate that pB1 promotes keratinocyte migration by activating the epidermal growth factor receptor (EGFR)-mitogen-activated protein kinase (MAPK) signaling pathway [9]. These findings

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were corroborated in vivo using a rat corneal alkali-burn model, where topical administration of pB1 accelerated re-epithelialization, reduced corneal opacity, and minimized neovascularization, achieving full closure by day 7 [10]. Notably, the inherent protease resistance of the pB1 cystine-knot scaffold offers a distinct therapeutic advantage over endogenous growth factors like EGF, which are rapidly degraded in the protease-rich microenvironment of chronic wounds [9,10]. Consequently, bleogens represent a promising class of stable, bioactive scaffolds for the development of novel topical therapeutics.

7. Synergistic Mechanisms

While modern pharmacology often isolates single active constituents, the traditional efficacy of *L. bleo* likely stems from the concerted action of its diverse chemical arsenal. A comparative analysis of its small-molecule and peptide profiles suggests a bipartite mechanism of action, particularly relevant to its wound-healing and anti-inflammatory applications.

The small-molecule constituents such as phenolics, sterols, and fatty acids, likely provide immediate, broad-spectrum effects. For instance, phenolic antioxidants and sterols may rapidly neutralize reactive oxygen species and modulate acute inflammatory cytokines immediately upon application [5,32–34,40]. In contrast, the cysteine-rich peptides (bleogens) appear to function through specific receptor-mediated signaling, such as the activation of the EGFR-MAPK pathway by pB1 to drive cellular proliferation and re-epithelialization [9]. This suggests a temporal synergy: small molecules may create a favorable microenvironment by reducing inflammation and oxidative stress, thereby facilitating the regenerative signaling activities of the slower-acting but highly specific peptides.

Despite these logical inferences, experimental validation of synergistic interactions remains a significant gap. To date, most studies have tested crude extracts or isolated fractions independently. Future research must prioritize "entourage effect" studies, comparing the efficacy of whole extracts against reconstituted mixtures of isolates, to determine whether the combination of small molecules and peptides yields additive, synergistic, or antagonistic effects.

8. Future Directions

The comprehensive characterization of *L. bleo*, spanning from metabolic intermediates to gene-encoded microproteins, establishes a new paradigm for understanding the chemical ecology of the Cactaceae. While the dichotomy between its small-molecule and peptide constituents has been defined, bridging these two chemical worlds requires targeted investigation. Future research should prioritize the following areas to fully exploit this chemical diversity.

8.1. Expanding the Peptidomic Landscape

While the discovery of bleogens has shifted the analytical focus, current characterization is largely limited to the archetypal member, pB1. A priority for future chemical research is the structural and functional deorphanization of the remaining bleogen family members (pB2–pB15). Given the sequence variations in their variable loops, these uncharacterized isoforms may possess distinct target specificities or biological activities beyond the antifungal and wound-healing properties observed in pB1. Advanced peptidomic workflows combining *de novo* sequencing with high-throughput bioassays will be essential to mapping the full functional diversity of the *L. bleo* peptidome.

8.2. Elucidating Chemo-Biological Synergies

The coexistence of small-molecule metabolites and stable microproteins within the same tissue raises intriguing questions regarding their combined effects. Research must move beyond isolated constituent screening to investigate potential "entourage effects" where small molecules may modulate the pharmacokinetics or pharmacodynamics of the peptides. Specifically, mechanistic

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studies should explore whether the antioxidant milieu provided by phenolics and flavonoids protects the peptide scaffold from oxidative damage or if saponins and terpenoids enhance peptide permeation through biological membranes. Understanding these chemical interactions is crucial for rationalizing the efficacy of traditional crude preparations versus isolated compounds.

9. Conclusion

L. bleo illustrates the transformative power of modern analytical science in validating and demystifying traditional medicine. The recent discovery of bleogens, existing alongside a well-characterized spectrum of small-molecule metabolites, reveals a dual chemical identity that offers a coherent mechanistic rationale for the plant's diverse applications. The pharmacological data suggests a functional partition: small molecules likely drive the antioxidant, anti-inflammatory, and metabolic regulations, while the robust cysteine-rich peptides provide specific antifungal protection and tissue-regenerative signaling.

The exceptional stability of the bleogen family, resistant to the harsh conditions of traditional processing and the proteolytic environment of wounds, highlights their potential as scaffolds for next-generation peptide therapeutics. These findings suggest that the plant's historical reputation for "cooling" and "healing" is grounded in a sophisticated interplay of immediate biochemical modulation and sustained cellular signaling.

Ultimately, the study of *L. bleo* serves as a compelling model for natural product research, demonstrating that "old" medicinal plants may yet harbor novel chemical classes undetected by conventional screening. Moving forward, an integrated approach that respects traditional knowledge while employing rigorous chemical and biological validation will be essential to translate the therapeutic potential of this basal cactus into standardized, clinical applications for wound healing, metabolic health, and infectious disease management.

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