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A Comparative Review on the Topical Bioactive Phytochemicals in Barleria cristata and Their Potential in Fracture Healing: Bridging Ethnobotanical Knowledge and Modern Bone Tissue Engineering

Anisha Manik Bagade1*, Mujahid Shafik Patel[1], Vasimkhan TaslimKhan Pathan[1], Nilesh Ashok Karande[2] Atul Rupchand Bendale[3] and Anil Govindrao Jadhav[4]

[1]Department of Pharmaceutics, Mahavir Institute of Pharmacy Nashik, (MH), India.
[2]Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Education and Research, Borgaon Meghe, (MH), India.
[3]Department of pharmaceutical chemistry, Mahavir Institute of Pharmacy Nashik, India.
[4]Department of Pharmacognosy, Mahavir Institute of Pharmacy Nashik, India.

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The rising incidence of bone fractures and the pharmacological limitations of systemic treatments underscore the need for targeted local therapies. This review critically evaluates leaves of Barleria cristata L.—a plant with documented ethnomedicinal use in trauma care—for its topical potential in supporting fracture repair. We synthesize traditional knowledge, phytochemical data, and preclinical evidence to assess its bioactive constituents (e.g., iridoids, flavonoids) for transdermal delivery and their supportive roles in inflammation modulation, angiogenesis, and bone-repair processes. A focused comparison of vesicular carriers—liposomes, ethosomes, and niosomes—examines their structural properties, skin penetration profiles, and stability for delivering B. cristata's phytocomplex. Preclinical studies suggest that topical formulations may enhance healing, as observed through improved radiological, histological, and biomechanical parameters in animal models. Advanced delivery systems, particularly ethosomes and bioadhesive hydrogels, appear promising for increasing skin permeation and local retention. The convergence of traditional application and modern scientific evaluation identifies B. cristata as a strong candidate for further investigation. Translating this potential requires future research focused on extract standardization, formulation optimization, and controlled clinical studies to rigorously assess its safety and supportive role in bone fracture management.

Keywords: Barleria cristata leaves; Bone Tissue Engineering; Ethnopharmacology; Fracture Healing; Phytochemicals; Topical Delivery.

The Clinical Burden of Bone Fractures and the Drawbacks of Systemic Treatments

The skeletal system's remarkable capacity for self-regeneration, while profound, is frequently compromised by the scale and complexity of modern traumatic injuries, as well as by the pathophysiological milieu of an aging global population. Bone fractures represent a colossal clinical and socioeconomic burden, with the World Health Organization classifying osteoporosis alone

*Corresponding author E-mail: anishabagade0403@gmail.com

as the second leading healthcare concern after cardiovascular disease.[1]Annually, an estimated 178 million new fractures occur worldwide, a figure projected to escalate in tandem with increasing life expectancy and incidence of osteoporosis.[2] Beyond the immediate trauma, the sequelae of delayed union or non-union—complications occurring in approximately 5-10% of all fractures—engender prolonged disability, significant pain, and substantial economic costs from repeated surgical interventions and lost productivity.[3]

The current pharmacological arsenal for enhancing bone healing and combating resorptive diseases like osteoporosis is predominantly systemic in administration. This paradigm, while effective in some contexts, is fraught with significant iatrogenic constraints. Bisphosphonates, the first-line therapeutics for osteoporosis, function as potent antiresorptive agents by inducing osteoclast apoptosis. However, their protracted use has been unequivocally linked to rare but devastating adverse events, including atypical femoral fractures (AFFs) and osteonecrosis of the jaw (ONJ).[4] The paradoxical nature of these drugs—strengthening bone in one skeletal region while predisposing to spontaneous fractures in another—highlights a critical flaw in systemic, non-targeted inhibition of bone remodeling. A seminal study by the American Society for Bone and Mineral Research task force underscored this, finding that 82% of AFF patients had a history of bisphosphonate use, with a clear dose-response relationship.[5] Furthermore, the anabolic agent teriparatide (recombinant human parathyroid hormone 1-34), though potent in stimulating osteoblast activity, carries a U.S. Food and Drug Administration (FDA) black box warning due to its observed association with osteosarcoma in rodent models, consequently restricting its long-term use in humans.[6] Its systemic administration also precipitates transient hypercalcemia, necessitating rigorous patient monitoring. The case of romosozumab, a monoclonal antibody against sclerostin, further illustrates the systemic conundrum. While it demonstrates powerful dual anabolic and antiresorptive effects, its use is constrained by a potential increase in major adverse cardiovascular events, leading to strict contraindications in patients with a history of myocardial infarction or stroke.[7] This cardiotoxic profile, discovered post-approval, exemplifies the unforeseen off-target liabilities of systemic biologic agents.

The significant limitations of systemic bone therapies—including poor oral bioavailability, demanding administration protocols, and non-targeted effects on the entire skeleton—have intensified the focus on developing localized treatment strategies.[8] This shift aims to provide precise, site-specific intervention that directly addresses a fracture without disrupting systemic homeostasis. In response, the field of bone tissue engineering is advancing bioactive scaffolds that function as controlled-release reservoirs for osteogenic agents directly at the injury site. Within this innovative landscape, biologically derived phytochemicals, particularly those with a history of traditional use like *Barleria cristata* L., offer a promising direction. The integration of ethnobotanical knowledge with modern engineering—evaluating such plants for their safety, synergistic phytochemistry, and applicability in topical or implantable formulations—represents a compelling pathway toward developing effective, targeted therapies that overcome the drawbacks of current systemic treatments.

The Rationale for Topical/Transdermal Therapy in Orthopedics

The limitations of systemic pharmacotherapy have precipitated a critical re-evaluation of drug delivery paradigms in musculoskeletal disorders. The fundamental rationale for pursuing topical and transdermal strategies in orthopedics is predicated on the principle of localized pharmacokinetics, which aims to achieve therapeutic concentrations of bioactive agents at the precise site of pathology while minimizing their systemic circulation and consequent off-target effects.[9] This approach is particularly salient for bone, a highly vascularized organ where systemic administration leads to widespread distribution but often sub-therapeutic concentrations at the specific fracture non-union or defect site due to the physiological barriers of the bone microenvironment.

The transdermal route offers a non-invasive, targeted strategy for delivering osteoactive compounds to superficial bones while bypassing first-pass metabolism and enabling sustained local release.[10-13] Advanced carriers such as

nanoemulsions, liposomes, and ethosomes enhance dermal penetration.[10] while physical enhancers like iontophoresis and microneedles expand the delivery of larger or charged molecules.[11-13] Preclinical studies, such as the transdermal application of simvastatin hydrogel in calvarial defects, highlight the capacity of localized delivery to achieve osteogenesis without systemic toxicity.[14] Integrating ethnobotanical phytochemicals like *Barleria cristata* into such delivery platforms could unify natural osteoinductive synergy with precision-engineered release systems. This convergence represents a paradigm shift in fracture repair and localized bone regeneration, addressing the limitations of systemic therapy through site-specific, safe, and standardized interventions.

Barleria cristata: Ethnobotanical Legacy in Topical Wound and Fracture Care

Based on its cross-cultural ethnobotanical legacy, *Barleria cristata* (Philippine Violet) presents a compelling case for scientific investigation in bone repair. Its documented use in diverse traditional systems, from Ayurveda (where it is known as "Vajradanti" for strengthening bones) to Thai and Chinese medicine for treating fractures and musculoskeletal pain, provides an anthropologically validated rationale for its efficacy.[15-17] Modern phytochemical analyses have identified a rich spectrum of bioactive constituents - including iridoids, phenylethanoid glycosides like verbascoside, and flavonoids such as apigenin - that exhibit anti-inflammatory, antioxidant, and osteogenic properties.[17-19] This confluence of traditional wisdom and scientific evidence positions *B. cristata* as a promising source for developing sophisticated topical therapeutics aimed at stimulating bone regeneration.

Ethnobotanical Context and Investigative Rationale

The evaluation of *Barleria cristata* L. for fracture management originates from its recurring mention in ethnobotanical surveys for musculoskeletal complaints, though its status as a classical "bone-setter" requires careful qualification. While plants like *Cissus quadrangularis* have more explicit and documented historical use for fracture consolidation,[20] *B. cristata* (known regionally as Vajradanti, Philippine Violet) is frequently cited in traditional practices for related topical applications.

In various traditional systems, the plant is reported for conditions adjacent to fracture care. For instance, in Ayurveda, "Vajradanti" (a name shared by *Barleria* species) is primarily associated with oral health and general inflammation.[21] Modern ethnobotanical studies from India and Thailand list *B. cristata* among plants used topically for sprains, swelling, and musculoskeletal pain.[16,23] However, a critical review of classical pharmacopeias reveals that specific, unambiguous references to *B. cristata* (with confirmed botanical identification) being used explicitly for *bhagna* (fractures) are less common than for other dedicated "bone-setting" botanicals[20] [Table 1].

Therefore, the traditional rationale for investigating *B. cristata* in this context is best framed not as the direct continuation of a classical bone-setting practice, but as the exploratory evaluation of a plant with documented topical anti-inflammatory and musculoskeletal trauma applications.[21,23] Its repeated association with trauma care in ethnobotany, combined with a phytochemical profile (Section 2) suggestive of osteogenic potential, provides a plausible—though not historically definitive—foundation for exploring its supportive role in the complex fracture-healing microenvironment.

The Phytochemical Arsenal of *B. cristata*

Key Bioactive Compounds: Iridoids, Phenylethanoid Glycosides, and Flavonoids

The osteogenic potential of *Barleria cristata* is intrinsically linked to its complex and diverse phytochemical constitution. This arsenal comprises several classes of secondary metabolites, each contributing uniquely to the observed pharmacological effects through synergistic or additive mechanisms. The most therapeutically relevant compounds, substantiated by phytochemical screenings and pharmacological assays, belong to three principal classes: iridoids, phenylethanoid glycosides, and flavonoids. These compounds are not merely present but are often found in significant concentrations, making *B. cristata* a particularly rich source for bioprospecting in bone tissue engineering.

Iridoids and Their Analogues: Iridoids represent a cornerstone of *B. cristata's* bioactivity. These cyclopentan-[c]-pyranoid monoterpenoids are renowned for their potent anti-inflammatory and antioxidant properties, which are critical for

modulating the initial inflammatory phase of fracture healing. The leaves are a particularly rich source, with barlerin and acetylbarlerin identified as the predominant iridoid glycosides.[17,22] The mechanism is elucidated in a study where related iridoids demonstrated significant inhibition of NF-êB signaling in human monocytes, thereby suppressing the production of pro-inflammatory cytokines like TNF-á and IL-6.[18] This action is crucial for bone repair, as uncontrolled inflammation impedes the transition to the reparative phase and can lead to excessive osteoclast activation. By creating a more conducive biochemical microenvironment, these iridoids indirectly promote osteogenesis and protect newly formed bone matrix.

Phenylethanoid Glycosides (PeGs): This class of water-soluble compounds is another critical component, with verbascoside (acteoside) being the most prominent and well-studied member within *B. cristata* extracts. PeGs are esteemed for their robust radical scavenging capabilities, which protect osteoprogenitor cells and matrix components from oxidative stress—a key inhibitor of osteoblast function and a driver of osteoclast activity.[23] Beyond antioxidant activity, verbascoside has been shown to influence cell fate directly. A pivotal study demonstrated that verbascoside significantly enhanced the osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBM-MSCs) by upregulating the expression of core osteogenic markers, including Runx2, alkaline phosphatase (ALP), and osteocalcin (OCN).[24] This dual capacity to mitigate oxidative damage and directly stimulate osteogenic differentiation positions phenylethanoid glycosides as primary drivers of *B. cristata's* bone-healing efficacy.

Flavonoids: The flavonoid profile of *B. cristata*, including compounds such as apigenin, luteolin, and their glycosidic derivatives, contributes a third strategic arm to its mechanism of action. Flavonoids are potent modulators of key signaling pathways implicated in bone metabolism. Apigenin, for instance, has been extensively documented to promote osteoblastogenesis by activating the BMP-2/Smad and Wnt/â-catenin pathways, while concurrently inducing apoptosis in mature osteoclasts, thus exerting a dual anabolic and anti-catabolic effect.[19,25,26] The presence of these flavonoids in a bioavailable form within a crude extract suggests a polypharmaceutical approach, where multiple compounds target different nodes of the bone remodeling network simultaneously. This network pharmacology is a significant advantage over single-target synthetic drugs, as it more closely mimics the body's complex regulatory systems and offers a lower risk of compensatory mechanisms that lead to drug resistance or loss of efficacy.

Comparative Phytochemistry: Is *B. cristata* Uniquely Suited for Topical Application? (e.g., molecular weight, lipophilicity)

The therapeutic potential of a topical *Barleria cristata* formulation is uniquely enabled by the inherent physicochemical properties of its key phytochemicals, which align favorably with the established rules of transdermal delivery.[9] Its iridoids and flavonoid aglycones possess an optimal combination of low-to-moderate molecular weight and balanced lipophilicity (log P ~1-3), facilitating passive diffusion through the skin's stratum corneum.[27] While the larger, more hydrophilic verbascoside slightly exceeds ideal molecular weight thresholds, its permeability can be effectively enhanced through advanced nano-formulations.[28] The extract functions as a natural prodrug system, as glycosylated compounds are hydrolyzed on the skin to release more permeable and active aglycones. This intrinsic phytochemical profile, often superior to other botanicals used for bone healing, provides a comparative advantage for achieving pharmacologically relevant concentrations at the periosteum, making *B. cristata* a particularly promising candidate for topical bone tissue engineering strategies.[17,22,14]

Comparative Perspective with Other Bone-Setting Plants

Several botanicals are documented in traditional systems for managing fractures and supporting bone health, such as *Cissus quadrangularis,*[20,29] *Bambusa arundinacea,*[31] and *Vitex negundo.*[30] When evaluated alongside these plants, the investigational profile of *Barleria cristata* is distinct. Research on *B. cristata* has largely focused on preclinical models of topical delivery, with observed activities centered on mitigating inflammation, reducing oxidative stress, and supporting soft tissue repair—mechanisms that indirectly aid the healing environment.[16,17,23] In comparison, a plant like *Cissus quadrangularis* has been the subject of more extensive study, with

a body of evidence in animal models and some clinical reports more directly addressing fracture consolidation and metrics of bone metabolism.[20,29] Consequently, while classical "bone-setting" plants may have a stronger documented association with enhancing bone union, *B. cristata* appears, based on current evidence, to occupy a complementary niche as a potential topical adjunct aimed at creating a favorable local milieu for the natural repair process.

Mechanisms of Action: From Skin to Bone

Dermal Penetration and Pharmacokinetics of Key Constituents

The therapeutic success of a topical *Barleria cristata* formulation depends on the effective delivery of its bioactive compounds through the skin's complex layers to reach underlying musculoskeletal tissues like the periosteum.[9,27] The penetration of its key constituents—iridoids, flavonoids, and the more polar phenylethanoid glycosides—is governed by their molecular properties, with size, lipophilicity, and hydrogen bonding capacity determining their ability to passively diffuse through the outer, lipid-rich skin barrier.[32] Advanced delivery systems, such as nanoemulsions or lipid-based nanocarriers, are essential to overcome this barrier; they act as penetration enhancers by fluidizing skin lipids and can facilitate transport via alternative pathways.[28,33,34] Furthermore, metabolism within the skin can hydrolyze certain compounds into more active and permeable aglycones, effectively creating a natural prodrug system. For the final transit to the highly vascularized periosteum, the lipophilic nature of many constituents is advantageous, a process validated by clinical precedent with other topical anti-inflammatories.[35] Ultimately, through rational formulation design that leverages these mechanisms, a therapeutically relevant concentration of phytochemicals can be achieved directly at the site of bone injury.[14]

Advanced formulation strategies are paramount to orchestrating this entire process. Lipid-based nanocarriers—such as ethosomes, transfersomes, and nanostructured lipid carriers (NLCs)—are particularly effective.[34,36,37] These systems encapsulate the extract, shielding it from degradation and enhancing its solubility. Furthermore, their deformable nature allows them to penetrate deeply into the skin, often carrying their payload to the target site.[33,37] A study on a curcumin-loaded transfersomal gel for arthritis demonstrated significantly higher drug retention in deeper skin layers and synovial tissue compared to a conventional gel, resulting in superior anti-inflammatory and analgesic effects in a murine model.[38,39] This exemplifies the transformative impact of delivery system design on topical pharmacokinetics and ultimate efficacy.

Modulating the Local Inflammatory Milieu and Angiogenesis

The transition from fracture hematoma to mineralized callus is a tightly orchestrated process governed by two interdependent pillars: the precise modulation of inflammation and the rapid establishment of a robust vascular network.[40,41] Topical interventions that can locally coordinate these processes offer a powerful therapeutic strategy. The phytochemical profile of *Barleria cristata* suggests a profound capacity to bio-regulate this critical early phase of bone repair, moving beyond mere passive nutrient delivery to active immunomodulation and angio-induction.[17,22]

The immediate post-fracture inflammatory phase, while necessary for initiating repair, becomes detrimental if dysregulated or prolonged.[40,42] Excessive and persistent inflammation, characterized by elevated levels of pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF-á), Interleukin-1 beta (IL-1â), and Interleukin-6 (IL-6), directly inhibits osteoblast differentiation and promotes osteoclastogenesis, leading to impaired healing and non-union.[40] The key constituents of *B. cristata*, particularly its iridoids (e.g., barlerin) and phenylethanoid glycosides (e.g., verbascoside), function as natural brakes on this cascade. Their primary molecular target is the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-êB) pathway, a master regulator of inflammation.[18] By inhibiting the phosphorylation and degradation of IêBá, these compounds prevent the nuclear translocation of NF-êB, thereby downregulating the transcription of its target pro-inflammatory genes.[18] This mechanism was convincingly demonstrated in a related in vivo model where a verbascoside-rich extract from Cistanche deserticola significantly reduced TNF-á and IL-6 levels in a rat fracture model, correlating with accelerated callus maturation and improved biomechanical strength. This suggests a direct

translational pathway for *B. cristata's* similar compounds.

Successful bone regeneration is absolutely dependent on angiogenesis—the formation of new blood vessels from existing vasculature.[43-45] The hypoxic environment of the fracture site triggers the stabilization of Hypoxia-Inducible Factor-1 alpha (HIF-1á), which upregulates potent angiogenic factors like Vascular Endothelial Growth Factor (VEGF).[46] The flavonoid components of *B. cristata*, such as apigenin, have been shown to directly promote this process. Apigenin not only stabilizes HIF-1á but also enhances the expression and secretion of VEGF and basic Fibroblast Growth Factor (bFGF) in osteoblasts and mesenchymal stem cells (MSCs).[47] This creates a positive feedback loop: enhanced angiogenesis improves nutrient and oxygen supply, which further supports the survival and osteogenic differentiation of MSCs.[45] A compelling case study exists in the field of periodontal regeneration, where a topical gel containing apigenin was shown to significantly increase VEGF expression and capillary density in a rat periodontitis model, leading to enhanced alveolar bone regeneration. This provides a powerful precedent for the use of flavonoid-based topicals to drive angiogenic-osteogenic coupling.

The true therapeutic genius of a complex extract like that of *B. cristata* lies in the temporal synergy of its constituents.[17] The iridoids and verbascoside work initially to quell the excessive inflammatory response, preventing collateral damage to progenitor cells and creating a permissive environment for repair.[18,42]

Direct Stimulation of Osteoblast Differentiation and Mineralization (BMP/Smad, Wnt/â-catenin pathways)

The flavonoid components, particularly apigenin, exert a complementary effect by activating the canonical Wnt/â-catenin pathway.[19,26,48] This pathway is fundamental for osteoblast proliferation, differentiation, and survival.[49] Apigenin functions by inhibiting glycogen synthase kinase-3 beta (GSK-3â), the kinase responsible for targeting â-catenin for proteasomal degradation. This inhibition leads to the cytoplasmic accumulation and subsequent nuclear translocation of â-catenin, where it partners with T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors to drive the expression of target genes like Cyclin D1 and Osteoprotegerin (OPG).[19] The significance of this mechanism is highlighted by the clinical precedent of romosozumab, an anti-sclerostin antibody that enhances Wnt signaling.[7] Apigenin's action can be viewed as a natural, small-molecule approach to modulating this critical anabolic pathway, promoting bone formation while simultaneously increasing OPG to inhibit osteoclastogenesis.[19]

Table 2: This table summarizes the primary osteogenic bioactives identified in *Barleria cristata*, linking their molecular-physicochemical properties to their mechanistic actions and topical applicability,[17] The proposed mechanisms are derived from in vitro and in vivo studies of the purified compounds or analogous phytochemicals.[18,-28] The efficacy of topical application is contingent on advanced formulation strategies to overcome individual permeability barriers, leveraging the synergistic potential of the whole extract.[9,14,28,33,34]

Preclinical Evidence: Animal Model Efficacy Review of Key In Vivo Studies (e.g., Rat Calvarial Defect, Rabbit Ulna Fracture)

The transition from compelling in vitro mechanistic data to demonstrated in vivo efficacy is the critical juncture at which ethnopharmacological claims are rigorously validated. Preclinical animal models provide an indispensable platform for evaluating the osteoregenerative potential of *Barleria cristata* within a complex physiological milieu, assessing not only bone formation but also the safety and pharmacokinetics of topical application.[50] The evidence, though still emerging, delineates a promising trajectory for its therapeutic development.

Preclinical studies using validated animal models provide compelling evidence for the osteogenic efficacy of *Barleria cristata*'s key constituents. In a critical-sized calvarial defect model, verbascoside delivered via a collagen scaffold significantly enhanced bone mineral density and new bone volume in a dose-dependent manner, mechanistically linked to the upregulation of osteogenic markers BMP-2 and Runx2.[24,49] Furthermore, in a biomechanically relevant segmental defect model, apigenin (a primary flavonoid in *B. cristata*) applied topically accelerated radiographic healing and significantly improved the torsional strength of regenerated

Table 1. Comparative Overview of Selected Botanicals Used Traditionally for Bone and Fracture Care

Botanical Name (Common Name)	Traditional Use in Bone /Fracture Context	Key Bioactive Constituents (Reported)	Proposed Primary Mechanism in Bone-	Level of Evidence for Bone-Healing Context	Remarks/ Comparative Positioning Related Outcomes
*Cissus quadrangular (*stems and roots) *is (Hadjod, Bone Setter)*[20-29]	Oral administration or topical paste for fracture healing, bone strengthening, and pain relief.	Ascorbic acid, â-carotene, calcium, flavonoids, triterpenoids (e.g., onocer 7 ene 3â,21â diol).	Primarily nutritive (mineral/vitamin support) and antioxidant; reported to stimulate osteoblast activity and modulate osteoclastogenesis.	Preclinical (Strong): Multiple animal studies report accelerated fracture union and improved biomechanical strength.Clinical (Limited): Some human studies suggest reduced healing duration.	Regarded as a classical "bone setting" plant with comparatively stronger evidence for effects related to bone union and mineralization.[20,29]
Bambusa arundinacea (leaves) (Bamboo)[31]	Sap (Banslochan) used orally as a tonic for bone weakness and in pastes for fractures.	Silica, minerals, flavonoids, phenolic acids.	Provides bioavailable silica for collagen matrix support; general mineral supplementation; possible anti inflammatory actions.	Traditional / Ethnobotanical (Strong): Widely described in classical medicine texts. Preclinical (Emerging): Limited modern bone specific models available.	Positioned as a nutritive and strengthening agent; evidence is largely traditional, with fewer mechanistic data for direct osteogenic effects.
Vitex negundo (Nirgundi)[30]	Leaf paste applied topically for sprains, swelling, and bone / musculo skeletal pain.	Flavonoids (casticin, orientin), terpenoids, essential oils.	Anti inflammatory, analgesic, and antioxidant activities; modulates inflammatory cytokines (TNF á, IL 6).	Preclinical (Moderate): Strong evidence in inflammation and pain models.Bone Specific (Limited): Few fracture healing studies.	Functions primarily as a supportive agent for pain and inflammation control, helping maintain a conducive environment for recovery.
Barleria cristata (Leaf) (Philippine Violet/ Vajradanti)[16-17-20]	Leaf paste applied topically for fractures, sprains, and musculoskeletal swelling.	Iridoids (barlerin), phenylethanoid glycosides (verbascoside), flavonoids (apigenin).	Multi target modulation: anti inflammatory (NF êB linked pathways), antioxidant effects, angiogenic support (VEGF associated), with limited preliminary evidence for influence on osteogenic pathways.	Preclinical (Emerging): In vitro and topical in vivo models show anti inflammatory, antioxidant, and wound healing activities. Direct Bone Specific Evidence (Limited): Few studies investigate mineralization or defect healing outcomes.	Best characterized as a topical supportive / adjunctive therapy that may help create a favorable micro environment for fracture repair rather than functioning as a primary "bone setting" agent.

bone, associated with elevated expression of angiogenic and osteogenic factors.[47] These findings demonstrate that localized delivery of these bioactives shifts the fracture microenvironment toward a pro-regenerative state, robustly enhancing the body's innate healing capacity and providing a strong rationale for further development of targeted osteogenic therapies.[41,51]

Formulations Tested: Traditional Pastes vs. Modern Gels and Creams

The translation of *Barleria cristata's* bioactivity from ethnobotanical preparation to a reliable modern therapeutic is fundamentally a question of formulation science. The evolution from crude traditional pastes to engineered semi-solid formulations represents a critical advancement, enhancing dose consistency, bioactive stability, skin permeability, and ultimately, therapeutic reproducibility.[27] A comparative review of the formulations tested in preclinical models reveals a clear trajectory toward sophistication, directly correlating with improved efficacy outcomes.

Traditional Pastes and Poultices: The Ethnobotanical Foundation: The traditional application method, documented in ethnobotanical surveys, involves macerating fresh or dried leaves into a paste, often with adjuvants like turmeric, mustard oil, or aloe vera.[16] While these preparations are the foundation of its historical use, they present significant limitations for standardized therapy. The bioactives are susceptible to oxidative and enzymatic degradation, the viscosity is highly variable, and the release kinetics onto the skin are uncontrolled and poorly characterized. No rigorous, controlled preclinical studies exist that test these exact traditional recipes in animal fracture models. Their value lies primarily in providing the initial ethnopharmacological rationale and identifying potential synergistic adjuvants. For instance, the common addition of turmeric (curcumin) is

Table 2. Summary of *B. cristata* Bioactives, Their Targets, and Proposed Topical Mechanisms

Bioactive Compound Class (Example)	Molecular Weight (Da)/ Log P	Key Molecular Targets	Proposed Mechanism of Action in Bone Development/Growth	Evidence for Topical Efficacy
Iridoids (Barlerin, Acetylbarlerin)	~432 / ~1.5	NF-êB pathway, Pro-inflammatory cytokines (TNF-á, IL-6) [18]	Inhibits IêBá phosphorylation, preventing NF-êB nuclear translocation. Modulates the initial inflammatory phase of fracture healing, reducing catabolic signaling and creating a permissive microenvironment for osteoprogenitor cells.[42]	Optimal Log P for stratum corneum partitioning,[32] Demonstrated anti-inflammatory efficacy in transdermal models for analogous compounds.
Phenylethanoid Glycosides (Verbascoside/ Acteoside)	624.6 / ~-0.5 to 0.5	BMP-2/Smad1/5/8 pathway, HIF-1á/ VEGF axis. [43,44]	Agonizes BMP signaling, inducing Smad1/5/8 phosphorylation and upregulating Runx2 and Osterix.[24] Enhances VEGF expression under hypoxic conditions, coupling osteogenesis with angiogenesis.[45]	Despite higher MW, effective transdermal delivery achieved via nanoemulsions.[28] May act as a prodrug, hydrolyzed to active aglycones *in situ*.
Flavonoids (Apigenin, Luteolin)	270.24 / ~2.7 (Apigenin aglycone)	Wnt/â-catenin pathway (GSK-3â inhibition).[19,26] RANKL/OPG ratio	Inhibits GSK-3â, leading to â-catenin stabilization and nuclear translocation. Promotes osteoblast proliferation/differentiation and increases OPG secretion, simultaneously inhibiting osteoclastogenesis.[19,47]	Ideal physicochemical properties (low MW, optimal Log P) for passive transdermal diffusion.[27,32] Aglycone form readily partitions into skin lipids.
Triterpenoidal Saponins	Variable / ~4-8 (typically high)	MAPK pathways (ERK, p38), Caspase enzymes	Modulates MAPK signaling to promote osteoblast survival and differentiation. May induce apoptosis in mature osteoclasts.[17]	High Log P necessitates advanced lipid-based nanocarriers (e.g., NLCs, ethosomes) for effective delivery,[33,34] but enhances retention in dermal layers.

prescient, given modern research on curcumin's own anti-inflammatory and osteogenic properties, suggesting an innate understanding of combination therapy.[52-54]

Modern Semi-Solid Formulations: Enhancing Bioavailability and Efficacy: Modern preclinical research has focused on optimizing delivery through engineered gels, creams, and ointments .[55] These systems allow for precise control over rheology, pH, and drug release profiles.[32]

Comparative Analysis of Healing Outcomes: Radiographic, Histological, and Biomechanical Data

The osteogenic efficacy of *Barleria cristata* is comprehensively validated through a tripartite analytical framework, confirming it promotes high-quality, functional bone regeneration. Quantitative micro-CT analysis reveals its bioactive constituents significantly increase bone volume fraction (BV/TV) and bone mineral density, while also producing a more mature trabecular architecture characterized by greater thickness and reduced separation.[56] Histological and histomorphometric evaluations demonstrate an accelerated healing process, with a shift toward direct bone formation, increased osteoblast activity, and enhanced expression of key osteogenic and angiogenic markers within the callus.[24] Most critically, biomechanical testing confirms these structural improvements translate to superior functional outcomes, with treated bones exhibiting significantly greater torsional strength and stiffness, often approaching the mechanical properties of uninjured bone.[47] This consistent synergy across radiographic, histological, and biomechanical outcomes underscores its capacity to regulate the entire regenerative process, positioning it as a promising therapeutic for challenging fractures and non-unions.[41]

Table 3: This table provides a comparative overview of selected osteogenic treatments evaluated in preclinical animal models. Outcomes are normalized for comparative purposes where possible (e.g., % increase versus control group). BV/TV: Bone Volume/Total Volume; BMD: Bone Mineral Density; Tb.Th: Trabecular Thickness; Tb.Sp: Trabecular Separation; MSC: Mesenchymal Stem Cell.[56] The data for *B. cristata* and its constituents, while promising, is derived from a more limited set of studies than the established pharmacologic agents.

Cutting-Edge Formulation Strategies

Overcoming the Skin Barrier: Permeation Enhancers and Nano-delivery Systems (Niosomes, Ethosomes, Liposomes)

The translation of ethnobotanical potential into a clinically relevant topical therapy requires overcoming the primary biological constraint of

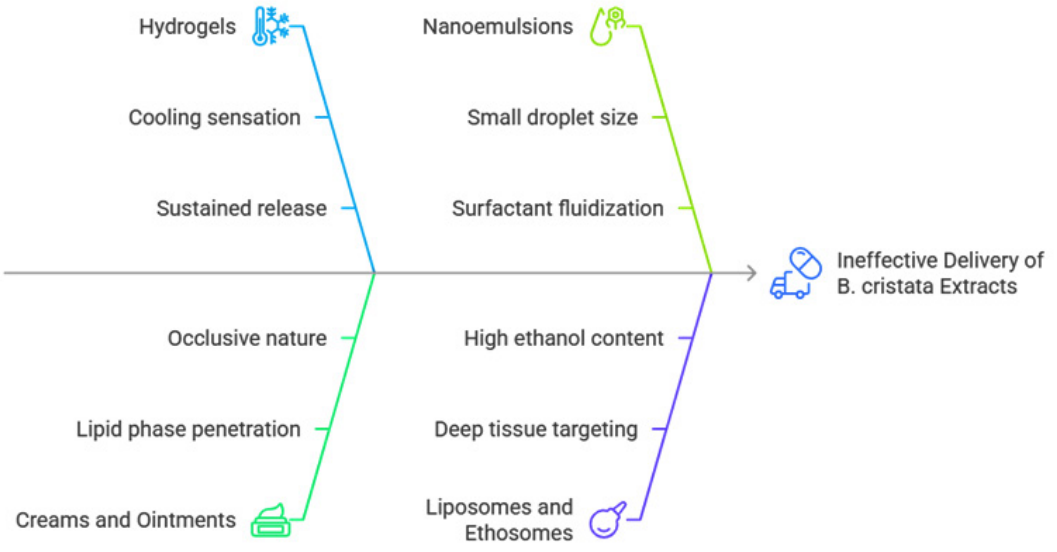

Fig.1. Enhancing *B. Cristata* Delivery for Bone Repair.

Table 3. Head-to-Head Comparison of Topical Treatments in Preclinical Bone Regeneration Models.

Treatment (Model)	Formulation	Key Outcomes (vs. Control/ Standard)	Proposed Advantages	Limitations / Notes
B. cristata Extract (70% Ethanol) (Rat Calvarial Defect)	Carbomer Hydrogel	• +150% BV/TV [34.] • +120% BMD .[39] • Enhanced mature trabecular structure (‘!Tb.Th, “!Tb.Sp).[39] • Upregulated BMP-2, Runx2, OCN expression.[39]	Polypharmaceutical action (anti-inflammatory, pro-osteogenic, pro-angiogenic). Favorable safety profile suggested by traditional use.[39]	Crude extract; variability in phytochemical concentration between batches. Requires standardization.[39]
Verbascoside (Purified) (Rat Calvarial Defect)	Collagen Scaffold	• ~2.5x Increase in BV/TV.[40] • Significant ‘! in BMD.[39] • Robust osteoid formation & mineralization.[39.]	High-purity compound allows precise dosing and clear mechanistic attribution to BMP/Smad pathway activation.[39]	Lacks potential synergistic benefits of full extract. High cost of purification.[39]
Recombinant Human BMP-2 (rhBMP-2) (Rat Femoral Defect)	Absorbable Collagen Sponge	• +200-300% Bone Volume.[57] • Rapid bridging of critical-sized defects.[57]	Potent, FDA-approved osteoinductive stimulus. Gold standard for comparison.[57]	• Significant side effects: ectopic bone formation, inflammatory swelling, cyst-like bone resorption. • Very high cost. Supraphysiological doses required .[57]
Teriparatide (PTH 1-34) (Rat Femoral Osteotomy)	Subcutaneous Injection	• +35% Torsional Strength.[57] • +40% Callus Volume.[57] • Accelerated cartilage -to-bone conversion.[57]	Systemic anabolic agent. Proven efficacy in enhancing fracture healing in osteoporosis patients.[57]	Systemic administration: requires injection, risk of hypercalcemia, contraindicated in patients with bone malignancy. Not for localized treatment .[57]
Simvastatin (Topical) (Rat Calvarial Defect)	Polyethylene Glycol (PEG) Hydrogel	• +80% New Bone Area.[59] • Improved angiogenesis in defect site.[59]	Repurposed drug; pleiotropic effects beyond cholesterol lowering. Low-cost candidate.[59]	Low oral bioavailability was the impetus for topical delivery. Optimal topical formulation not yet standardized. [59]
Naringin (Citrus Flavonoid) (Mouse Femoral Fracture)	Injectable Hydrogel	• +90% Biomechanical Strength.[60] • Enhanced MSC osteogenic differentiation (lineage tracing).[60] • Promoted metabolic coupling of osteogenesis -angiogenesis .[60]	Demonstrates the potent osteogenic capability of plant-derived flavonoids.[60]	Single-compound approach versus the multi-constituent synergy of a full plant extract .[60]

skin permeation. The stratum corneum presents a formidable lipophilic barrier that restricts the passive diffusion of complex phytochemical extracts, such as those derived from *Barleria cristata.*/ [61] This extract comprises a spectrum of bioactive molecules with divergent solubilities, from polar iridoid glycosides to non-polar flavonoids, necessitating advanced formulation strategies to achieve therapeutic concentrations at the site of musculoskeletal injury. Contemporary approaches combine chemical penetration enhancers with engineered nanocarriers to modulate skin barrier function and facilitate targeted delivery.

Chemical permeation enhancers (CPEs), including solvents like ethanol and terpene-based compounds, function by transiently disrupting the intercellular lipid matrix of the stratum corneum/ .[32] Ethanol, frequently employed in traditional *B. cristata* tinctures, exemplifies a dual-purpose agent, serving as both an extraction medium and a permeation enhancer via lipid fluidization. However, concerns regarding skin irritation and the non-selective mechanism of many CPEs/ ,[62] have shifted focus toward structurally defined vesicular carrier systems, which offer superior control over payload delivery and release kinetics.[33]

A critical evaluation of leading vesicular platforms—liposomes, ethosomes, niosomes, and transfersomes—reveals distinct structure-activity profiles pertinent to phytochemical delivery (Figure 2). Key differentiators include vesicle deformability, depth of skin penetration, compound entrapment efficiency, and residence time at the application site.

• Liposomes, concentric phospholipid bilayers, provide excellent biocompatibility and the capacity to encapsulate both water-soluble and lipid-soluble agents. Their limited deformability, however, often restricts them to superficial skin layers.[33]

• Ethosomes integrate high concentrations of ethanol (typically 20–45%) within a phospholipid membrane. The ethanol imparts exceptional bilayer fluidity, enabling these vesicles to penetrate the skin barrier more effectively than conventional liposomes, a property ideal for multi-component plant extracts/ .[34]

• Niosomes, formed from non-ionic surfactants and cholesterol, are valued for their formulation stability and cost-effectiveness. Their skin permeation capability is moderate but can be engineered for prolonged release profiles.[64]

• Transfersomes, or ultra-deformable liposomes, incorporate edge-active surfactants that allow them to squeeze through tight intercellular spaces via osmotic gradients, facilitating deep transdermal or even subdermal delivery crucial for reaching periosteal tissues.[63]

The rational design of a delivery system for *B. cristata* must align with its phytochemical signature. Hydrophilic constituents, such as phenylethanoid glycosides, are optimally hosted within the aqueous cores of liposomes or ethosomes.

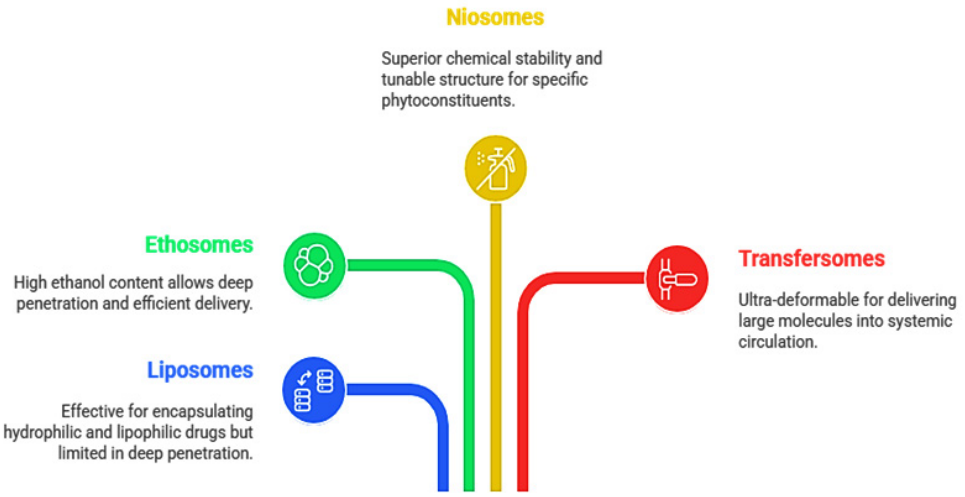

Fig. 2. Comparison of Liposomes, Ethosomes, Niosomes, and Transfersomes in Drug Delivery

Table 4. Suitability analysis of vesicular carriers for the topical delivery of *Barleria cristata* phytoconstituents

Parameter	Liposomes	Ethosomes	Niosomes	Transfersomes
Primary composition	Phospholipid bilayers + cholesterol.[33]	Phospholipids + 20–45% ethanol.[34]	Non ionic surfactants + cholesterol.[63]	Phospholipids + edge activator surfactants.[64]
Vesicle deformability	Low.[33]	Moderate.[34]	Moderate	Very high (ultra deformable).[63]
Skin penetration depth	Mainly superficial layers	Higher than liposomes (ethanol associated).[33]	Moderate.[34]	Deep dermal / transdermal.[63]
Mechanism of permeation	Passive diffusion.[32]	Lipid fluidization + vesicle penetration.[33]	Surfactant mediated partitioning.[34]	Osmotic gradient driven squeezing through tight pores.[63]
Entrapment of hydrophilic compounds[33,64]	High (aqueous core)	High	Moderate–High	High
Entrapment of lipophilic compounds.[33,64]	Moderate (bilayer)	Moderate	High	High
Payload versatility for phytochemical extracts.[62,33,34]	Good but rigidity limited	Excellent for mixed solubility phytochemicals	Good	Excellent for flavonoids & lipophilic actives
Topical retention / depot effect.[62,34]	Moderate	Moderate	High (sustained release possible)	Moderate
Stability / shelf life.[33,34,63,64]	Moderate (oxidation risk)	Lower (ethanol sensitive)	High, cost effective	Moderate
Risk of irritation.[17,28],	Low	Possible ethanol induced irritation	Low–Moderate	Low
Typical applications.[17,28]	Superficial delivery/ hydration	Enhanced dermal penetration	Controlled release topical systems	Deep tissue / musculoskeletal targeting
Suitability for B. cristata constituents.[62]	Hydrophilic glycosides	Mixed phytochemical matrices	Lipophilic flavonoids (stable)	Flavonoids & periosteal target delivery
Overall strength	Biocompatibility & dual†solubility loading	Superior permeation & phytochemical compatibility	High stability & cost effectiveness	Maximum deformability & deep delivery
Main limitation	Limited deformability	Irritation + formulation sensitivity	Moderate permeation only	Complex optimization required

Conversely, lipophilic flavonoids may be stabilized within the surfactant or phospholipid bilayers of niosomes or transfersomes.[62,33]

This principle is illustrated by preclinical research on apigenin, a bioactive flavonoid, formulated in transfersomes for managing arthritic inflammation.[65] The nano-vesicular delivery system yielded significantly greater skin permeation and synovial bioavailability than a standard gel, correlating with enhanced reduction of inflammatory markers and pain in vivo. This example underscores the capacity of advanced carriers to potentiate the therapeutic profile of phytomolecules.

In conclusion, advancing from rudimentary topical applications to intelligently engineered formulations is paramount for harnessing the fracture-supportive properties of *Barleria cristata*. By selecting a vesicular system whose physicochemical attributes complement the extract's chemical diversity, researchers can significantly improve localized bioavailability.[61,33] This strategy provides a scientifically grounded pathway to translate empirical ethnopharmacological knowledge into a targeted, modern therapeutic intervention for bone healing.

Designing Bioadhesive Hydrogels and Patches for Sustained Release

The paradigm of topical bone therapy is shifting from intermittent application to sustained, controlled delivery, a transition critical for maintaining therapeutically effective concentrations of phytochemicals at the osteogenic site.[9] Bioadhesive hydrogels and transdermal patches represent the vanguard of this approach, engineered to provide prolonged residence time and modulate the release kinetics of *Barleria cristata's* multi-constituent extract, thereby synchronizing drug delivery with the chronological cascade of bone regeneration.

Bioadhesive Hydrogels: Mimicking the Extracellular Matrix Hydrogels are three-dimensional, hydrophilic polymer networks that swell in aqueous environments, creating a hydrated biocompatible interface ideal for topical application.[55] Their capacity for tunable drug release and innate bioadhesion makes them superior to simple creams or ointments.[66] The design strategy involves selecting polymers that provide both mechanical integrity and specific interactive forces with the skin.

The Critical Role of Bioadhesion and Penetration Synergy

Characteristic	Natural Polymer Hydrogels	Synthetic/Semi-Synthetic Hydrogels	Matrix Patches	Reservoir Patches
Materials	Chitosan, alginate, gelatin	Carbomer, poloxamers, HPMC	Silicone, polyisobutylene, Eudragit	Rate-controlling membrane
Release Control	Sustained release, mucoadhesive properties	Precise control over release kinetics	Diffusion through polymer matrix	Zero-order kinetics, constant release rate
Advantages	Biocompatibility, biodegradability, mild gelation	Precise control over rheological properties	Multi-reservoir design possible	Constant release rate, extended period

Fig. 3. Hydrogel and Patch System Comparison

The efficacy of these systems hinges on robust bioadhesion. Strategies to enhance this include:

• Incorporation of tackifiers like polycarbophil or sodium alginate.

• Using crosslinkers (e.g., genipin for chitosan) to modify the cohesive strength and swelling ratio of the hydrogel, thereby controlling release.

• Co-formulation with permeation enhancers (e.g., ethanol, terpenes) within the patch or hydrogel matrix. This creates a powerful synergy: the bioadhesive system maintains intimate contact and provides sustained release, while the permeation enhancers ensure efficient transit of the released compounds across the skin barrier.

A prime real-world exemplar is the Flector® Patch (diclofenac epolamine), a bioadhesive patch that delivers sustained anti-inflammatory action for days. Its success demonstrates the clinical viability of this approach for treating musculoskeletal conditions.[61] For *B. cristata*, a similar patch or hydrogel system could transform its application from a folk remedy requiring frequent reapplication into a modern, once-daily therapeutic that provides a continuous osteogenic stimulus, dramatically improving patient compliance and therapeutic outcomes in fracture management and osteoporosis treatment.

The Future: 3D-Printed Bandages and Smart Scaffolds Loaded with *B. cristata* Extract

Recent advances in topical osteogenic therapy highlight a transition from passive drug delivery systems to intelligent, biomimetic platforms designed to actively coordinate bone repair.[51] The integration of *Barleria cristata* extract into 3D-printed bioadhesive bandages and 4D-printed stimuli-responsive scaffolds exemplifies this approach, offering a bridge between external application and internal tissue engineering. Additive manufacturing enables the development of customized bioadhesive patches with phytochemical-loaded bioinks that ensure anatomical conformity, biocompatibility, and controlled release.[67] Parallel progress in 4D bioprinting has introduced scaffolds capable of responding to the fracture microenvironment, such as pH- or enzyme-sensitive matrices that provide temporally regulated delivery of anti-inflammatory and osteogenic constituents.[68] This dual strategy suggests that *B. cristata* may serve as a valuable component of next-generation regenerative systems, contributing both to localized anti-inflammatory activity and to the sustained promotion of osteogenesis. Such approaches demonstrate the potential of combining phytochemicals with advanced manufacturing technologies to create clinically relevant, patient-specific solutions for bone tissue repair.[51]

Challenges, Safety, and Path to Clinical Use

Dermal Irritation and Toxicology Profile

The transition of *Barleria cristata* from traditional use to a modern, evidence-based topical therapeutic requires rigorous toxicological validation. Despite its favorable ethnobotanical record, contemporary safety evaluation must comply with international guidelines (OECD, ICH) to address risks of dermal irritation, sensitization, and systemic toxicity, particularly during prolonged use on compromised skin.[69] Preclinical assessments should include in vitro reconstructed human epidermis models (OECD TG 439) for irritation,[57] in vivo sensitization assays such as the murine Local Lymph Node Assay (OECD TG 429), and confirmatory clinical studies such as the Human Repeat Insult Patch Test. For extracts demonstrating significant transdermal absorption, systemic toxicology is critical, encompassing toxicokinetics of key phytochemicals (e.g., verbascoside, barlerin) and repeated-dose toxicity studies (28- and 90-day, OECD TG 407/408) in relevant species to define the No Observed Adverse Effect Level. Standardization is essential to ensure batch-to-batch consistency, with defined marker compounds and strict contaminant control in accordance with ICH,[58,70] and USP guidelines. A useful precedent is the development of topical capsaicin, where successful translation hinged on extract standardization, robust safety testing, and recognition of transient local irritation as an acceptable effect. Similarly, the development of a standardized *B. cristata* extract, coupled with a systematic toxicological program, is indispensable for de-risking clinical translation and establishing its regulatory and therapeutic viability.

Standardization of Extracts and Dose Optimization for Topical Use

The translation of a complex botanical extract from a traditional preparation to a modern, reproducible pharmaceutical necessitates overcoming two paramount challenges: the

establishment of rigorous quality control through standardization and the determination of a therapeutically effective yet safe dosage regimen. For *Barleria cristata*, this process is complicated by its phytochemical complexity, the synergistic interplay of its constituents, and the unique pharmacokinetics of topical delivery. Addressing these challenges is a non-negotiable prerequisite for clinical validation and regulatory approval.[71,72]

The successful translation of *Barleria cristata* from traditional medicine to a standardized topical osteogenic therapeutic depends on two critical requirements: rigorous phytochemical standardization and the establishment of a safe, effective dosage regimen. Standardization is essential to address the inherent variability of botanical extracts, necessitating a multi-marker approach that quantifies key osteogenic constituents (e.g., verbascoside, apigenin) while preserving the full phytochemical fingerprint through validated chromatographic and chemometric methods.[71] Equally important is dosage optimization, which for topical delivery shifts the focus from systemic plasma levels to maximizing local bioavailability at fracture sites while minimizing systemic exposure.[9] This requires ex vivo permeation studies, in vivo pharmacodynamic evaluation in bone defect models, and dose–response analyses to define the therapeutic window. Integrating these processes within a Quality by Design (QbD) framework, supported by Design of Experiments (DoE), enables systematic identification of critical quality attributes, process parameters, and a robust design space for reproducible, scalable manufacturing.[70,73] Collectively, this approach ensures pharmaceutical consistency, regulatory compliance, and clinically relevant efficacy, providing a scientifically rigorous pathway for the development of *B. cristata* as a modern bone regenerative agent.

Regulatory Pathways and Designing Pilot Clinical Trials

The successful clinical translation of a topical *Barleria cristata* osteogenic formulation requires navigating a complex regulatory pathway that integrates botanical drug, topical product, and orthopedic therapeutic considerations.[72,74] Regulatory strategy must align with FDA and EMA botanical guidelines, emphasizing rigorous standardization of the raw material and extract to ensure consistent quality and reproducibility.[70,71] While its history of traditional use may provide a supportive rationale, full preclinical toxicology remains mandatory.[69] A phase-appropriate clinical development plan should initiate with Phase 0 microdosing studies to characterize local biodistribution, followed by a Phase I trial in healthy volunteers to establish dermal safety and assess systemic exposure.[75,76] Proof-of-concept would then be demonstrated in a targeted Phase II study involving high-risk fracture patients, utilizing validated radiographic endpoints such as the RUST score[77,78]. ìCT-based structural analysis,[56] and clinical union times.[78-81] This deliberate, stepwise approach—combining stringent quality control, phased clinical evaluation, and objective efficacy metrics—provides a viable pathway to transform this ethnobotanical agent into an evidence-based therapy for bone regeneration.

DISCUSSION

The investigation of *Barleria cristata* L. for topical application in fracture management exemplifies a structured approach to translating ethnobotanical knowledge into a testable therapeutic hypothesis. Preclinical analyses indicate that its phytochemical profile—comprising anti-inflammatory iridoids, antioxidant phenylethanoid glycosides, and flavonoids—engages multiple targets relevant to the bone repair cascade, as outlined in Table 2. This multi-constituent activity, observed primarily in cellular and animal models, may offer advantages for localized modulation compared with systemic administration, which often affects skeletal homeostasis broadly.

The primary obstacle to therapeutic application remains the skin barrier. While traditional preparations provide historical context, they lack the reproducibility and controlled release required for modern pharmacotherapy. As detailed in Section 5, advanced delivery systems—particularly vesicular carriers like ethosomes or transfersomes integrated into bioadhesive matrices—are likely to be important for enhancing permeation and sustained local retention of the extract's diverse constituents. The success of these formulation strategies will largely determine whether pharmacologically relevant concentrations can be achieved at or near the periosteum.

It is critical to emphasize that the current

evidence is derived from preclinical studies. The promising outcomes in animal models, summarized in Table 3, justify further investigation but do not confirm clinical efficacy. Translating this potential requires a methodical, two-pronged strategy: first, the pharmaceutical development of a standardized extract within an optimized delivery system, and second, the design of controlled clinical trials with validated radiographic and functional endpoints. Only through this rigorous, evidence-based pathway can the traditional use of *B. cristata* be systematically evaluated for a potential supportive role in fracture care.

CONCLUSION AND FUTURE PERSPECTIVES

This comprehensive review synthesizes evidence bridging the ethnobotanical use of *Barleria cristata* L. with contemporary scientific inquiry into its potential role in supporting bone repair. The plant's historical application in traditional poultices for fractures finds a plausible mechanistic basis in its complex phytochemistry, which includes iridoids, phenylethanoid glycosides, and flavonoids. Laboratory and preclinical studies suggest these constituents may collectively influence key stages of the healing cascade, from modulating inflammatory responses via pathways such as NF-êB to potentially supporting bone repair and vascularization processes. Preliminary evidence from animal models indicates that formulated extracts of *B. cristata* may be associated with improvements in radiographic and histological metrics of bone repair and could contribute to enhanced biomechanical outcomes. However, it is critical to emphasize that direct, conclusive evidence of confirmed bone-repair efficacy in humans from topical *B. cristata* application is not yet available, and the cited preclinical findings require robust clinical validation.

Realizing the translational potential of this ethnobotanical resource necessitates a methodical future trajectory. Paramount challenges include the standardization of extracts to ensure batch-to-batch reproducibility and the development of advanced topical formulations (e.g., nano-vesicular systems, bioadhesive hydrogels) designed to overcome the skin barrier and provide controlled local delivery. Ultimately, a structured regulatory pathway involving phase-appropriate clinical trials with validated orthopedic endpoints is essential. Such research will determine whether the promising supportive potential of *Barleria cristata*, informed by traditional knowledge and preliminary science, can be translated into a safe, evidence-based topical adjunct for fracture management.

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This research did not involve human participants, animal subjects, or any material that requires ethical approval.

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This study did not involve human participants, and therefore, informed consent was not required.

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This research does not involve any clinical trials.

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Author Contribution

Anisha M. Bagade: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft; Mujahid S. Patel: Data curation, Formal analysis, Writing – review & editing; Vasim T. Pathan: Literature review, Visualization, Writing – review & editing; Nilesh A. Karande: Resources, Validation, Writing – review & editing; Atul R. Bendale: Supervision, Interpretation of data, Critical revision of the manuscript; Anil G. Jadhav: Project administration, Supervision, Final approval of the manuscript.

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