Review Article

Shikonin-Rich *Arnebia benthamii*: Evidence Synthesis on Phytochemistry, Bioactivity, and Critically Endangered Status in the Northwestern Himalayas

Lubna Andleeb, Saduf Nisar, Faisal Mushtaq, Peerzada Arshid Shabir, Sihbaan Majeed, Sadaf Rasool
Department of Botany, Govt. College for Women M.A. Road Srinagar, Jammu and Kashmir, India.
***Corresponding author's E-mail:** peerzadarshid@gmail.com

Received: 02-07-2026; **Revised:** 25-09-2026; **Accepted:** 30-09-2026; **Published online:** 20-10-2026.

ABSTRACT

Arnebia benthamii (Boraginaceae), a high-altitude Himalayan herb, is traditionally used as "Ratanjot" for fever, wounds, inflammation and cardiac conditions. This review synthesises published evidence on its botany, ethnopharmacology, phytochemistry, pharmacology, safety and conservation status. The roots contain the naphthoquinone pigment shikonin and its derivatives, and extracts of the plant show antioxidant, antibacterial, cytotoxic and antidepressant-like activity in experimental studies, whereas anti-inflammatory, wound-healing, anti-diabetic, anticancer and antiviral effects are documented mainly for shikonin and for *Arnebia euchroma*. Aerial parts, commonly used in decoctions, contain the hepatotoxic pyrrolizidine alkaloids europine, heliotrine, lycopsamine and echimidine, and one rodent study reported aggravation of chemically induced hepatotoxicity. The species is described as critically endangered in the Kashmir Himalaya because of overexploitation and a restricted habitat. Sustainable use requires standardised, alkaloid-tested preparations, toxicological and clinical studies, *in vitro* propagation and community-based conservation.

Keywords: *Arnebia benthamii*, shikonin, pyrrolizidine alkaloids, hepatotoxicity, critically endangered, conservation biology.

1. INTRODUCTION

The Northwestern Himalayas, recognized as a global biodiversity hotspot, host a rich diversity of high-altitude medicinal plants adapted to extreme conditions such as intense UV radiation, low temperatures and hypoxia, many of which are threatened by harvesting.[1,2] These conditions favour complex secondary metabolism. Within this region, the genus *Arnebia* Forssk. (Boraginaceae), comprising approximately 30 species,[3,4] holds ethnobotanical value across traditional Asian medicinal systems for its red-purple root pigments, which are naphthoquinones, and its wide-ranging therapeutic applications.[5,6]

Arnebia benthamii (Wall. ex G. Don) I.M. Johnst. is a perennial herb of alpine and sub-alpine slopes of the North-West Himalaya (Jammu & Kashmir, Himachal Pradesh, Uttarakhand and Pakistan), distinguished by its phytochemical richness and precarious conservation status.[7-9] In traditional medicine *A. benthamii* is known locally as "Ratanjot", "Laljari", "Kahzaban" or "Gao-zaban".[3,10] Its thick roots exude a red or purplish dye, and the plant is used for fever, kidney and liver problems in the Neelum Valley of Azad Kashmir,[3,11] and for jaundice, cough, fever, blood purification and chronic constipation in Indian Kashmir.[12] Whole plants, aerial parts and leaves are used mainly as oral decoctions,[3] the flowers are reported to have a comforting effect on heart patients, and the roots antiseptic and antibiotic properties.[8]

Experimental studies have begun to substantiate some of these traditional claims. Extracts of *A. benthamii* show radical-scavenging, DNA-protective, antibacterial and cytotoxic activity,[7,10,13,14] and shikonin has been identified and quantified in the roots by chromatographic methods.[14,15] Phytochemical screening of aerial and root extracts indicates alkaloids, anthraquinones, flavonoids and phenolic compounds, with composition depending on plant part and solvent.[10,16,17]

Despite its therapeutic potential, *A. benthamii* is described as critically endangered in the Kashmir Himalaya and is listed in the Indian Red Data Book because of overexploitation, a restricted habitat and difficult regeneration.[8,9,18] Seed germination and in vitro propagation have been investigated as conservation tools,[8,19,20] but genetic data on natural populations remain limited, which constrains conservation planning.

Prior reviews on *Arnebia* species have focused mainly on *A. euchroma*,[6,21] and a genus-level chapter covers *A. benthamii* only briefly,[22] giving limited attention to safety issues such as the presence of hepatotoxic pyrrolizidine alkaloids (PAs) in aerial parts.[3] This review addresses these gaps by integrating traditional ethnomedicinal knowledge with analytical and bioassay data, drawing on the peer-reviewed literature up to December 2025. We distinguish evidence obtained on *A. benthamii* itself from evidence extrapolated from shikonin or from *A. euchroma*, identify knowledge gaps (e.g., quantitative PA content, in vivo pharmacology and clinical data), and propose a framework for sustainable development combining in vitro propagation and community-based conservation. Through this approach, we aim to facilitate the responsible translation of *A. benthamii* from an endangered species to standardised phytopharmaceuticals or nutraceuticals.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

2. MATERIALS AND METHODS

This review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure a comprehensive, reproducible and unbiased synthesis of the existing literature on *Arnebia benthamii*.[23]

Information was gathered from major scientific databases, including Scopus, Web of Science, PubMed/MEDLINE, and ScienceDirect. The search was performed using the keywords "*Arnebia benthamii* ", "Ratanjot", "Laljari", "shikonin", and related phytochemical and pharmacological terms. The search period encompassed literature from 1990 to December 2025. No language restrictions were initially applied, though non-English articles without accessible abstracts or key data in English were excluded. This exclusion criterion may introduce language bias, as it potentially overlooks valuable non-English literature; future reviews should incorporate multilingual databases such as CNKI to capture Asian-language studies. Additional relevant studies were identified from the reference lists of retrieved articles and specialized regional databases.

A total of 1245 records were initially identified up to December 2023. After removing 370 duplicates, 875 unique records were screened by title and abstract, resulting in the exclusion of 720 records that were irrelevant or did not meet preliminary criteria. Subsequently, 155 full-text articles were assessed for eligibility. Of these, 85 were excluded for the following reasons: 40 were reviews without novel data, 30 focused on other Arnebia species without separate A. benthamii results, and 15 had insufficient methodological detail. Thus, 70 peer-reviewed articles met the inclusion criteria (Figure 1).

Studies were included if they provided original data on the botany, traditional use, phytochemistry, pharmacology, or conservation of A. benthamii. To ensure quality, studies were evaluated using the Newcastle-Ottawa Scale (NOS) for observational studies and SYRCLE's risk of bias tool for animal intervention studies. Only studies scoring moderate to high quality (NOS ≥6/9; SYRCLE low risk in ≥5 domains) were retained for synthesis.

The selected studies were categorized by theme (botany, ethnopharmacology, phytochemistry, pharmacology, conservation) using NVivo software for qualitative data analysis. Data were extracted, tabulated, and synthesized narratively to provide a state-of-the-art overview.

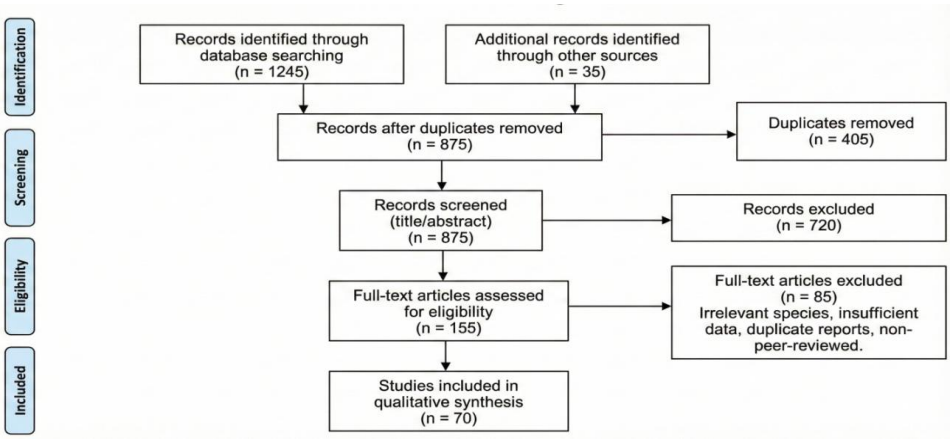


Figure 1: PRISMA flow diagram of the study selection process.

3. RESULTS

3.1.1 Taxonomic Position and Nomenclature

Arnebia benthamii (Wallich ex G. Don) I.M. Johnston is classified within the family Boraginaceae Juss., tribe Lithospermeae Dumort. It was first described as *Echium benthamii* Wall. ex G. Don (Gen. Hist. 4: 333, 1837)[24] and combined in *Arnebia* by Johnston (J. Arnold Arbor. 35: 56, 1954). Synonyms include *Lithospermum benthamii* (Wall. ex G. Don) I.M. Johnst., *Macrotomia benthamii* (Wall. ex G. Don) DC. ex Meisn. and *Leptanthe macrostachya* Klotzsch.[25,26] *Arnebia* and related genera of Lithospermeae have been analysed using micromorphology, plastid genomes and DNA sequence data,[4,27,28] and the Indian species of *Arnebia* have been revised taxonomically.[29]

3.1.2. Botanical Description and Habitat Ecology

A. benthamii is a monocarpic perennial herb up to about 80 cm tall with a swollen base and thick roots that exude a red or purplish dye; the simple, hollow stem arises from a cluster of large basal leaves covered with long hairs.[3] Detailed morphological descriptions are available in taxonomic revisions of Indian *Arnebia*.[29]

A. benthamii is an alpine and sub-alpine species of the North-West Himalaya. Plants used in experimental studies were collected at Sinthan Top, Kashmir (3748 m a.s.l.)[8] and in the Neelum Valley (2313 m a.s.l.),[3] illustrating a wide altitudinal occurrence that should be documented more precisely for each population.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

3.1.3. Geographical Distribution

A. benthamii exhibits a fragmented distribution in the Northwestern Himalayas. In India it has been recorded in Jammu & Kashmir (including Gurez, Anantnag, Doda, Kargil and Ladakh), Himachal Pradesh (including Kinnaur, Spiti and Chamba) and Uttarakhand (including Badrinath, Gangotri and Milam);[9] in Pakistan it occurs in the Neelum and Leepa valleys of Azad Kashmir, Dir and Hazara,[3,11] and its original global distribution includes India, Afghanistan, Pakistan and Nepal.[9] Occurrence records are available from GBIF (Figures 2 and 3).[30] This restricted range contributes to its threatened status.

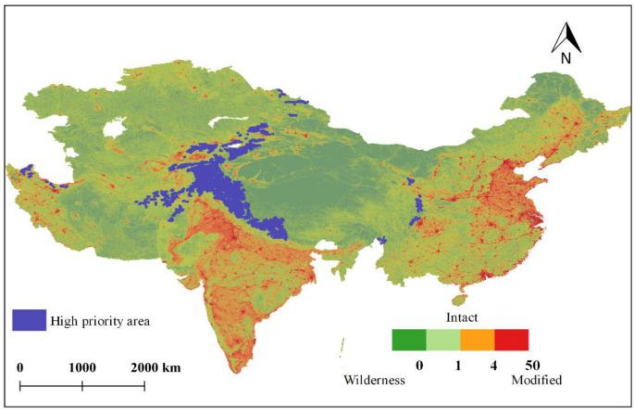


Figure 2: Global distribution of *Arnebia benthamii* based on GBIF occurrence data, showing concentrations in the Western Himalayas (India, Pakistan, Afghanistan).

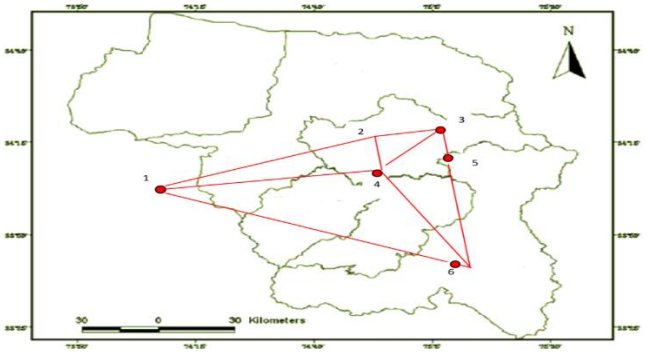


Figure 3: Detailed distribution map of *Arnebia benthamii* populations in the Western Himalayas, highlighting fragmented sites in Jammu & Kashmir, Himachal Pradesh, and northern Pakistan.

3.1.4. Ethnopharmacological Significance

A. benthamii, known as "Ratanjot", "Laljari", "Kahzaban" or "Gao-zaban", is used in Himalayan ethnomedicine (Table 1). In an interview survey of 30 respondents in the Neelum Valley (Azad Kashmir), fever, kidney and liver problems were the three principal uses; aerial parts were the part most often preferred (43% of respondents), followed by whole plants and leaves, and decoction was the main preparation.[3] Earlier surveys in the Neelum and Leepa valleys recorded leaf and flower juice and root decoction for navel pain, fever, burning urine and thirst,[11,31] and a survey in Bandipora (Indian Kashmir) recorded use for jaundice, cough, fever, blood purification and chronic constipation.[12] Quantitative indices such as use value and fidelity level have not been reported for this species.

Table 1: Ethnomedicinal Uses of *Arnebia benthamii* Across Himalayan Communities.

Community / Region	Local Name	Plant Part Used	Preparation & Administration	Therapeutic indication	Use Value (UV)	Relative Freq. of Citation (RFC)	Reference(s)
Neelum Valley, Azad Kashmir	Gao-zaban	Aerial parts (most preferred), whole plant, leaves	Decoction, oral (2–3 teaspoons of powder in 250–350 mL boiling water, sometimes with honey or sugar)	Fever, kidney and liver problems	Not reported	Not reported	3
Neelum and Leepa valleys, Azad Kashmir (earlier surveys)	Not reported	Leaves and flowers (juice); root (decoction)	Oral	Navel pain, fever, burning urine, thirst	Not reported	Not reported	11,31
Bandipora district, Jammu & Kashmir	Not reported	Not specified	Not specified	Jaundice, cough, fever, blood purification, chronic constipation	Not reported	Not reported	12

3.2. Phytochemical Profile

Phytochemical screening of aerial and root extracts of *A. benthamii* indicates alkaloids, anthraquinones, flavonoids and phenolic compounds, with differences between plant parts and solvents.[10,16,17] The characteristic red pigments of the roots are naphthoquinones, chiefly shikonin and its esters, which have been determined by chromatographic

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

methods in this species.[14,15] Phenolic compounds and flavonoids contribute to the radical-scavenging properties of the extracts.[7,8,13]

3.2.1. Naphthoquinones and Their Biosynthesis

Naphthoquinones are the signature class of *Arnebia* roots. Shikonin and β,β-dimethylacrylshikonin have been quantified in *A. benthamii* by normal-phase HPTLC,[15] and the biological efficacy and radical-scavenging potential of shikonin from this species have been demonstrated.[14] Other shikonin derivatives reported for *Arnebia* include acetylshikonin, deoxyshikonin, β-hydroxyisovalerylshikonin, teracrylshikonin, isovalerylshikonin and arnebin-7.[5,10] Extractable phytochemicals differ markedly with solvent and plant part in *A. benthamii*,[16] so standardised extraction protocols are needed. Quantitative comparison with *A. euchroma* and the effect of altitude on shikonin content have not been established for *A. benthamii*.

Shikonin is biosynthesised from p-hydroxybenzoic acid (shikimate/phenylpropanoid pathway) and geranyl pyrophosphate (mevalonate pathway), which condense to 3-geranyl-4-hydroxybenzoic acid before cyclisation and hydroxylation steps (Figure 4).[5] Elicitation with fungal extracts has been used to stimulate shikonin production in cell suspension cultures of *A. euchroma*,[32] and in vitro culture with plant growth regulators has been applied to *A. benthamii*.[8]

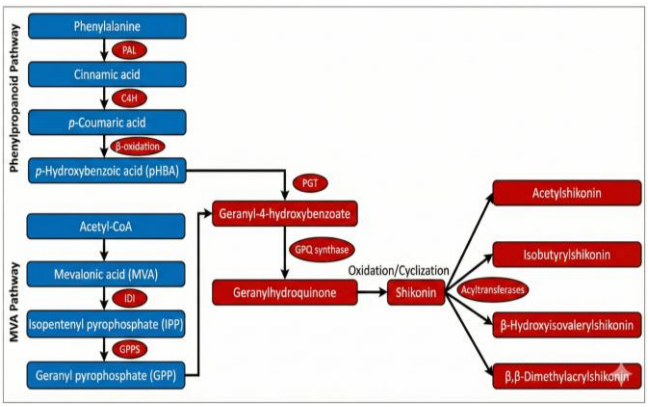


Figure 4: Biosynthetic pathway of shikonin in *Arnebia benthamii*

3.2.2. Phenolic Compounds and Flavonoids

Aerial parts and roots of *A. benthamii* are rich in phenolic compounds and flavonoids; the total phenolic content of methanolic aerial-part and root extracts was about 200 and 210 µg gallic acid equivalents per mg dry weight, respectively.[10] Phenolic acids such as rosmarinic acid and flavonoid glycosides are typical constituents of *Arnebia* species.[6]

Phenolic compounds arise from the phenylpropanoid pathway and contribute to antioxidant activity through radical scavenging and metal chelation. Methanol, ethyl acetate, ethanol and aqueous extracts of *A. benthamii* scavenge DPPH and hydroxyl radicals in vitro.[7,13]

3.2.3. Hepatotoxic Pyrrolizidine Alkaloids (PAs) — A Critical Safety Profile

A significant safety concern is the presence of pyrrolizidine alkaloids (PAs) in *A. benthamii*. HPLC analysis of aerial parts collected in the Neelum Valley (Azad Kashmir) identified four hepatotoxic PAs: europine, heliotrine, lycopsamine and echimidine (Table 2).[3] That study was qualitative and did not report concentrations or root content, and PAs have also been detected in roots of *A. euchroma*.[33] The PA content of *A. benthamii* roots and aerial parts should therefore be quantified before either is considered safe.

PAs with a 1,2-unsaturated necine base are bioactivated in the liver by cytochrome P450 enzymes to reactive pyrrolic esters, which alkylate DNA and proteins and cause hepatocyte necrosis, hepatic veno-occlusive disease (HVOD) and genotoxicity.[34] Heliotrine is a heliotridine-type PA associated with chromosome damage and hepatic carcinogenicity, and lycopsamine has been reported to be hepatotoxic.[3] European regulatory limits for herbal products containing PAs are very low (a transitional limit of 1 µg/day set by the European Medicines Agency, and 0.007 µg/kg body weight per day), so regular consumption of aerial-part decoctions needs to be assessed against them; the authors of the survey recommended that the plant be excluded from local markets until PA levels are tested.[3] PAs have also been reported in *A. euchroma* and *A. guttata*,[35] so a quantitative cross-species comparison is needed to establish species-specific safety profiles.

Table 2: Pyrrolizidine alkaloids detected in *Arnebia benthamii* aerial parts.

PA	Detection	Retention time (min)	Plant material	Reference
Europine	Detected (HPLC-UV, 280 nm)	10.21	Aerial parts, Neelum Valley	3
Heliotrine	Detected (HPLC-UV, 280 nm)	10.77	Aerial parts, Neelum Valley	3
Lycopsamine	Detected (HPLC-UV, 280 nm)	37.16	Aerial parts, Neelum Valley	3
Echimidine	Detected (HPLC-UV, 280 nm)	46.64	Aerial parts, Neelum Valley	3

3.3. Pharmacological Activities and Molecular Mechanisms

Pharmacological investigations of *A. benthamii* remain limited. Extracts show antioxidant, DNA-protective, antibacterial and cytotoxic effects in vitro,[7,10,13,14] and an aqueous root extract showed antidepressant-like activity in rats.[36] Evidence for anti-inflammatory, wound-healing, anti-diabetic, anticancer and antiviral activity comes largely from studies on shikonin or on *A. euchroma* and is summarised in Table 3, with the source of each finding made explicit.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Table 3: Reported pharmacological activities of *Arnebia benthamii* extracts and of shikonin/related *Arnebia* preparations.

Activity	Key Active Principle	Model / Assay	Potency / Outcome	Evidence base	Reference
Antioxidant	Crude extracts; shikonin	DPPH and hydroxyl-radical scavenging; DNA protection (in vitro)	Radical scavenging; protection of DNA from hydroxyl radical damage	*A. benthamii* (in vitro)	7,13,14
Antimicrobial	Methanol extracts; naphthazarins	Antibacterial assays	Antibacterial activity of extracts; antimicrobial naphthazarins from *A. euchroma*	*A. benthamii* (in vitro); *A. euchroma*	10,13,37
Anti-inflammatory	Shikonin; hydroxynaphthoquinone fraction	LPS-induced lung injury (mouse); osteoarthritis (rat); experimental arthritis	Reduced inflammation via NF-κB and PI3K/Akt signalling	Shikonin / *A. euchroma* (not tested in *A. benthamii*)	38-40
Wound healing	*A. euchroma* preparations	Rat excisional wounds; clinical trial in second-degree burns	Accelerated healing	*A. euchroma*	41,42
Anti-diabetic / anti-obesity	Shikonin; *A. euchroma* constituents	3T3-L1 adipocytes; FFA4 receptor assays	Reduced fat accumulation; FFA4 agonism	Shikonin / *A. euchroma*	43,44
Anticancer / cytotoxic	Shikonin and derivatives; *A. benthamii* extracts	Cancer cell lines	Apoptosis induction (ROS, MAPK, NF-κB); cytotoxicity of extracts	Shikonin (reviews); *A. benthamii* (in vitro)	7,45,46
Antiviral	Caffeic acid tetramers	Anti-HIV assays	Anti-HIV activity	*A. euchroma*	47

3.3.1. Antioxidant and Cytotoxic Activity

Methanol, ethyl acetate, ethanol and aqueous extracts of *A. benthamii* scavenge free radicals, including hydroxyl radicals, in vitro,[7,13] and shikonin from the roots scavenges radicals and protects DNA from hydroxyl radical damage.[14] Cytotoxic activity of the extracts has also been reported.[7] These findings give a plausible basis for traditional use in fever and inflammatory conditions, but in vivo antioxidant studies on *A. benthamii* are lacking.

No study demonstrating hepatoprotection by *A. benthamii* could be verified. In contrast, *A. benthamii* aggravated potassium dichromate-induced hepatotoxicity in Wistar rats,[48] and aerial parts contain hepatotoxic PAs.[3] Claims of hepatoprotection therefore require cautious interpretation.

3.3.2. Antimicrobial Activity

Methanolic extracts of aerial parts and roots of *A. benthamii* show antibacterial activity,[10,13] and naphthazarin pigments from *A. euchroma*, including shikonin derivatives, are antimicrobial.[37] Activity of *A. benthamii* against multidrug-resistant pathogens or biofilms, and nanoparticle formulations, were not found in the verified literature and remain open research topics.

3.3.3. Anti-inflammatory Activity of Shikonin

Shikonin has been reported to reduce inflammation in several models, including NF-κB-dependent acute lung injury in mice[38] and chondrocyte inflammation through PI3K/Akt signalling in a rat osteoarthritis model,[39] and a hydroxynaphthoquinone fraction of *A. euchroma* was active in experimental arthritis.[40] The pharmacology of shikonin has been reviewed.[49] Whether these effects occur with *A. benthamii* preparations has not been tested (Figure 5).

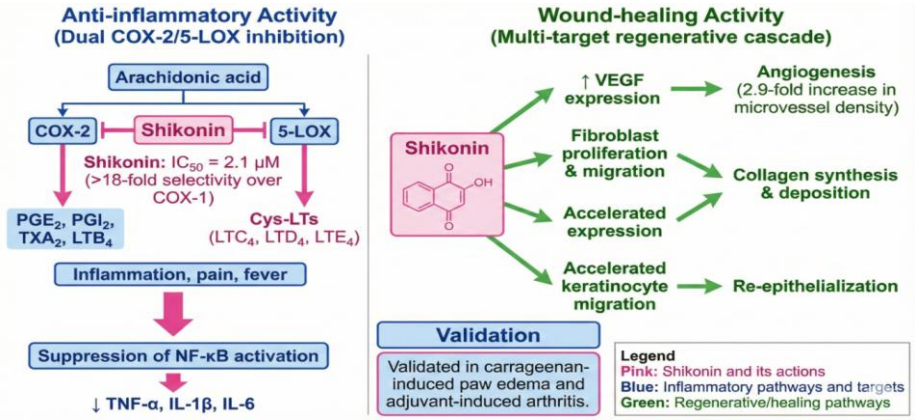


Figure 5: Proposed molecular mechanism of shikonin's anti-inflammatory and wound-healing action.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

3.3.4. Wound-Healing and Tissue-Regenerative Potential

Arnebia euchroma preparations accelerated the healing of excisional wounds in rats[41] and of second-degree burn wounds in a randomized clinical trial.[42] These data support the traditional use of "Ratanjot" for wounds and burns, but equivalent studies on *A. benthamii* are needed.

3.3.5. Anti-diabetic and Anti-obesity Potential

Shikonin inhibits fat accumulation in 3T3-L1 adipocytes,[43] and constituents of *A. euchroma* activate the free fatty acid receptor FFA4 with anti-diabetic activity.[44] These findings suggest metabolic effects of shikonin-type compounds, but no verified study has tested *A. benthamii* in diabetic models.

3.3.6. Anticancer Activity of Shikonin and Derivatives

Shikonin and its derivatives have been extensively investigated as anticancer agents and have been reviewed.[45,46] Extracts of *A. benthamii* showed cytotoxic activity in vitro.[7]

Mechanisms of apoptosis reported for shikonin in cancer cells:[45,46]

1. ROS Burst: Shikonin rapidly induces a cytotoxic surge of intracellular ROS.

2. MAPK Activation: This is followed by sustained activation of stress kinases c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK).

3. Mitochondrial Pathway: Leads to the dysregulation of Bcl-2 family proteins (increased Bax, decreased Bcl-2), resulting in mitochondrial membrane depolarization, cytochrome c release, and caspase-3 activation.

4. NF-κB Inhibition: Concurrently, shikonin inhibits the pro-survival nuclear factor-kappa B (NF-κB) signalling pathway.[38,45] An aqueous root extract of *A. benthamii* also showed antidepressant-like activity in rats.[36]

3.3.7. Antiviral Activity

Anti-HIV activity has been reported for caffeic acid tetramers from *A. euchroma*.[47] No verified antiviral study on *A. benthamii* itself was identified.

3.3.8. Hepatic Safety Considerations

Roots and aerial parts of *A. benthamii* have not been compared for PA content or hepatotoxicity. Hepatotoxic PAs were detected in aerial parts,[3] aerial parts are the plant part most preferred for local decoctions,[3] and an earlier rat study reported aggravation of chemically induced hepatotoxicity.[48] Any clinical development should therefore start with standardised extracts tested for PAs and with formal toxicity studies.

3.4 Conservation Biology

A. benthamii is described as critically endangered in the Kashmir Himalaya and is listed in the Indian Red Data Book.[8,10] The FRLHT regional assessment places Jammu & Kashmir and Himachal Pradesh in its highest population-reduction category (≥80% over 10 years).[9] Overexploitation for medicinal use is the main threat.[8,18] Seeds of *A. benthamii* have no chilling requirement for germination[20] and treatments to improve germination have been tested,[19] and in vitro propagation on MS medium with plant growth regulators has been established, with phytochemical and DNA-protection evaluation of the plantlets,[8] offering an alternative to wild harvest. Conservation priorities for medicinal plants in the Indian Himalaya, including community involvement, have been discussed,[1,2] and climate-change effects are being studied in the related *A. euchroma*.[50,51]

4. DISCUSSION

This section synthesises the findings, distinguishing evidence generated on *A. benthamii* from evidence extrapolated from shikonin or *A. euchroma*. Overall, the evidence for *A. benthamii* is limited and preliminary: it consists mainly of in vitro antioxidant, antimicrobial and cytotoxic assays, phytochemical screening, one qualitative PA analysis and a small number of rodent studies, whereas clinical evidence is absent.

4.1 Botanical and Ethnopharmacological Insights

The nomenclature of *A. benthamii* is settled (*Echium benthamii* Wall. ex G. Don → *Arnebia benthamii*),[24,25] but its placement within *Arnebia* has been addressed only in broader molecular studies of the genus.[4,27,28] Distribution records indicate a fragmented range in Jammu & Kashmir, Himachal Pradesh, Uttarakhand and Pakistan.[3,9] Ethnopharmacological information comes mainly from small interview surveys (e.g., 30 respondents in the Neelum Valley), with potential recall bias and without quantitative indices of consensus;[3] quantitative ethnobotanical studies are needed.

4.2 Phytochemical Profile: Strengths and Safety Concerns

Shikonin and β,β-dimethylacrylshikonin are confirmed in *A. benthamii*,[14,15] but comparative quantitative data against *A. euchroma* and altitude-related variation are lacking. Phenolic compounds and flavonoids contribute to antioxidant activity.[7,10,13] The main safety concern is PA content: hepatotoxic PAs were detected in aerial parts[3] and cause hepatic veno-occlusive disease.[34] PAs have also been found in other *Arnebia* species,[33,35] so quantitative, species- and plant-part-specific analysis is a priority.

4.3 Pharmacological Activities: Evidence Evaluation and Mechanisms

Antioxidant, antimicrobial and cytotoxic activities of *A. benthamii* extracts are supported by in vitro studies,[7,10,13,14] and an antidepressant-like effect by one rat study.[36] Anti-inflammatory, wound-healing, anti-diabetic, anticancer and antiviral activities are known from shikonin and *A. euchroma* studies[41-45,47,49] but require confirmation in *A. benthamii*. Hepatoprotection is not supported, and an adverse hepatic effect was reported.[48] Most findings come from crude, non-standardised extracts, and clinical evidence is absent.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

4.4 Conservation Implications and Framework

A. benthamii is described as critically endangered in the Kashmir Himalaya, with regional population reductions of 80% or more reported by FRLHT.[8,9] Overharvesting and a restricted habitat threaten the species.[8,18] Ex situ approaches include seed-germination protocols[19,20] and in vitro propagation.[8] In situ monitoring, community involvement and cultivation should be combined with PA-tested, standardised preparations for sustainable use.[1,2] Socio-economic analyses of trade and ecological niche modelling for climate adaptation remain to be done.

Overall, substantiation of the ethnomedicinal claims for *A. benthamii* is preliminary. Clinical trials, toxicological evaluation (including PAs) and integrated conservation are priorities for sustainable use of this species. Future directions include well-controlled studies of standardised *A. benthamii* extracts in wound, inflammation and metabolic models, and socio-ecological modelling to reconcile traditional use with biodiversity conservation.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Kala CP. Indigenous uses, population density, and conservation of threatened medicinal plants in protected areas of the Indian Himalayas. Conserv Biol. 2005;19(2):368-378.
2. Hamilton AC. Medicinal plants, conservation and livelihoods. Biodivers Conserv. 2004;13(8):1477-1517.
3. Ahmad L, He Y, Hao JC, Semotiuk A, Liu QR, Mazari P. Toxic pyrrolizidine alkaloids provide a warning sign to overuse of the ethnomedicine *Arnebia benthamii*. J Ethnopharmacol. 2018;210:88-94. Doi: 10.1016/j.jep.2017.08.009;
4. Coppi A, Cecchi L, Nocentini D, Selvi F. Arnebia purpurea: a new member of formerly monotypic genus Huynhia (Boraginaceae-Lithospermeae). Phytotaxa. 2015;204(2):123-136
5. Papageorgiou VP, Assimopoulou AN, Couladouros EA, Hepworth D, Nicolaou KC. The chemistry and biology of alkannin, shikonin, and related naphthazarin natural products. Angew Chem Int Ed Engl. 1999;38(3):270-300.
6. Hosseini A, Mirzaee F, Davoodi A, Jouybari HB, Azadbakht M. The traditional medicine aspects, biological activity and phytochemistry of Arnebia spp. Med Glas. 2018;15(1).
7. Ganie SA, Dar TA, Hamid R, Zargar O, UlAbeer S, Masood A, et al. In vitro antioxidant and cytotoxic activities of *Arnebia benthamii* (Wall ex. G. Don): a critically endangered medicinal plant of Kashmir valley. Oxid Med Cell Longev. 2014;2014:792574. Doi: 10.1155/2014/792574;
8. Parray JA, Kamili AN, Jan S, Mir MY, Shameem N, Ganai BA, et al. Manipulation of plant growth regulators on phytochemical constituents and DNA protection potential of the medicinal plant *Arnebia benthamii*. Biomed Res Int. 2018;2018:6870139. Doi: 10.1155/2018/6870139
9. FRLHT ENVIS Centre. *Arnebia benthamii* (Wall. ex G.Don) Johns. Threatened taxa database [Internet]. Bengaluru: Foundation for Revitalisation of Local Health Traditions. Available from: https://envis.frlht.org/tds/225_6.htm
10. Shameem N, Kamili AN, Parray JA, Hamid R, Bandh SA. Antimicrobial and antioxidant activity of methanol extracts of *Arnebia benthamii* (Wall ex. G. Don) Johnston - a critically endangered medicinal plant of North Western Himalaya. J Anal Sci Technol. 2015;6:1-8. Doi: 10.1186/s40543-015-0076-z.
11. Mahmood A, Mahmood A, Malik RN. Indigenous knowledge of medicinal plants from Leepa valley, Azad Jammu and Kashmir, Pakistan. J Ethnopharmacol. 2012;143(1):338-346.
12. Lone PA, Bhardwaj AK, Bahar FA. A study of some locally available herbal medicines for the treatment of various ailments in Bandipora district of J & K, India. Int J Trad Nat Med. 2013;2(3):164-178.
13. Ganie SA, Jan A, Muzaffar S, Zargar BA, Hamid R, Zargar MA. Radical scavenging and antibacterial activity of *Arnebia benthamii* methanol extract. Asian Pac J Trop Med. 2012;5(10):766-772. Doi: 10.1016/S1995-7645(12)60140-0.
14. Parray JA, Hamid R, Kamili AN, Shameem N, Jan S, Ganai BA. Biological efficacy and radical scavenging potential of shikonin in *Arnebia benthamii* (Wall ex. G. Don) Johnston. Ind Crops Prod. 2015;74:434-439. Doi: 10.1016/j.indcrop.2015.04.040.
15. Katoch P, Rana S, Kumar D, Kumar S, Bhushan S. Concurrent NP-HPTLC determination of shikonin and beta,beta-dimethylacryl shikonin in *Arnebia benthamii*. J Chromatogr Sci. 2016;54(8):1421-1427.
16. Fayaz M, Bhat MH, Kumar A, Jain AK. Comparative studies on different solvents used for the extraction of phytochemicals from the plant parts of *Arnebia benthamii* (Wall ex. G. Don) Johnston. J Chem Pharm Res. 2017;9(1):220-224.
17. Rather MA, Pandey DP, Kumar S, Per TS, Rashid Y. Phytochemical investigation of *Arnebia benthamii* (Wall. ex G. Don) I.M. Johnst. Int J Innov Res Rev. 2018;6:21-26.
18. Manjkhola S, Dhar U. Conservation and utilization of *Arnebia benthamii* (Wall. ex G. Don) Johnston - a high value Himalayan medicinal plant. Curr Sci. 2002;83(4):484-488.
19. Manjkhola S, Dhar U, Rawal RS. Treatments to improve seed germination of *Arnebia benthamii*: an endangered medicinal herb of high altitude Himalaya. Seed Sci Technol. 2003;31(3):571-577.
20. Khursheed AG, Shabana A, Nawchoo IA. No chilling obligation for germination in seeds of *Arnebia benthamii*: a critically endangered alpine medicinal plant of North-West Himalaya. Int J Biodivers Conserv. 2011;3(5):155-159.
21. Kumar A, Shashni S, Kumar P, Pant D, Singh A, Verma RK. Phytochemical constituents, distributions and traditional usages of *Arnebia euchroma*: a review. J Ethnopharmacol. 2021;271:113896. Doi: 10.1016/j.jep.2021.113896
22. Dulta K, Kaur G, Kumar K, Mazhar M, Bhardwaj G, Sharma A. Species of Arnebia genus found in the Western Himalayas: *Arnebia euchroma* (Royle ex Benth.), *Arnebia benthamii* (Wall. ex G Don) Johnston, Arnebia guttata Bunge. In: Sharma A, Nayik GA, editors. Immunity boosting medicinal plants of the Western Himalayas. Singapore: Springer; 2023. Doi: 10.1007/978-981-19-9501-9_4.
23. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097. Doi: 10.1371/journal.pmed.1000097; PMID: 19621072.
24. Don G. A general history of the dichlamydeous plants. Vol. 4. London: J.G. and F. Rivington; 1837. p. 333.
25. World Flora Online. *Arnebia benthamii* (Wall. ex G.Don) I.M.Johnst. Available from: https://list.worldfloraonline.org/wfo-0000549455
26. Plants of the World Online. Lithospermum benthamii (Wall. ex G.Don) I.M.Johnst. (synonym of *Arnebia benthamii*). Richmond: Royal Botanic Gardens, Kew. Available from:

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:117965-1

27. Park I, Yang S, Song JH, Moon BC. Dissection for floral micromorphology and plastid genome of valuable medicinal borages Arnebia and Lithospermum (Boraginaceae). Front Plant Sci. 2020;11:606463. Doi: 10.3389/fpls.2020.606463.

28. Sun J, Wang S, Wang Y, Wang R, Liu K, Li E, et al. Phylogenomics and genetic diversity of Arnebiae Radix and its allies (Arnebia, Boraginaceae) in China. Front Plant Sci. 2022;13:920826. Doi: 10.3389/fpls.2022.920826.

29. Ambrish K, Srivastava SK. Taxonomic studies on the genus Arnebia Forssk. (Boraginaceae) in India. Taiwania. 2014;59(4):315-325.

30. GBIF.org. *Arnebia benthamii* (Wall. ex G.Don) I.M.Johnst. Global Biodiversity Information Facility. Available from: https://www.gbif.org/species/4062105

31. Mahmood A, Malik RN, Zabta KS, Aqeel M. Ethnobotanical survey of plants from Neelum, Azad Jammu and Kashmir, Pakistan. Pak J Bot. 2011;43:105-110.

32. Fu XQ, De-Wei L. Stimulation of shikonin production by combined fungal elicitation and in situ extraction in suspension cultures of *Arnebia euchroma*. Enzyme Microb Technol. 1999;24(5-6):243-246.

33. Smyrska-Wieleba N, Wojtanowski KK, Mroczek T. Comparative HILIC/ESI-QTOF-MS and HPTLC studies of pyrrolizidine alkaloids in flowers of Tussilago farfara and roots of *Arnebia euchroma*. Phytochem Lett. 2017;20:339-349.

34. EFSA Panel on Contaminants in the Food Chain (CONTAM). Risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements. EFSA J. 2017;15(7):4908. Doi: 10.2903/j.efsa.2017.4908.

35. Zan K, Wang Z, Hu XW, Li YL, Wang Y, Jin HY, et al. Pyrrolizidine alkaloids and health risk of three Boraginaceae used in TCM. Front Pharmacol. 2023;14:1075010. Doi: 10.3389/fphar.2023.1075010.

36. Kumar N, Eloziia N, Kothiyal P. Evaluation of antidepressant activity of aqueous root extract of *Arnebia benthamii* in rats. Indo Am J Pharm Sci. 2017;4(7):1956-1964.

37. Shen CC, Syu WJ, Li SY, Lin CH, Lee GH, Sun CM. Antimicrobial activities of naphthazarins from *Arnebia euchroma*. J Nat Prod. 2002;65(12):1857-1862

38. Liang D, Sun Y, Shen Y, Li F, Song X, Zhou E, et al. Shikonin exerts anti-inflammatory effects in a murine model of lipopolysaccharide-induced acute lung injury by inhibiting the nuclear factor-kappaB signaling pathway. Int Immunopharmacol. 2013;16(4):475-480.

39. Fu D, Shang X, Ni Z, Shi G. Shikonin inhibits inflammation and chondrocyte apoptosis by regulation of the PI3K/Akt signaling pathway in a rat model of osteoarthritis. Exp Ther Med. 2016;12(4):2735-2740.

40. Fan H, Yang M, Che X, Zhang Z, Hui X, Liu K, et al. Activity study of a hydroxynaphthoquinone fraction from *Arnebia euchroma* in experimental arthritis. Fitoterapia. 2012;83(7):1226-1237.

41. Mohsenikia M, Khakpour S, Azizian Z, Ashkani-Esfahani S, Razavipour ST, Toghiani P. Wound healing effect of *Arnebia euchroma* gel on excisional wounds in rats. Adv Biomed Res. 2017;6:2.

42. Nasiri E, Hosseinimehr SJ, Hosseinzadeh A, Azadbakht M, Akbari J, Azadbakht M. The effects of *Arnebia euchroma* ointment on second-degree burn wounds: a randomized clinical trial. J Ethnopharmacol. 2016;189:107-116.

43. Lee H, Kang R, Yoon Y. Shikonin inhibits fat accumulation in 3T3-L1 adipocytes. Phytother Res. 2010;24(3):344-351.

44. Xu F, Wang P, Zhang X, Hou T, Lala Q, Wang C, et al. Identification and target-pathway deconvolution of FFA4 agonists with anti-diabetic activity from *Arnebia euchroma* (Royle) Johnst. Pharmacol Res. 2021;163:105173.

45. Boulos JC, Rahama M, Hegazy MEF, Efferth T. Shikonin derivatives for cancer prevention and therapy. Cancer Lett. 2019;459:248-267.

46. Wang F, Yao X, Zhang Y, Tang J. Synthesis, biological function and evaluation of shikonin in cancer therapy. Fitoterapia. 2019;134:329-339.

47. Kashiwada Y, Nishizawa M, Yamagishi T, Tanaka T, Nonaka G, Cosentino M, et al. Anti-AIDS agents, 18. Sodium and potassium salts of caffeic acid tetramers from *Arnebia euchroma* as anti-HIV agents. J Nat Prod. 1995;58(3):392-400.

48. Hamid Z, Zahoor I, Haq E. *Arnebia benthamii* aggravates potassium dichromate induced hepatotoxicity in Wistar rats. Int J Pharm Chem Biol Sci. 2014;4(2):234-240. Doi: to be added; PMID: to be added.

49. Andújar I, Ríos JL, Giner RM, Recio MC. Pharmacological properties of shikonin - a review of literature since 2002. Planta Med. 2013;79(18):1685-1697.

50. Sofi II, Zargar SA, Ganie AH, Shah MA. Distribution dynamics of *Arnebia euchroma* (Royle) IM Johnst. and associated plant communities in trans-Himalayan Ladakh region in relation to local livelihoods under climate change. Trees For People. 2022;7:100213.

51. Singh KN, Lal B, Chand G, Todaria NP. Ecological features and conservation of *Arnebia euchroma*, a critically endangered medicinal plant in Western Himalaya. Int J Conserv Sci. 2012;3(3):189-198.

For any questions related to this article, please reach us at: globalresearchonline@rediffmail.com

New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.