

Nutraceutical Potential and Bioactive Properties of *Cicer microphyllum* Benth. from the Trans-Himalayan Region of Ladakh, India

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Abstract

The present study evaluates the nutraceutical profile and bioactivity of *Cicer microphyllum* Benth., a wild legume widely consumed in Ladakh. Seeds, flowers and leaves were analyzed for macronutrients (protein, carbohydrates), secondary metabolites and antioxidant and antibacterial activities. Seeds exhibited the highest protein content (1.06 mg/mL), whereas flowers contained maximum carbohydrates (15.18 mg/mL). The extract showed high levels of total phenols (57.5 mg/mL; 5750 mg/g GAE) and flavonoids (34.80 mg/mL; 3480 mg/g QE), accompanied by strong DPPH scavenging activity (66.8%). Antibacterial evaluation revealed prominent, dose-dependent inhibition zones ranging from 2.2–3.8 cm at 10 μ L and 2.0–4.4 cm at 15 μ L against *E. coli*. The convergence of macronutrient richness, antioxidant capacity and antibacterial efficacy validates the traditional use of *C. microphyllum* as a functional food and underscores its potential as a high-altitude nutraceutical resource.

Introduction

Nutraceuticals derived from plants have gained global attention as safe, accessible options for health promotion and disease prevention, particularly in communities with limited access to conventional healthcare (1). High-altitude cold desert ecosystems such as the Ladakh Trans-Himalaya depend heavily on wild edible plants to supplement staple diets and provide traditional remedies for a range of ailments (2). In such fragile environments, indigenous knowledge system (IKS) have historically played a crucial role in ensuring nutritional security and enhancing resilience against climatic stress.

Among the wild edible flora of Ladakh, *Cicer microphyllum* Benth. (Fabaceae) is recognized as highly valued seasonal food resource. It is widely recognized in the Trans-Himalayan region of Ladakh as a nourishing wild food plant. Young leaves and flowers are eaten raw or mixed with traditional dishes, while seeds are boiled, roasted, or ground into flour practices documented in ethnobotanical surveys of wild edible plants from Leh district. (3) The species is also used as livestock fodder and its dried stems serve as fuel in rural communities (4). Traditional healers recommend *C. microphyllum* to alleviate weakness and fatigue, in line with its perceived nutritional and health-promoting value (5).

Despite its cultural prominence and frequent citation in ethnobotanical surveys, there is very limited experimental work substantiating its nutraceutical and pharmacological properties.

Given the increasing recognition of wild legumes as rich sources of proteins, complex carbohydrates and bioactive secondary metabolites (6), the present study was designed to quantify macronutrients in different plant parts of *C. microphyllum* from Ladakh. The study also designed to characterize phytochemicals (phenols, flavonoids), assess antioxidant capacity (DPPH scavenging); and evaluate antibacterial activity against *E. coli*. By integrating ethnobotanical knowledge with biochemical analysis, this study aim to provide a scientific basis for the traditional use of *C. microphyllum* and assess its potential as a high-altitude nutraceutical.

Materials and Methods

2.1 Sample collection

The sample was collected from Barsoo (Sankoo region), Kargil district, Ladakh, India. Mature seeds, flowers and leaves of *C. microphyllum* were collected in triplicate during the active growing season. Species identity was confirmed using regional floras and matched with authenticated herbarium specimens housed at the University of Ladakh. Voucher specimens were prepared and deposited in the University of Ladakh herbarium (LAD) under voucher number (LAD-701) for future reference.

2.2 Nutritional Analysis

2.2.1 Protein

Crude protein was detected qualitatively using Biuret and xanthoproteic tests. Quantitative estimation was carried out by the Lowry method, with absorbance recorded at 660 nm against an appropriate blank. Bovine serum albumin was used for the standard curve, and results were expressed as mg of protein per mL of extract (Table 1).

2.2.2 Carbohydrates

Total carbohydrates were estimated using the phenol–sulphuric acid method. Absorbance of the reaction mixture was measured at 490 nm, and concentrations were calculated from a glucose standard curve and expressed in mg/mL (Table 1).

2.3 Phytochemical Screening and Quantification

For quantitative analyses, total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent and expressed as mg gallic acid equivalents (GAE) per g dry weight. Total flavonoid content (TFC) was estimated by the aluminium chloride method and expressed as mg quercetin equivalents (QE) per g.

2.4 Antioxidant Activity (DPPH Assay)

Antioxidant capacity of the methanolic extract was assessed by DPPH radical scavenging. An aliquot of extract was mixed with DPPH solution, incubated in the dark, and absorbance was recorded spectrophotometrically. Radical scavenging activity was calculated as percentage inhibition of DPPH relative to control (Table 2).

2.5 Antibacterial Assay

Antibacterial activity was evaluated against *Escherichia coli* using the agar disc diffusion method. Sterile filter paper discs were impregnated with 10 μ L and 15 μ L of *C. microphyllum* methanolic extract and placed on nutrient agar plates inoculated with standardized bacterial cultures. After 24 h incubation, zones of inhibition (ZOI) were measured in centimeters (Table 3).

Results

Cicer microphyllum Royle ex Benth. (Fig. 1)

Botanical name

Cicer microphyllum

Common name

Himalayan chickpea

Local name

Sari

Family: **Fabaceae**

Distribution

The native range of this species is Afghanistan, China, Nepal, Pakistan, and India (Uttar Pradesh, Himachal Pradesh, Punjab, J&K, and Ladakh). In Ladakh it's found in Nubra, Zaskar, Leh, Kargil, Sankoo, Barsoo, and Drass.

Habitat

On pasture and dry hill slopes.

Description

It is a very diffuse, erect, spreading shrublet, glandular herb. Stem up to 50 cm, much branched and hairy. Leaves pinnate, ending in a coiled tendril, sessile, cuneate.

Flowering and fruiting

June to August.

3.2 Nutritional Composition

Protein and carbohydrate contents varied among plant parts (Table 1). Seeds exhibited the highest protein concentration (1.06 mg/mL), supporting their traditional use as an energy-rich food. Carbohydrates were most abundant in flowers (15.18 mg/mL), followed by seeds (11.47 mg/mL) and leaves (8.41 mg/mL). This differential distribution suggests that floral tissues act as major carbon sinks during reproductive development, while seeds accumulate both proteins and carbohydrates for germination and metabolic demands.

3.3 Phytochemical and Antioxidant Profile

Quantitatively, *C. microphyllum* showed very high levels of total phenols (57.5 mg/mL; 5750 mg/g GAE) and flavonoids (34.80 mg/mL; 3480 mg/g QE) (Table 2). Correspondingly, DPPH radical scavenging activity reached 66.8%, indicating strong antioxidant potential.

3.4 Antibacterial Activity

Methanolic extracts of *C. microphyllum* showed marked inhibitory effects against *E. coli* (Table 3). At 10 µL extract volume, zones of inhibition ranged from 2.2–3.8 cm, while at 15 µL they increased up to 4.4 cm, reflecting a clear dose-dependent response

Table 1
Nutraceutical and Bioactive Properties of *Cicer microphyllum*
(Seeds, Leaves, and Flowers)

Parameter	Plant Part / Condition	Result
Protein Content	Seeds	1.06 mg/mL
Carbohydrate Content	Flowers	15.18 mg/mL
	Seeds	11.47 mg/mL
	Leaves	8.41 mg/mL

Table 2
Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and DPPH Radical Scavenging Activity of *Cicer microphyllum* (Whole Plant)

Assay	Plant Part/ Extract	Results
Total Phenols	Methanolic Extract	57.5 mg/mL / 5750 mg/g GAE
Total Flavonoids	Extract	34.80 mg/mL / 3480 mg/g QE
DPPH Radical Scavenging Activity	Extract	66.8%

Table 3
Antibacterial Activity of methanolic extract of *Cicer microphyllum* (Whole Plant)

Assay	Solvent/Quantity	Inhibition Zone
Antibacterial Activity	Methanolic extract – 10 µL	2.2–3.8 cm
	Methanolic extract – 15 µL	2.0–4.4 cm

Discussion

Earlier phytochemical investigations have identified several bioactive compounds from ethanolic extracts of seeds and aerial parts of *C. microphyllum* including the sterol stigmasterol; triterpenoids such

as oleanolic acid and oleanolic acid-3-acetate; isoflavonoids including biochanin A, genistein and pratensein; and flavones such as chrysoeriol and luteolin (7). Experimental studies further demonstrate that alcoholic extracts can modulate immune responses enhancing Th1-mediated immunity in both normal and chronically stressed mice (8). These findings provide a scientific support for the traditional use of *C. microphyllum* as a tonic and health-promoting plant.

The present represents one of the first attempts to evaluate the nutritional and bioactive properties of *Cicer microphyllum* from the Trans-Himalayan region of Ladakh. The high protein content detected in its seeds showcase its potential as a locally important high-altitude pulse. Comparable nutritional significance has been reported in related legumes such as *Cicer arietinum* (chickpea), which serves as a major protein source for mountain populations (6).

The strong antioxidant activity, as evidenced by high DPPH radical scavenging activity, suggests that *C. microphyllum* accumulates protective secondary metabolites. Such a trend is commonly observed among high-altitude medicinal plants exposed to intense UV radiation and low-temperature stress (9). Comparable antioxidant potential has been widely reported in Himalayan species such as *Hippophae rhamnoides* and *Rheum emodi* (10, 11).

The antibacterial activity observed against *E.coli* is consistent with findings from other phenolic-rich high-altitude plants, where bioactive compounds, particularly flavonoids and tannins, exert antimicrobial effects through multiple mechanisms. These include disruption of microbial cell membrane integrity, increased permeability leading to leakage of intracellular constituents, and interference with key cellular metabolic processes (12). In *Cicer microphyllum*, such activity may be attributed to the synergistic action of diverse secondary metabolites, highlighting its potential as a natural source of antibacterial agents adapted to extreme environmental conditions.

These findings, together with previous reports on the immunomodulatory and anti-leishmanial activities of extracts from *C. microphyllum* extracts (8, 13), highlighting its promising nutraceutical potential. So far, only a limited phytochemical studies have been conducted on the trans-Himalayan plants (2, 4, 5, 14, 15). However, further researches are necessary to comprehensively characterize its phytochemical profile, evaluate its activity against a broader spectrum of pathogens, and assess its safety and efficacy through in vivo investigations.

Overall, the integration of nutritional richness, antioxidant capacity, and antibacterial properties positions *C. microphyllum* as a promising nutraceutical candidate. This becomes especially relevant for high-altitude communities which frequently experience nutritional deficiencies, oxidative stress from hypoxia and intense UV exposure, and limited access to conventional pharmaceuticals (16). Future research should focus on comprehensive profiling of amino acid and mineral composition, isolation and characterization of specific bioactive components, and pharmacological validation through in vivo studies. Additionally, the development of sustainable harvesting practices and community-based cultivation strategies are essential to ensure conservation of the species while supporting local livelihoods.

Declarations

Author Contribution

Mohd Shabir conceptualized and planned the research work, analyzed the data, prepared the original draft of the manuscript, and carried out manuscript revision. Harron Ali conducted field collection and laboratory experiments. Konchok Dorje contributed to editing and improvement of the manuscript. All authors read and approved the final version of the manuscript.

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Data Availability

All data presented in this study were generated by the authors themselves during the course of the research.

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Figures

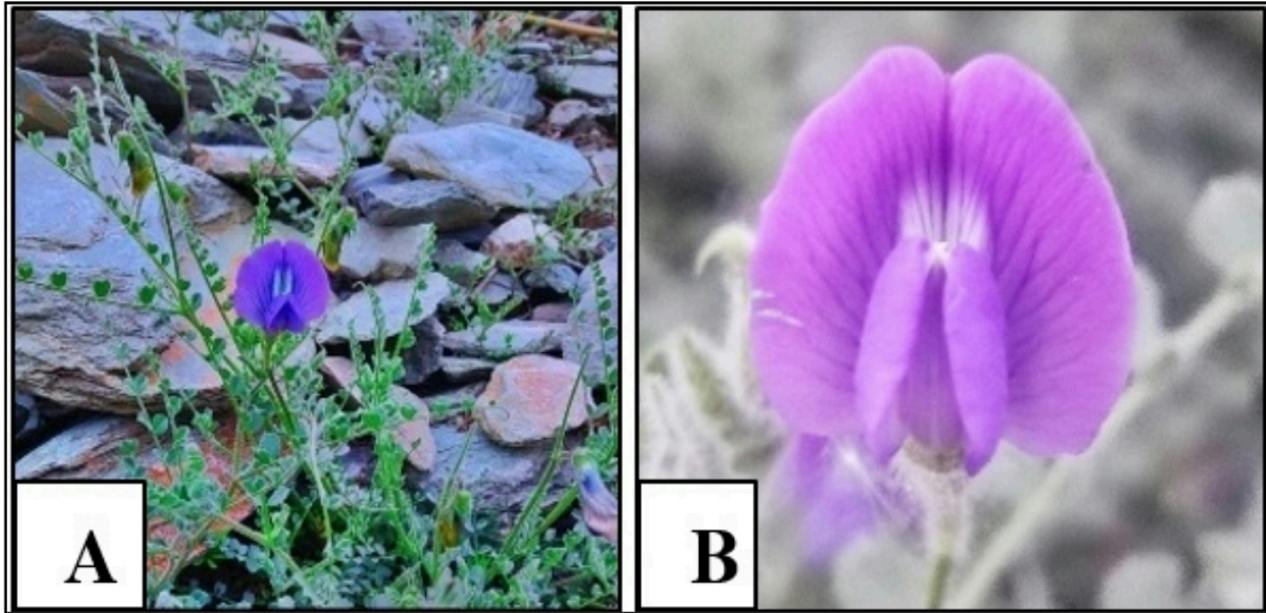
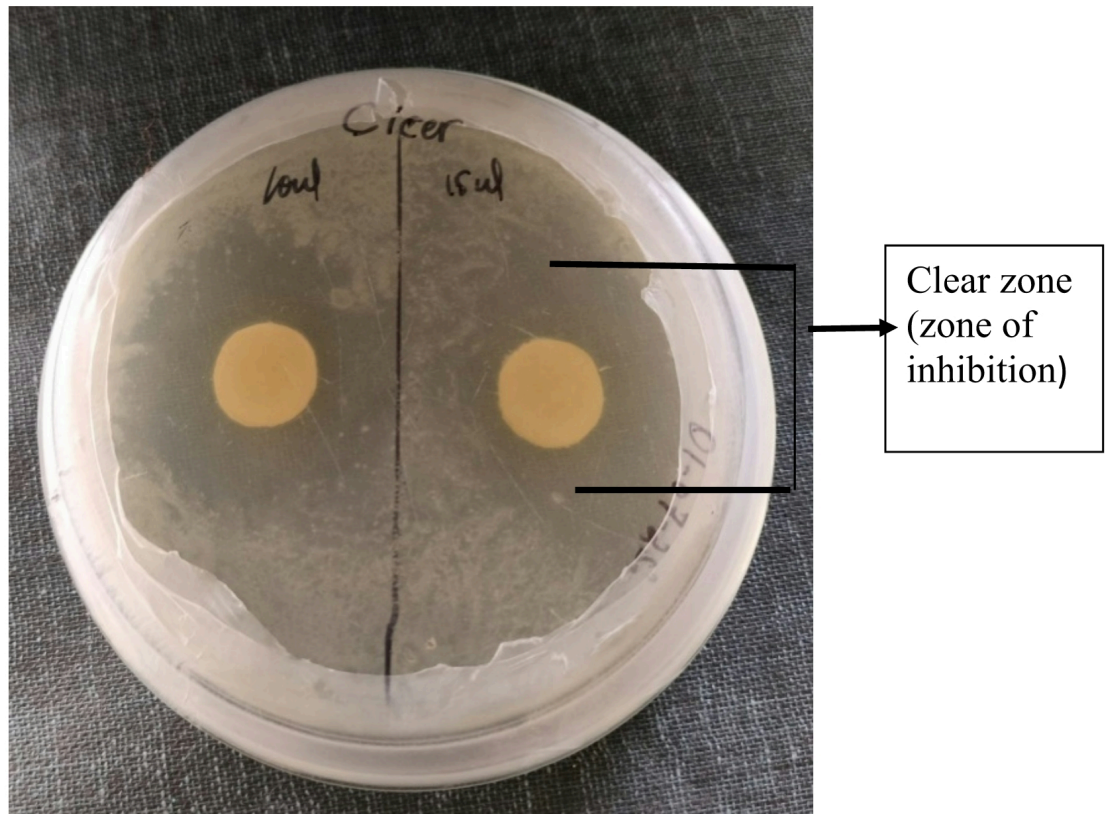


Figure 1

(A) Habitat (B) Close-up of flower



Clear zone (zone of inhibition)

Figure 2

Antibacterial activity of *Cicer microphyllum* against *E.coli*.