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Research article

Unveiling the drought adaptations and pharmacological potential of *Blepharis sindica* for arid land restoration and medicinal uses

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Abstract

Blepharis sindica T. Anders, an endangered xerophytic plant from the Indian Thar Desert, possesses adaptation traits essential for survival in dry environments and shows potential as a nutraceutical fodder. This research investigated the morpho-phytochemical characteristics by utilizing field emission scanning electron microscopy (FESEM), energy dispersive X-ray spectroscopy (EDS), and high-performance liquid chromatography (HPLC). SEM examination identified xeromorphic adaptations, including thick cuticles, elongated glandular trichomes, and hygroscopic seed hairs, which contribute to drought resistance and water-mediated seed dissemination. Elemental analysis by EDS revealed that leaf tissues included intricate mineral compositions, including Ca, K, Mg, and Si, which underpin their metabolic and ecological roles. The seed coat and fruit capsule had a more basic structure, indicating their primary function as protective entities. Phytochemical analysis confirmed the presence of the anticarcinogenic compound asiaticoside, a bioactive triterpenoid, with concentrations of 8.37% in the seeds and 3.87% in the leaves. Current findings confirmed *B. sindica* as a dual-purpose plant for the regeneration of dry rangelands and the sustainability of cattle.

Keywords: Arid-zone adaptation, Asiaticoside, Phytochemical profiling, Rangeland restoration, Xeromorphic traits

Introduction

Blepharis sindica T. Anders (Acanthaceae) is an endangered xerophytic shrub predominantly found in the arid and sandy regions of Rajasthan, India. It is commonly known as 'Bhangari' and its natural populations are rapidly declining due to habitat degradation and unsustainable harvesting (Tripathi and Arya, 2002; Mathur, 2005). It is mainly found in different parts of the country, such as Rajasthan, Gujarat, Punjab, Haryana, and the Indus delta region of India (Apurva *et al.*,2015). Recognized for its ecological importance, *B. sindica* has been categorized as "Vulnerable" (A2cd) by the IUCN Red List under the UNDP-CCF II Project (UNDP, 2008). It plays a crucial role in dune stabilization and regulating aeolian processes, contributing significantly to the maintenance of desert ecosystems (Mathur and Sundaramoorthy, 2013; Lal and Mohammed, 2020). Aziz and Khan (1993) classified their population dynamics as a 'Deevey Type III survivorship curve', which exhibits high juvenile mortality, indicating the species' vulnerability in early life stages. The deep-rooted, xerophytic nature, occurrence in sand dunes, inherent drought hardiness and seed persistence make it suitable for sand dune stabilization and revegetation efforts in degraded arid zones.

B. sindica emerges as a promising non-conventional forage source in arid landscapes, where traditional fodder is extremely limited. Bhatt *et al.* (2017) investigated the use of Fagonia herbage in sheep diets by incorporating it into complete feed blocks at inclusion levels of 30% and 45%, replacing Cenchrus hay. The results indicated enhanced digestibility of dry matter, organic matter, and crude protein, alongside a decrease in acid detergent fiber and cellulose digestibility. With 7.71% crude protein and a fiber profile rich in hemicellulose and cellulose (though high in lignin), the herbage was deemed nutritionally adequate for maintenance-level feeding of ruminants. Microbial protein synthesis and nitrogen utilization also improved, demonstrating that up to 45% of conventional forage can be replaced with processed *B. sindica* herbage, especially in feed-scarce seasons.

The reproductive strategy of *B. sindica* includes aerial seed retention on senesced maternal plants, likely an adaptation to desert conditions where seed predation is intense, and favorable germination windows were brief and unpredictable (Narita and Wada, 1998). However, population viability analysis (PVA) conducted by Mathur (2014) predicts the extinction of local populations within two decades due to mismatches between reproductive output (e.g., delayed seed release) and prevailing harsh ground conditions. Further investigations by Mathur (2015, 2018) emphasized that phenotypic plasticity, reproductive traits, and soil attributes (texture, pH) significantly influence the survival and distribution of the species, suggesting that any conservation or relocation strategy must carefully account for ecological site specificity.

B. sindica is also valued for its medicinal properties. Phytochemical studies have identified bioactive compounds with aphrodisiac, antifungal, and antibacterial properties, showing activity against *Streptomyces*, *Trichoderma reesei*, *Penicillium notatum*, and *Fusarium oxysporum* (Mathur and Sundaramoorthy, 2006; Sharma and Roy, 2018a, 2018b). Pharmacological safety of *B. sindica* has also been affirmed in studies evaluating organ toxicity. Recent advances in histochemical analysis and propagation techniques are providing insights into tissue-level biochemistry and enabling in vitro conservation approaches (Sharma and Roy, 2023a, 2023b). These findings underscore the plant's dual ecological and pharmacological value.

To elucidate the adaptive and metabolic traits of *B. sindica*, a detailed morphological and phytochemical study was conducted using scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and high-performance liquid chromatography (HPLC). HPLC, a robust method for the separation and identification of non-volatile and polar compounds (Bidlingmeyer, 1992), was employed to characterize leaf and fruit extracts. This approach enabled the precise profiling of triterpenes and steroids, supporting both ecological physiology and pharmacological investigations. This integrated approach provides baseline data essential for species restoration and sustainable utilization. Based on the objectives of evaluating the ecological, phytochemical, and forage utility of *B. sindica*, the proposed hypothesis is: "The morphological and phytochemical adaptations of *B. sindica* enhance its resilience to arid conditions, and its integration into managed forage systems can simultaneously improve livestock nutrition and support ecosystem functions such as sand dune stabilization and biodiversity conservation." Accordingly, the study aims to examine the surface ultrastructure of *B. sindica* leaf, fruit, and seed using SEM coupled with energy dispersive X-ray spectroscopy (EDX); extract and quantify the phytochemical content, particularly asiaticoside, from the leaf and fruit of *B. sindica* using HPLC and compare the retention time and concentration of asiaticoside in the leaf and fruit extracts. Asiaticoside is a bioactive triterpenoid saponin known for its role in promoting collagen synthesis, facilitating wound healing, and tumor necrosis (Huang *et al.,* 2004; Fatima, 2013).

Materials and Methods

Collection and identification of plant material: Fresh and healthy specimens of *B. sindica* were collected from Ramsisar village (Latitude: 28.08073, Longitude: 74.82559), Churu district, Rajasthan, India. Three plants were collected and carefully packed in aerated open bags along with the native soil to maintain viability during transport. One specimen was preserved and submitted to the Herbarium of the Department of Botany, University of Rajasthan, Jaipur, for taxonomic authentication. The plant sample was authenticated and assigned the voucher number RUBL 211345. Authenticated plant material was used for all subsequent analyses, with two biological replicates included in the study. The plant samples were collected with the permission of the relevant authorities. The plant collection and use were in accordance with all the relevant guidelines.

Preparation of samples for ultra-structural analysis: Representative leaf, fruit, and seed samples were processed according to standard SEM (scanning electron microscope) protocols. After air-drying and dehydration, the samples were mounted on stubs and sputter-coated with gold. The surface structure of the gold-coated tissues was observed under a field emission scanning electron microscope (FESEM), the Nova Nano SEM 450 model from FEI, operated at 10–20 kV. The stage was maintained at a temperature of 20°C with a chamber pressure of 6 Torr. Elemental composition of the samples was simultaneously assessed using an attached EDX system, following established protocols (Ogura *et al.*, 1989; Pathana *et al.*, 2009).

Preparation of leaf and fruit extracts for HPLC analysis: Plant materials (leaves and fruits) were dried, powdered, and separately subjected to Soxhlet extraction. Approximately 5 grams of each powdered sample was extracted with 500 mL of methanol for 20 hours. The methanolic extracts were filtered and dried in petri dishes, then stored at −20°C for further analysis. These crude extracts were intended for the quantitative estimation of asiaticoside, a biologically active triterpenoid saponin.

Chromatographic conditions and HPLC analysis: The quantification of asiaticoside in the methanolic extracts was carried out using an HPLC system HP Agilent 1200 Infinity Series equipped with a quaternary pump and a diode array detector (DAD). Separation was achieved on a reverse-phase C18 column (ZOBRAX SB-C18, 4.6 × 250 mm, 5 µm). The mobile phase consisted of three solvents: Milli-Q water (Solvent A), acetonitrile (Solvent B), and methanol (Solvent C), delivered at a flow rate of 1.0 mL/min. Each injection consisted of 5 µL of either sample or standard solution. Chromatographic data were processed using Agilent software. The system pressure ranged from 37.9 to 38.2 bar throughout the run. The identification of asiaticoside was confirmed by comparing sample retention times with those of the standard.

Preparation of standards and calibration curve: A stock solution of asiaticoside reference standard was prepared by dissolving 50 µg/mL in methanol and subsequently diluting it to prepare working solutions of 10 µg/mL and other concentrations as needed. For sample preparation, 10 grams of powdered leaves and fruits were separately extracted using 500 mL of 90% methanol in a Soxhlet apparatus for 20 hours. The extracts were filtered, concentrated at 50°C, and stored in 10 mL vials. For HPLC measurement, 50 ppm solutions were prepared by appropriate dilution of the extracts with methanol. A calibration curve was plotted by analyzing standard solutions of asiaticoside, enabling quantitative estimation in the plant extracts (Mothana *et al.*, 2009; Sood, 2014; Mukherjee, 2019).

Results and Discussion

Morphological characteristics

Leaf micro-morphology and xeromorphic adaptations: SEM analysis revealed distinct xeromorphic traits in *B. sindica* that contribute significantly to its drought resistance. The abaxial surface of the leaf, particularly along the prominent midrib, was densely covered with two types of trichomes: long, slender non-glandular types and short glandular trichomes with a verrucose, swollen base and tapering apex (Fig 1A-C). The high-resolution SEM micrographs (Fig. 1E) confirmed the glandular nature of the short trichomes and their role in reducing water loss by acting as barriers to transpiration. Stomata were also evident, with a characteristic arrangement of two guard cells flanked by two subsidiary cells oriented perpendicularly, suggesting functional regulation of gas exchange under water-limited conditions (Fig 1C-D). Additionally, wax crystals observed on the leaf surface (Fig 1F) reinforce the plant's adaptations to arid environments by minimizing water evaporation, consistent with xerophytic traits documented in other desert flora (Zhang *et al.*, 2021; Soliman *et al.*, 2019).

***Fruit capsule and seed morphology*:** SEM imaging of young fruit capsules revealed photosynthetically active green to yellow coloration, further corroborated by the presence of stomata on the capsule sheath (Fig 2A-C). During maturation, the fruit became brown and dried, displaying stomatal patterns that closely resembled those of the leaves (Fig 2D-E). A distinct hilum structure, acting as a hygroscopically sensitive valve, was clearly visible (Fig 2F), serving as a critical trigger for water-induced dehiscence. Under simulated monsoonal moisture conditions, capsule rupture was observed from one end (Fig 2G), releasing two seeds per capsule (Fig 3A).

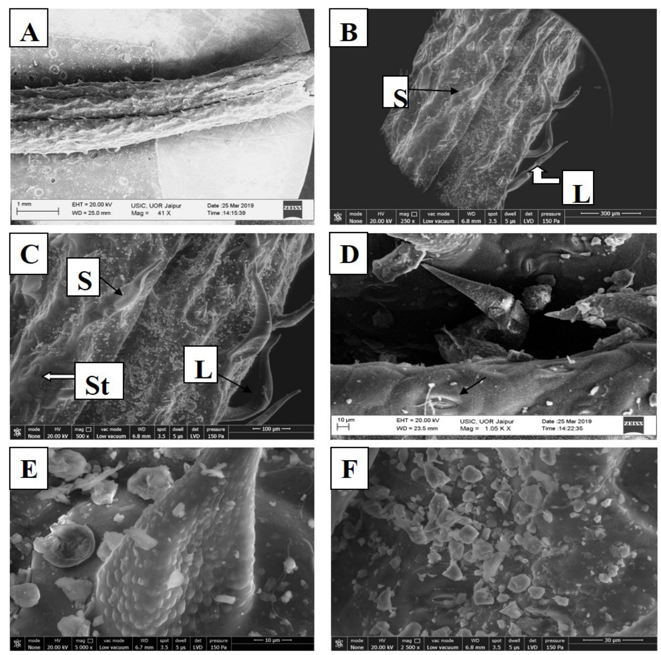

Fig 1. SEM analysis of the leaf of *Blepharis sindica* [**A:** SEM picture of the raised midrib of the leaf on the abaxial surface showing trichomes; **B:** SEM showing enlarged view of two types of trichomes on the midrib, long slender (L), and short with swollen base and tapering tip (S); **C:** Enlarged view of the SEM picture showing long smooth slender trichomes (L) and short trichomes having broad swollen base with verrucose surface and thin smooth tapering tip (S). Stomata (St) are also visible on the surface of the leaf; **D:** SEM picture of enlarged view of stomata showing two guard cells, and two subsidiary cells at right angles to guard cells (Arrow); **E:** Enlarged view of short trichome showing its swollen glandular base with verrucose surface and relatively smooth upper tapering part and scattered wax; **F:** SEM picture of leaf surface showing scattered wax crystals]

The seeds were enveloped in hygroscopic hairs with a specialized three-part structure: a broad basal attachment, a cylindrical shaft, and a bifurcated apex with digitiform projections (Fig 3B-D). These hairs, firmly attached at the radicular end and loosely arranged near the cotyledons (Fig 3E-F), facilitated both seed anchorage and moisture responsiveness. Upon hydration, the seed hairs became erect, generating pressure that led to seed coat rupture and radicle emergence (Fig 3G). Moisture-triggered germination was further validated in controlled Petri dish experiments, which showed the emergence of seedlings from split capsules (Fig 3H). These rain-responsive mechanisms make *B. sindica* particularly suitable for desert revegetation, erosion control, and rangeland stabilization (Martínez-Berdeja *et al.*, 2015; Li *et al.*, 2021).

Elemental composition: tissue-specific functions: EDS analysis (Table 1; Figs 4.1-4.3) highlighted tissue-specific elemental profiles correlating with physiological roles. The fruit capsule exhibited the highest carbon content (80.92%), emphasizing its structural function. In contrast, the leaf showed the most complex elemental spectrum, with significant concentrations of calcium (7.17%), potassium (2.64%), silicon (2.26%), aluminium (1.11%), magnesium (0.69%), iron (0.73%), and chlorine (2.16%), suggesting multifaceted involvement in photosynthesis, osmoregulation, and stress tolerance (de Bang *et al.*, 2021). The seed coat, although primarily composed of carbon (44.55%) and oxygen (52.65%), also contained potassium (2.14%) and magnesium (0.66%), potentially contributing to membrane stability and early germination processes. Sulfur was exclusively detected in the capsule (0.15%), indicating localized biochemical activities.

Phytochemical profiling and Asiaticoside quantification: HPLC confirmed the presence of asiaticoside, a bioactive triterpenoid saponin, in both fruit and leaf tissues. The retention time of the standard compound (2.338 min) closely matched the peaks observed in seed (2.332 min) and leaf (2.320 min) extracts (Table 2). Quantitative analysis revealed asiaticoside contents of 8.37% (w/w) in fruit and 3.87% (w/w) in leaves, equivalent to 43 and 20% relative to a 1 mg/mL standard, respectively. This identifies the fruit as a substantially

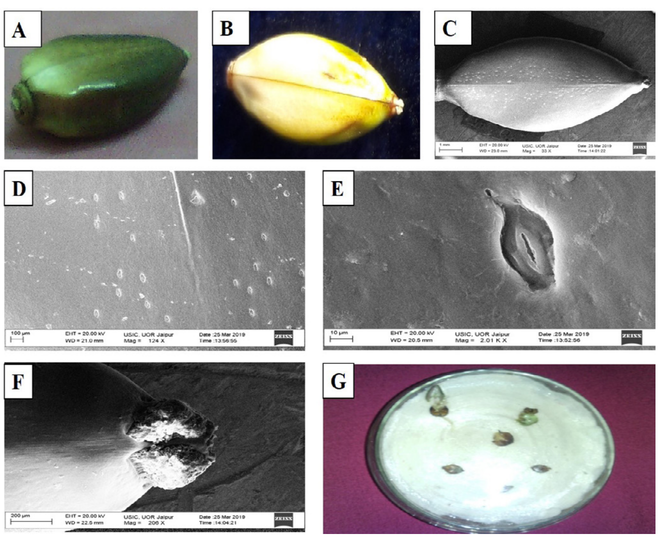

Fig 2. Morphology of fruit and seed of *Blepharis sindica*; [**A:** Green immature fruit of *B. sindica*; **B:** Maturing fruit of *B. sindica*; **C:** Surface analysis of dried fruit with FESEM showing the presence of stomata's on the fruit surface; **D:** Little close-up of stomata's on the fruit surface; **E:** Enlarged SEM picture of the stomata clearly showing guard cells, and two subsidiary cells at right angles to the guard cells; **F:** SEM picture of hilum of fruit; **G:** Petri plate showing splitting of fruit shell (capsule) from apex to base to separate segments known as valves to release the seeds (Arrow) by absorbing moisture]

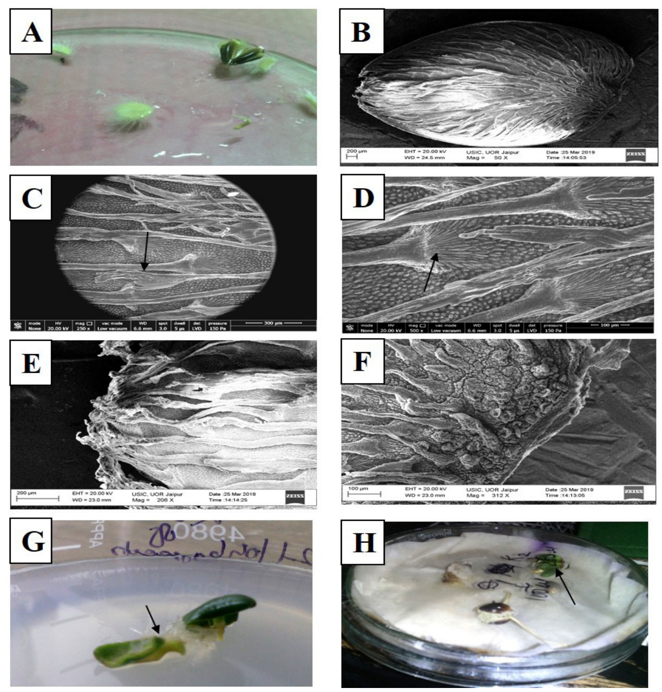

Fig 3. Morphology of the seed of *Blepharis sindica*;[**A:** The capsule splits open, releasing the two seeds present in it, when water enters the fruit through the hilum. The seeds are covered with dense, thin hygroscopic hairs standing erect in moisture; **B:** SEM Surface view of the dried seed showing hairs on its surface; **C:** SEM enlarged view of the long seed coat hairs showing their basal attachments, narrow long body, and branched apical portions into many finger-like projections (arrow); **D:** Enlarged view of broad basal attachments of seed coat hairs having numerous thin fibrous spreading branches attached to the seed coat (arrow); **E:** SEM of plumular end of the seed coat showing loose apical ends of the hairs; **F:** Enlarged SEM picture of the radicular end of the seed coat showing firm attachment of the hairs; **G:** The thin, nearly transparent seed coat with numerous hygroscopic hairs (arrow) after being soaked burst open to expose young seedlings with a radical, two thick dark green cotyledons and plumule. From each fruit, two seeds are released, each producing a young seedling; **H:** Petri plate showing cracking of fruit and release of two young seedlings from a fruit (arrow)]

Table 1. Table showing elemental distribution (%) in different plant parts of *B. sindica*

Plant parts	Elemental distribution (%)					
	O	C	Ca	K	Si	Cl
Leaf	46.25	36.99	7.17	2.64	2.26	2.16
Fruit	18.93	80.92	-	-	-	-
Seed	52.65	44.55	-	2.14	-	-

richer source and positions *B. sindica* as a novel herbal reservoir of asiaticoside. Given asiaticoside's well-documented roles in wound healing, anti-inflammatory, and anti-tumor activity (Huang *et al.,* 2004; Fatima, 2013), these findings warrant further investigation into its pharmacological potential and therapeutic applications.

Can be used as fodder resource in dryland ecosystem: In addition to its medicinal and ecological roles, *B. sindica* has demonstrated considerable promise as a fodder resource in arid ecosystems. The presence of structural features such as a resilient cuticle and trichomes (as revealed by SEM) may influence forage digestibility, while the leaf's mineral richness—including potassium, calcium, and magnesium- offers additional nutritional benefits for ruminants, especially in mineral-deficient grazing areas. The detection of asiaticoside in forage tissues suggests potential nutraceutical roles in enhancing gastrointestinal health, immune response, and overall metabolism in livestock. This positions *B. sindica* as a promising candidate for dual-purpose (forage and pharmacological) applications in sustainable dryland agriculture. Integration of *B. sindica* into silvipastoral systems can help address seasonal fodder shortages, promote biodiversity, and rehabilitate degraded lands (Kumar *et al*., 2025). Bhatt *et al*. (2017) found that inclusion of 30 to 45% *B. sindica* in complete feed blocks for sheep improved the digestibility of dry matter, organic matter, and crude protein. The herbage contains 7.71% crude protein and a favorable fiber profile rich in hemicellulose and cellulose, with moderate lignin levels that support slow ruminal degradation and sustained nutrient release. Field-based observations by Kaushish and Patel (1997) in Jaisalmer support its fodder utility, where goats grazing on Blepharis -Dhaman grass- lemon grass-sewan grass communities showed average body weight gains of 9.8 kg (females) and 13.5 kg (males) over 10.5 months, reinforcing the productivity of such xeric plant communities.

Table 2. Table showing comparison of availability% asiaticoside in the plant parts of *Blepharis sindica* with the standard

S.No.	Sample	Ret time (min)	Area (mAU*s)	Amount/Area	Amount (%)
1.	Standard asiaticoside	2.338	5184.95166	1.88849e-4	19.58
2.	Seed extract	2.332	2218.35327	1.88849e-4	8.378695
3.	Leaf extract	2.320	1026.16570	1.88849e-4	3.875816

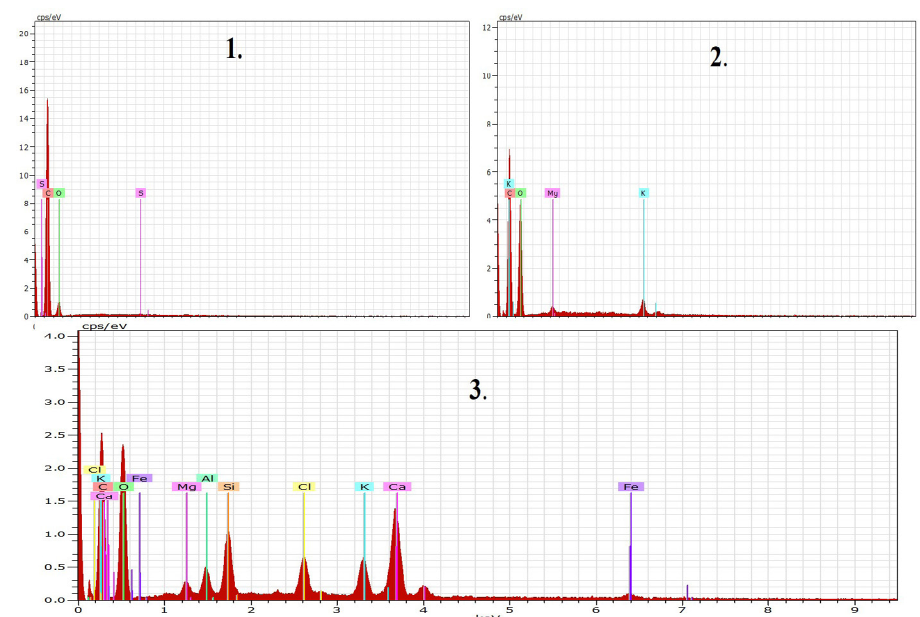

Fig 4. Graph-based EDS analysis of different parts of *Blepharis sindica***; [**1: Fruit capsule; 2: Seed coat; 3: Leaf)

Conclusion

This study presents the first comprehensive characterization of *B. sindica*, integrating micro-morphological features, elemental composition, and phytochemical profiling to elucidate its ecological adaptations, medicinal value, and potential as a forage resource. The identification of substantial levels of asiaticoside in both leaves and fruits highlights its pharmacological significance, while its anatomical resilience and favorable digestibility parameters support its suitability for arid-zone livestock systems. Moreover, its rain-triggered seed dispersal and adaptive structural traits make it a promising candidate for sand dune stabilization. Collectively, these findings offer valuable insights for species conservation, advocate its use in sustainable fodder development, and reinforce its role in enhancing the ecological stability and economic resilience of desert rangelands. Despite providing critical insights into the morphology, microanatomy, and bioactive potential of *B. sindica*, several research gaps remain. Genetic diversity studies using molecular markers (e.g., SSR, RAPD) are needed to identify superior ecotypes. Long-term field trials assessing effects on livestock performance, immunity, and gut microbiota are essential for validating its use as a nutraceutical forage. Integrating *B. sindica* into community-based conservation strategies and traditional veterinary practices may enhance rangeland resilience and support rural livelihoods across arid regions.

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