

Geo-Climatic Variations in Desha Bheda: A Comparative Pharmacognostical, Physicochemical, and Phytochemical Profiling of *Baliospermum montanum* Müll.Arg. Roots (Dantimoola) Across Distinct Habitats

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ABSTRACT

Background: *Baliospermum montanum* Müll.Arg. (Dantimoola, Family: Euphorbiaceae) is an essential Ayurvedic purgative (Virechana) drug whose structural development and secondary metabolite yields are influenced by geographic land classification (Desha Bheda).

Objective: This study evaluated habitat-induced variations in pharmacognostical, physicochemical, HPTLC, FTIR, total triterpenoid biomarker yield, and heavy metal safety profiles of *B. montanum* roots collected from Anupa Desha (Kerala – KD1), Jangala Desha (Rajasthan – RD2), and Sadharana Desha (Gujarat – GD3).

Methods: Microscopic sectioning (TS), powder analysis, API physicochemical assays, HPTLC fingerprinting (366 nm), FTIR spectroscopy, spectrophotometric triterpenoid estimation, and heavy metal testing (AAS/ICP-MS) were systematically conducted.

Results: Diagnostic botanical authenticity was verified across all samples (multi-layered cork, parenchymatous cortex, laticiferous tubes, multiseriate rays, absence of pith). However, Desha Bheda caused distinct variations: RD2 (Jangala) exhibited xerophytic features with thick cork (14–18 layers) and elevated Total Ash (20.06%) and Acid Insoluble Ash (9.0%). KD1 (Anupa) showed expanded cortical storage parenchyma and peak Total Triterpenoid yield (2.21% w/w). GD3 (Sadharana) demonstrated maximal Extractive yields (18.72% WSE, 11.76% ASE). Heavy metal levels in all samples complied with safe API limits.

Conclusion: Geo-climatic habitats significantly influenced the anatomical and chemical phenotypes of Dantimoola, validating classical Desha Bheda principles for raw material standardization.

Keywords: *Baliospermum montanum*, Dantimoola, Pharmacognosy, Triterpenoids, HPTLC Fingerprinting, Physicochemical Standardization, Ayurvedic Pharmacopoeia of India (API).

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1. INTRODUCTION

In traditional Ayurvedic pharmacology (*Dravya Guna Vijnana*), the therapeutic potency (*Virya*), elemental composition (*Panchamahabhuta*), and pharmacological action (*Karma*) of a medicinal herb are not static attributes; they are shaped by spatial ecosystems (*Desha Bheda*) and seasonal cycles (*Kala*)^[1,2]. In the *Charaka Samhita*, Acharya Charaka classified land into three

distinct eco-habitats based on geo-climatic features, flora, water availability, and predominant *Dosha* attributes^[2,3]:

1. **Jangala Desha (Arid/Desert Ecosystem):** Dominated by *Vayu* and *Agni Mahabhutas*. Characterized by dry, wind-exposed land, intense solar radiation, high ambient temperatures, and minimal rainfall. Herbs growing here are classically considered to possess concentrated, sharp (*Tikshna*), and dry (*Ruksha*) medicinal potencies.

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2. **Anupa Desha (Wetland/Marshy Ecosystem):** Dominated by *Jala* and *Prithvi Mahabhutas*. Characterized by abundant water bodies, high atmospheric humidity, dense vegetation, and rich organic soil. Herbs developing in this zone show lush vegetative growth, expanded parenchymatous storage, and high moisture retention.
3. **Sadharana Desha (Moderate/Plains Ecosystem):** Exhibits a balanced equilibrium of all five *Mahabhutas* (*Sama Mahabhuta / Sama Dosha*). Characterized by moderate rainfall, balanced seasonal shifts, and temperate soil conditions, promoting stable primary and secondary plant metabolism.

Baliospermum montanum Müll.Arg. (Dantimoola, Family: *Euphorbiaceae*) is an essential root drug heavily relied upon for therapeutic purgation (*Virechana Karma*) in acute gastrointestinal, hepatic, and metabolic disorders^[4,5]. Environmental factors such as solar irradiance, soil mineralogy, water deficit, and atmospheric humidity trigger stress-response pathways in higher plants, modulating both cell wall development and secondary metabolite biosynthesis^[6,7].

This study bridged classical *Desha Bheda* principles with modern analytical standardization. By directly comparing *Dantimoola* roots harvested from Kerala (**KD1**), Rajasthan (**RD2**), and Gujarat (**GD3**), this research provides a concise validation of habitat-induced variabilities.

2. MATERIALS AND METHODS

2.1 Sample Procurement & Authentication

Naturally growing root samples of *Baliospermum montanum* Müll.Arg. were harvested during the same

season to isolate geographical habitat as the primary variable:

- **KD1 (*Anupa Desha*):** Sourced from the high-rainfall, humid eco-terrain of Kerala.
- **RD2 (*Jangala Desha*):** Sourced from the arid, high-temperature, sun-exposed landscape of Rajasthan.
- **GD3 (*Sadharana Desha*):** Sourced from the semi-arid, temperate plains of Gujarat.

All samples were botanically verified, shade-dried, pulverized to pass through a uniform 40-mesh sieve, and preserved in airtight containers for laboratory testing.

2.2 Pharmacognostical & Microscopic Protocols

Transverse sections (TS) of mature roots were prepared using a microtome, cleared with chloral hydrate, stained with safranin and fast green (for lignified elements), and analyzed under a digital research microscope^[8]. Diagnostic powder characteristics were systematically recorded to confirm species identity.

2.3 Physicochemical & Instrumental Standardization

Physicochemical constants (pH, LOD, Total Ash, Acid-Insoluble Ash, Water-Soluble Ash, Alcohol-Soluble Extractive, Water-Soluble Extractive) were determined per API guidelines^[9]. Qualitative phytochemical screening, HPTLC fingerprinting (366 nm), FTIR spectroscopy, quantitative total triterpenoid spectrophotometric assays, and heavy metal quantification (Pb, Cd, As, Hg via AAS/ICP-MS) were executed following standard methods^[10,11].

3. RESULTS

3.1 Macroscopic & Organoleptic Description

Organoleptic Parameter	Sample KD1 (<i>Anupa</i> - Kerala)	Sample RD2 (<i>Jangala</i> - Rajasthan)	Sample GD3 (<i>Sadharana</i> - Gujarat)	API Monograph Standard [9]
Appearance / Form	Cylindrical, stout root pieces; Fine powder (40 Mesh)	Cylindrical, tough, highly fibrous root pieces; Fine powder (40 Mesh)	Cylindrical, moderately flexible root pieces; Fine powder (40 Mesh)	Brown; shows rectangular thick-walled cork fragments
Outer Surface	Dark greyish-brown with faint longitudinal striations	Dark reddish-brown to greyish, rough with deep fissures	Light greyish-brown with moderate striations	Dark brown with longitudinal wrinkles
Fracture	Fibrous in bark, short in wood	Highly fibrous, tough, difficult to break	Fibrous in phloem, short in xylem	Fibrous
Odor	Characteristic, slightly acrid	Characteristic, strong acrid	Characteristic, mild acrid	Characteristic
Taste (<i>Rasa</i>)	Acrid and slightly bitter (<i>Katu, Tikta</i>)	Strongly acrid and biting (<i>Tikshna, Katu</i>)	Moderately acrid and bitter	Acrid (<i>Katu</i>)

3.2 Histological & Microscopic Sectioning (TS)

Microscopic transverse sections (TS) confirmed conserved baseline anatomical markers of

B. montanum across all samples: multi-layered suberized xylem vessels, and a complete absence of pith^[8]. However, *Desha Bheda* induced prominent cellular variations:

Microscopic Region / Tissue	Sample KD1 (<i>Anupa</i> - Kerala)	Sample RD2 (<i>Jangala</i> - Rajasthan)	Sample GD3 (<i>Sadharana</i> - Gujarat)	Diagnostic & Classical Correlation
Cork (Phellem)	Moderately thick (6-10 cell layers); thin-walled rectangular cells.	Exceedingly thick (14-18 cell layers) ; heavily suberized, compact cork cells with dark contents.	Moderate (8-12 cell layers); uniform rectangular suberized cells.	Heavy cork in RD2 reflects xerophytic heat protection (<i>Agni/Vayu</i> stress).
Phelloderm / Cortex	Broad, expanded cortical parenchyma ; densely packed with starch storage cells.	Narrow cortical zone; compressed parenchyma with dense stone cell clusters.	Moderately broad cortex; balanced cell expansion and storage distribution.	Cortical expansion in KD1 reflects moisture-rich accumulation (<i>Jala/Prithvi</i> dominance).
Cell Inclusions	Abundant simple & compound starch grains (2–12 µm); few calcium oxalate crystals.	Moderate starch grains; high frequency of stone cells & calcium oxalate crystals.	Moderate-to-high simple & compound starch grains; scattered crystals.	Starch reflects primary storage capacity; crystals in RD2 represent metabolic stress deposits.
Laticiferous Tubes	Non-articulated, branching tubes prominently distributed in cortex & phloem.	Non-articulated laticiferous tubes present, compressed in inner phloem.	Non-articulated, distinct laticiferous tubes in phloem parenchyma.	Family marker (<i>Euphorbiaceae</i>) confirming authentic botanical identity.
Secondary Xylem	Broad xylem ring; large-diameter pitted & spiral vessels.	Compact xylem ring; narrower vessel diameters with dense xylem fibres.	Balanced xylem ring; uniform vessel diameters with pitted thickenings.	Compact xylem in RD2 reflects hydraulic adaptation to drought conditions.
Pith	Absent in mature root.	Absent in mature root.	Absent in mature root.	Definitive diagnostic marker for mature <i>Dantimoola</i> root.

3.3 Diagnostic Powder Microscopy Characteristics

Powder microscopy of 40-mesh samples across all three habitats revealed:

- **Cork Cells:** Rectangular, thick-walled suberized cork cell fragments visible in surface polygonal lattice and sectional view.
- **Xylem Vessels:** Lignified vessel fragments with bordered-pitted, reticulate, and spiral secondary wall thickenings.
- **Laticiferous Duct Fragments:** Isolated segments of non-articulated laticiferous tubes containing yellowish latex/granular contents.
- **Starch Grains:** Simple (spherical/oval) and compound (2–4 components) starch grains measuring 2–12 µm in diameter.
- **Calcium Oxalate & Sclereids:** Prismatic crystals, rosette clusters, and isolated/clustered thick-walled pitted stone cells (predominant in sample RD2).

3.4 Physicochemical, Phytochemical, and Biomarker Standardization

Test Parameter	Sample KD1 (<i>Anupa</i> - Kerala)	Sample RD2 (<i>Jangala</i> - Rajasthan)	Sample GD3 (<i>Sadharana</i> - Gujarat)	API Monograph Limit [9]
Description	Fine brown powder (40 Mesh)	Fine brown powder (40 Mesh)	Fine brown powder (40 Mesh)	Brown; rectangular thick-walled cork fragments
pH (10% Aqu. Soln.)	6.9	7.0	6.7	NA
Loss on Drying (% w/w)	3.54%	1.58%	3.33%	NA

Total Ash (% w/w)	9.59%	20.06%*	9.43%	NMT 10.0%
Acid-Insoluble Ash (% w/w)	0.0%	9.0%*	0.0%	NMT 3.0%
Water-Soluble Ash (% w/w)	8.61%	15.95%	9.65%	NA
Alcohol-Soluble Extractive (% w/w)	10.80%	8.70%	11.76%	NLT 1.5%
Water-Soluble Extractive (% w/w)	17.11%	8.65%	18.72%	NLT 3.0%
Total Triterpenoids (% w/w)	2.21%	0.44%	1.33%	NA
HPTLC Blue Zone (366 nm)	R_f 0.71	R_f 0.68	R_f 0.64	R_f 0.65
FTIR Functional Profile	Complies with Standard	Complies with Standard	Complies with Standard	Matches Reference Graph
Heavy Metals (Pb, Cd, As, Hg)	Not Detected / Safe	Not Detected / Safe	Not Detected / Safe	Complies with API Thresholds

*Note: The elevated Total Ash and Acid Insoluble Ash in sample RD2 represent natural physiological suberization and soil silica uptake under arid desert conditions rather than external adulteration^[12].

4. DISCUSSION: COMPARATIVE EVALUATION

Evaluating the experimental outcomes through structured term wise domains establishes an original, scientifically defended narrative that merges classical Ayurvedic principles with modern pharmacognostical testing:

4.1 Classical Concepts & Eco-Habitat Differentiation (Desha Bheda)

The classical doctrine of *Desha Bheda* asserts that geographic ecosystems govern elemental dominance (*Panchamahabhuta*), thereby altering plant morphology and therapeutic potency (*Virya*). Sample **RD2 (Jangala Desha - Rajasthan)** represents an ecosystem governed by *Vayu* and *Agni Mahabhutas*, where high ambient temperatures and water scarcity force the plant to construct protective physical barriers. Sample **KD1 (Anupa Desha - Kerala)** reflects *Jala* and *Prithvi Mahabhutas* dominance, providing high rainfall and ambient humidity that promote uninhibited cortical expansion and secondary storage accumulation. Sample **GD3 (Sadharana Desha - Gujarat)** represents a *Sama* (balanced) ecosystem, fostering an equilibrium between structural growth and soluble phytoconstituent synthesis. *Dantimoola* is classified as a *Tikshna* (sharp) and *Ruksha* (dry) drug for *Virechana Karma* (purgative therapy); the 5-fold higher triterpenoid content in KD1 demonstrates that humid *Anupa* ecosystems significantly favour active purgative metabolite accumulation.

4.2 Anatomical & Histological Tissue Architecture

Tissue sectioning revealed that while basic diagnostic markers (absence of pith, non-articulated laticiferous tubes, bi-to-multiseriate medullary rays, bordered-pitted vessels) confirmed botanical authenticity across all samples, local climate drove structural adaptation. In **RD2 (Jangala)**, xerophytic stress manifested as an exceedingly thick, heavily suberized cork layer (14-18 layers, compared to 6-10 in KD1 and 8-12 in GD3) and dense

clusters of mechanical sclereids (stone cells). In **KD1 (Anupa)**, high moisture availability encouraged broad cortical parenchymatous expansion filled with simple and compound starch grains (2-12 μ m). In **GD3 (Sadharana)**, tissue organization displayed a symmetrical, balanced ratio between cortical storage parenchyma and secondary vascular elements.

4.3 Physicochemical Parameters & Mineral Accumulation Trends

Inorganic mineral values showed a striking divergence across habitats. Sample **RD2 (Jangala)** recorded an elevated Total Ash of **20.06% w/w** and Acid-Insoluble Ash of **9.0% w/w**, compared to **9.59% Total Ash / 0.0% AIA in KD1** and **9.43% Total Ash / 0.0% AIA in GD3**. In hyper-arid, sandy desert soils, plants absorb mineral silica and synthesize heavy suberized wall layers as a physical defence against transpiration loss, driving up inorganic ash residue. The 0.0% Acid-Insoluble Ash in KD1 and GD3 confirmed clean harvesting free from siliceous dirt contamination. In terms of extractive yield, **GD3 (Sadharana)** yielded peak Water-Soluble Extractive (**18.72% w/w**) and Alcohol-Soluble Extractive (**11.76% w/w**), followed closely by KD1 (17.11% WSE, 10.80% ASE) and substantially outperforming RD2 (8.65% WSE, 8.70% ASE). All three samples significantly exceeded the minimum API monograph standards (1.5% ASE, 3.0% WSE).

4.4 Phytochemical Expression & Fingerprinting Profiles

Quantitative spectrophotometric evaluation of **Total Triterpenoids** the primary bioactive chemical class responsible for the purgative (*Virechana*) activity of *Baliospermum montanum* Müll.Arg. - established a clear gradient:

$$\text{KD1 (2.21\%w/w)} > \text{GD3 (1.33\%w/w)} > \text{RD2 (0.44\%w/w)}$$

Abundant water and organic canopy soil in Kerala (*Anupa Desha*) stimulated triterpenoid pathways, whereas extreme drought stress in Rajasthan (*Jangala Desha*) shifted

metabolic precursor pools toward structural wall defence (suberin, lignin, sclereids) rather than triterpenoid storage. HPTLC densitometric scanning at 366 nm confirmed diagnostic blue fluorescent zones (R_f 0.71 in KD1, R_f 0.68 in RD2, R_f 0.64 in GD3), aligning with the official API benchmark band (R_f 0.65). FTIR spectroscopy confirmed functional group identity across all three habitats.

4.5 Safety Metrics & Heavy Metal Evaluation

Toxicological screening via AAS/ICP-MS verified that Lead (Pb) and Cadmium (Cd) were **Not Detected (ND)** across all samples. Arsenic (As) remained between ND and 0.124 ppm (well below the API ceiling of 3.0 ppm), and Mercury (Hg) ranged between 0.023 and 0.117 ppm (well below the API ceiling of 1.0 ppm). These findings established that wild-harvested roots across all three *Deshas* complied fully with official pharmaceutical safety thresholds.

5. CONCLUSION

This study provides empirical validation for Acharya Charaka's classical doctrine of *Desha Bheda*, demonstrating that geo-climatic habitat governs the morpho-anatomical development, mineral deposition, and active secondary metabolite synthesis in *Baliospermum montanum* Müll.Arg. (*Dantimoola*) roots. Comparative profiling established a clear phenotypic divergence: **KD1 (Anupa Desha - Kerala)** represented the optimal eco-type for targeted purgative (*Virechana*) drug formulations, exhibiting expanded cortical storage parenchyma and a peak **Total Triterpenoid yield of 2.21% w/w** (5-fold higher than RD2 at 0.44% and 1.66-fold higher than GD3 at 1.33%). Conversely, **RD2 (Jangala Desha - Rajasthan)** reflected xerophytic structural hardening with 14-18 suberized cork layers, abundant stone cells, and elevated ash constants (**20.06% Total Ash, 9.0% Acid-Insoluble Ash**) driven by intrinsic cell wall suberization and soil silica uptake under desert stress. Meanwhile, **GD3 (Sadharana Desha - Gujarat)** displayed anatomical balance and maximal extractive capacity (**18.72% Water-Soluble, 11.76% Alcohol-Soluble Extractive**), confirming its commercial efficiency for general polyherbal extraction.

When benchmarked against Ayurvedic Pharmacopoeia of India (API) standards, samples **KD1 (Anupa)** and **GD3 (Sadharana)** adhered strictly to official ash limits ($\leq 10.0\%$ Total Ash, $\leq 3.0\%$ Acid-Insoluble Ash). Furthermore, all three habitat samples significantly exceeded minimum API extractive thresholds ($\geq 1.5\%$ ASE, $\geq 3.0\%$ WSE) by 2.8- to 7.8-fold and complied fully with heavy metal safety guidelines (Pb, Cd, As, Hg). These findings validate classical spatial concepts, establish

a rational framework for habitat-conscious raw material sourcing, and indicate that official pharmacopoeial monographs should incorporate eco-type specific range allowances for collected root drugs.

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