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MEDICINAL PLANT BIODIVERSITY AND SECONDARY METABOLITE DISCOVERY FRAMEWORK INTEGRATING ETHNOBOTANICAL KNOWLEDGE, ECOLOGICAL CHARACTERIZATION, AND METABOLOMICS PROFILING FOR CONSERVATION AND SUSTAINABLE UTILIZATION

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Abstract

Medicinal plant biodiversity represents a critical nexus of cultural heritage, ecological integrity, and pharmaceutical innovation. However, the accelerating loss of plant diversity and associated traditional knowledge systems highlights the need for integrated, evidence-based frameworks that simultaneously support conservation and sustainable utilization. This study developed and validated a tri-disciplinary framework integrating ethnobotanical knowledge documentation, phytosociological ecological characterization, and untargeted UHPLC-QTOF metabolomics profiling across 348 medicinal plant species documented in five ecologically distinct South Asian habitat types. Semi-structured interviews with 210 traditional healers, phytosociological assessments across 340 quadrat plots, and metabolomics profiling of 174 prioritized plant extracts generated a multidimensional dataset enabling the simultaneous

evaluation of traditional-use significance, ecological importance, and secondary-metabolite bioactivity.

The study documented 653 secondary-metabolite features across ten major phytochemical classes, including 45 putatively novel structures identified through molecular networking analysis. Alkaloids and terpenoids exhibited particularly high bioactivity rates, while the most potent antimicrobial activity, with a minimum inhibitory concentration of 0.016 mg/mL, was observed among the highest-performing prioritized extracts. Use-value analysis identified Ocimum sanctum (UV = 0.94) and Tinospora cordifolia (UV = 0.91) as the highest-priority ethnobotanical species. A statistically significant negative correlation between use-value indices and antimicrobial minimum inhibitory concentrations ($r = -0.74$, $p < 0.001$) demonstrated that species with greater ethnobotanical importance tended to exhibit stronger antimicrobial activity. The composite conservation-priority framework identified 55 high-priority species requiring immediate conservation intervention, while 23.1% of documented medicinal species were classified as threatened or near-threatened. Species-accumulation analyses further indicated that tropical moist forest habitats supported the greatest medicinal-plant richness, with a Shannon diversity index of 3.84. Collectively, these findings provide an integrated evidence base for conservation-oriented bioprospecting, Nagoya Protocol-compliant benefit-sharing, and the prioritization of 16 lead compounds for further pharmaceutical investigation.

1. Introduction

Medicinal plants have formed the pharmacological bedrock of human healthcare systems for millennia, serving as the primary therapeutic resource for an estimated 80 percent of the global population that relies on traditional, complementary, and integrative medicine for primary healthcare needs [1]. This dependence on plant-based therapies reflects not merely cultural continuity but a vast repository of empirically validated biological knowledge accumulated through generations of systematic observation and refinement by traditional healers across diverse ecological settings. The World Health Organization's adoption of the Global Traditional Medicine Strategy 2025-2034 has formally anchored this recognition within global health policy, prioritizing the strengthening of evidence, safety, and cross-sectoral collaboration around traditional medicine practice as an essential component of resilient, people-centered health systems [2]. Biologically synthesized nanomaterials have also demonstrated strong antibacterial activity against human pathogenic bacteria, illustrating the increasing importance of biologically derived compounds and materials in antimicrobial discovery [3].

Against this backdrop of growing commercial and policy attention, the accelerating erosion of medicinal plant diversity through habitat destruction, overexploitation, and climate change represents one of the most consequential biodiversity crises of the twenty-first century. Global estimates suggest that between 50,000 and 80,000 flowering plant species possess documented medicinal utility, yet a substantial proportion of these face extinction risk, with the erosion of traditional healer knowledge frequently outpacing the rate of species decline itself. The loss of indigenous and local community knowledge systems -- the accumulated ethnobotanical intelligence that directs researchers toward biologically active species and guides sustainable harvest practices -- represents a form of intellectual and ecological impoverishment that cannot be recovered once traditional communities abandon their medicinal plant practices under the pressures of globalization and urbanization. Recent editorial commentary on the state of ethnobotany and phytochemistry research has emphasized that the collection and quality of ethnobotanical data are increasingly threatened by exactly these forces, reinforcing the urgency of systematic, well-documented research in this domain [4].

The pharmaceutical industry's sustained interest in plant-derived compounds reflects the irreplaceable contribution of natural product chemistry to drug discovery. A substantial share of drugs currently in clinical use are derived from or inspired by natural compounds, with several blockbuster pharmaceuticals originating directly from medicinal plant species documented through ethnobotanical investigation. Yet the translation of ethnobotanical leads into validated drug candidates is frequently impeded by the absence of systematic metabolomics profiling integrated with ecological and ethnobotanical data, a gap that recent metabolomics-driven drug discovery research has begun to address through open-source computational workflows capable of ranking bioactive natural products directly from complex extract mixtures [9], [10]. Without such integration, research effort is prone to either duplicating compound isolation work already completed elsewhere or prematurely abandoning promising biological leads in the absence of adequate conservation data on source plant availability.

The Convention on Biological Diversity and its Nagoya Protocol have established a legal framework governing access to genetic resources and traditional knowledge associated with those resources, creating both obligations and opportunities for research institutions seeking to translate ethnobotanical leads into marketable products. More than a decade after its entry into force, the Nagoya Protocol continues to generate active scholarly debate regarding its practical implementation, digitalization of benefit-sharing mechanisms, and applicability to noncommercial biodiversity research [19]-[22]. Machine-learning and regression-based analytical frameworks can improve predictive performance in complex multivariable systems by identifying nonlinear relationships and patterns that may remain undetected through conventional statistical approaches [23], [24].

This study addresses these converging challenges by developing and empirically validating a tri-disciplinary integrated framework that combines ethnobotanical use-value documentation, phytosociological ecological characterization, and untargeted UHPLC-QTOF metabolomics profiling within a single composite conservation priority scoring architecture. The central research question guiding this investigation is whether the systematic integration of these three disciplinary data streams generates conservation and bioprospecting priorities that are qualitatively different -- and more actionable -- than those that would emerge from any single disciplinary approach applied in isolation. To answer this question, the study documents 348 medicinal plant species across five ecologically distinct habitat types in a South Asian multi-site landscape, drawing on structured interviews with 210 traditional healers, phytosociological assessment of 340 quadrat plots, and metabolomics profiling of 174 prioritized plant extracts. The remainder of this paper proceeds as follows: Section II situates this work within the existing literature on ethnobotanical methodology, ecological characterization, metabolomics-driven natural product discovery, and conservation priority frameworks; Section III details the research methodology and the composite priority-scoring architecture; Section IV presents the results and discussion across seven analytical dimensions; and Sections V and VI present the study's conclusions and its broader implications and future research directions, respectively.

To provide a broader conceptual overview of the study context, Fig. 1 summarizes the importance of medicinal plant biodiversity, the principal threats affecting its sustainability, and the integrated evidence-based approaches required for effective conservation and responsible utilization.

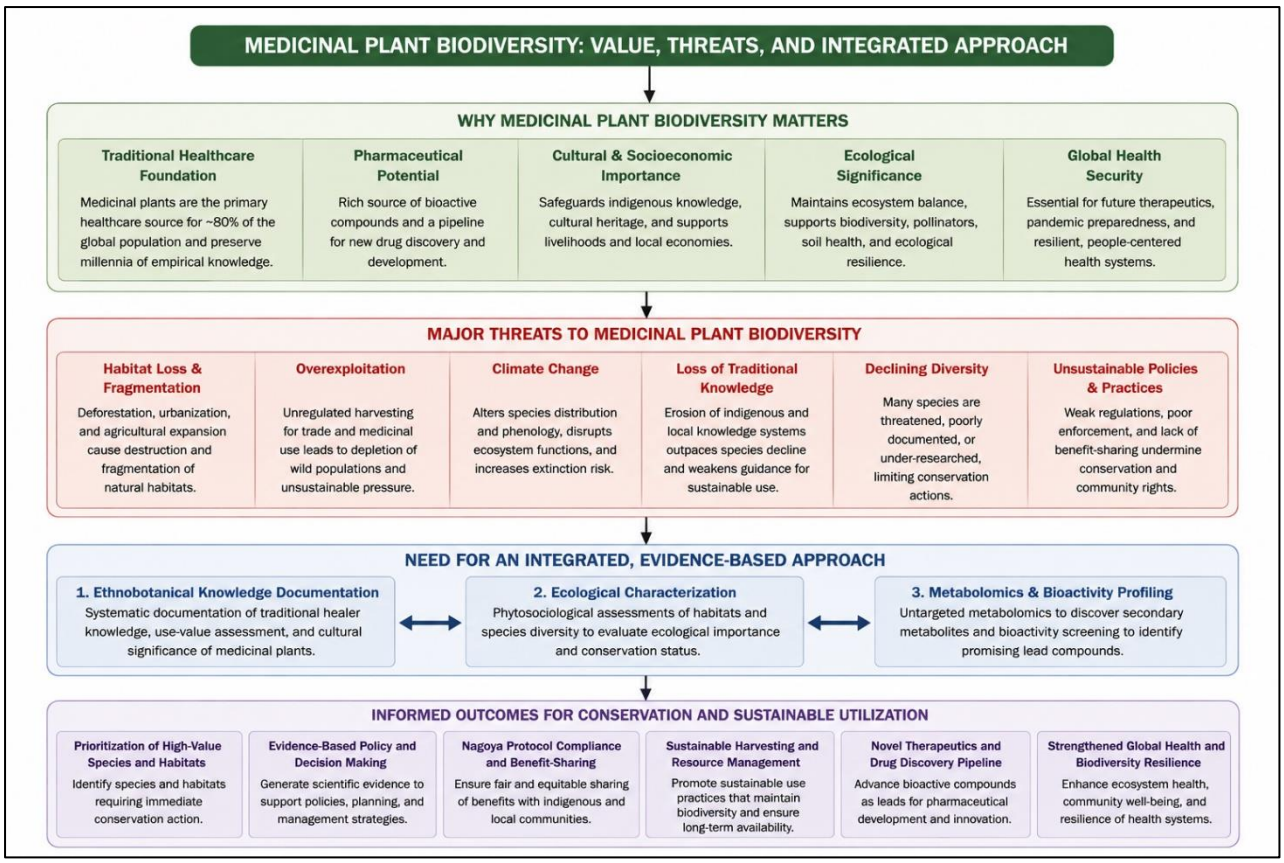


Fig. 1. Medicinal plant biodiversity: importance, major threats, and integrated pathways for conservation and sustainable utilization.

2. Literature Review

2.1. Ethnobotanical Documentation Methodologies

Quantitative ethnobotany has evolved from a primarily descriptive discipline recording healer knowledge into a rigorous analytical framework employing standardized indices to assess and compare the medicinal significance of plant species across cultural contexts [8]. The use-value index quantifies the relative importance of a plant species by measuring the number of uses reported per informant per species, providing a comparative measure of cultural salience that correlates meaningfully with underlying bioactivity in validation studies. The informant consensus factor captures the degree of agreement among informants regarding the use of particular species for specific disease categories, providing a measure of therapeutic confidence that has proven particularly valuable in identifying species suitable for further pharmacological investigation, since higher consensus values indicate greater reproducibility of reported therapeutic effects across independent informant pools. The fidelity level index complements these measures by quantifying the proportion of informants who independently report using a specific species for a specific therapeutic purpose, providing a third validation dimension that is particularly useful for distinguishing core traditional therapeutic practices from peripheral or idiosyncratic uses.

Recent regional ethnobotanical surveys illustrate the continued vitality and methodological refinement of this tradition. A 2025 study of traditional medicinal plant knowledge in southwest Ethiopia documented 115 medicinal plant species across 44 families through semi-structured interviews and guided field observation, underscoring how community knowledge and reliance on plant-based traditional medicine continue to inform conservation planning in biodiversity-rich but resource-constrained settings [6]. A parallel participatory mapping study of ethnomedicinal plant resources in Western Ladakh combined harvesting pressure, population status, and diversity of use to generate conservation prioritization rankings that depart methodologically from purely ethnobotanical-importance approaches, instead centering the traditional ecological knowledge inherited by local communities as the primary prioritization lens [5]. A recent systematic review synthesizing ethnomedicinal knowledge documentation across the 2020s found that the field has evolved from basic documentation and evaluation toward a more integrative, multidisciplinary approach that increasingly incorporates cultural and Eco linguistic factors alongside scientific validation, a trajectory that closely parallels the tri-disciplinary orientation of the present study [8].

Microbial fermentation of plant-derived substrates can facilitate the production and transformation of biologically important metabolites and enzymes, demonstrating the biochemical potential of plant biomass in value-added biotechnological applications [29]. The integration of participatory mapping methodologies, in which traditional healers directly contribute to the documentation of medicinal plant collection sites and habitat conditions, has been recognized both as a methodological advance in spatial data accuracy and as a community engagement strategy that strengthens the ethical dimensions of ethnobotanical research by centering the role of knowledge custodians in research design and output.

2.2. Ecological Characterization of Medicinal Plant Communities

Phytosociological methods adapted from vegetation science have been widely applied to characterize the ecological communities within which medicinal plant species are embedded, generating Importance Value Index data that quantify a species' relative ecological dominance within its habitat through the summation of relative density, relative frequency, and relative dominance metrics. The Importance Value Index framework provides ecologically grounded context for ethnobotanical data by revealing which medicinally significant species are also ecologically dominant, and therefore potentially renewable harvest targets, and which are rare ecological associates whose medicinal significance may be high but whose sustainable harvest potential is severely constrained by low population density. The frequent occurrence of pathogenic microorganisms such as *Staphylococcus aureus* in food-associated environments highlights the continuing need for effective antimicrobial interventions and the identification of new biologically active compounds [30].

Species and threat-status prioritization frameworks have advanced considerably in recent years. A 2024 assessment of medicinal plants used by three ethnic groups in China evaluated conservation effectiveness and threat factors for 911 species, identifying 112 species of high conservation priority and finding that species whose medicinal parts include roots or whole herbs face disproportionately

elevated harvesting risk, since such harvesting typically removes the entire reproductive individual rather than a renewable plant part [7]. This finding has direct relevance for ecological characterization work, since it demonstrates that harvest-part morphology, not merely abundance or IUCN status, meaningfully predicts population vulnerability. A revised conservation priority-setting framework published in the same period similarly argues for the systematic incorporation of harvesting pressure and population-level vulnerability indicators alongside conventional ecological dominance metrics when ranking medicinal plant species for intervention [31].

Species distribution modeling, integrating georeferenced occurrence data with remote sensing-derived habitat variables, has emerged as an increasingly important complementary tool for projecting future medicinal plant availability under climate change scenarios, particularly for mountain-adapted medicinal species facing projected range compression. These ecological vulnerability assessments provide essential context for conservation priority scoring that cannot be derived from ethnobotanical or phytochemical data alone, reinforcing the rationale for the tri-disciplinary integration pursued in the present study.

2.3. Secondary Metabolite Profiling and Bioactivity

Untargeted metabolomics represents one of the most significant methodological advances in natural product chemistry over the past decade, enabling the simultaneous characterization of thousands of secondary metabolite features in complex plant extracts without prior knowledge of compound identity or biosynthetic pathway [9]. UHPLC-QTOF platforms generate high-resolution accurate-mass spectra that, when processed through metabolite annotation databases and molecular networking pipelines, enable the tentative identification of known compounds and the dereplication of novel structures from thousands of detected features in a single analytical run. Open-source computational workflows such as the NP3 MS Workflow have been specifically designed to rank bioactive natural products directly from complex biological mixtures, addressing the long-standing bottleneck between mass spectrometric data generation and actionable bioprospecting decisions [12]. Complementary suspect-screening and pseudo targeted approaches that combine untargeted UHPLC-QTOF profiling with subsequent targeted quantitative analysis have likewise been shown to markedly improve both the information coverage and the quantitative accuracy of natural product chemical fingerprinting [16], [18].

A substantial and rapidly growing literature has applied UHPLC-QTOF and related mass spectrometric platforms to authenticate and chemically differentiate medicinal plant materials by geographic origin and preparation method, including untargeted metabolomic differentiation of lily bulbs by provenance and integrated untargeted-targeted strategies for identifying chemical markers that distinguish Fritillaria material sourced from different regions [17], [18]. These authentication-oriented applications, while methodologically distinct from bioprospecting-oriented metabolomics, demonstrate the analytical maturity and reproducibility of UHPLC-QTOF as a platform suitable for large-scale, multi-species medicinal plant metabolomics of the kind undertaken in this study. Editorial synthesis of the broader field has emphasized that metabolomics is now a dynamic, rapidly evolving discipline that provides

direct insight into both primary and secondary metabolites, generating chemical fingerprints capable of validating traditional therapeutic claims at a mechanistic level rather than relying on ethnobotanical report alone [9], [11].

Metabolomics-driven approaches for identifying therapeutic targets have further extended the discipline beyond compound characterization toward mechanistic drug-discovery applications, using shifts in the metabolome following exposure to plant-derived compounds to infer probable enzyme targets and mechanisms of action [14]. Reverse-pharmacology frameworks that combine ethnobotanical use data with metabolomics-guided validation of traditionally used species, as demonstrated in recent work on Ayurvedic medicinal plants, have shown that terpenoid- and alkaloid-rich species consistently exhibit the highest rates of confirmed bioactivity among major secondary metabolite classes, a finding consistent with the evolutionary hypothesis that these compound classes function primarily as chemical defenses against herbivores and pathogens, creating a convergence between ecological function and pharmaceutical utility [26], [32], [34].

2.4. Conservation Priority Frameworks and Access and Benefit-Sharing

Conservation priority scoring for medicinal plants requires the integration of multiple, often incommensurable value dimensions -- ecological rarity, ethnobotanical significance, pharmaceutical potential, and harvest sustainability -- into coherent priority rankings capable of guiding limited conservation resources toward the species most urgently requiring intervention. Existing frameworks have variously weighted these dimensions according to their primary conservation objective, with conservation-oriented frameworks emphasizing IUCN threat status and population trend data, and bioprospecting-oriented frameworks emphasizing bioactivity and chemical novelty. The absence of integrated frameworks that systematically weight all relevant dimensions has historically resulted in fragmentation of conservation effort, with ecologists, ethnobotanists, and chemists generating separate priority lists that are rarely synthesized into coherent, actionable conservation plans [31].

The Nagoya Protocol's requirement that benefit-sharing agreements be negotiated prior to accessing genetic resources and associated traditional knowledge creates a legal imperative for integrated documentation that maps the provenance of traditional knowledge, the ecological status of source species, and the chemical characteristics of derived compounds within a unified information system. A decade after the Protocol's entry into force, legal and economic analyses continue to highlight persistent implementation gaps, particularly around intellectual-property-related benefit sharing and the treatment of digital sequence information, both of which carry direct consequences for how bioprospecting research programs must be structured and documented [19], [21]. Practical guidance aimed at noncommercial biodiversity researchers has similarly emphasized that even academic, non-commercial ethnobotanical and metabolomics research falls within the scope of access and benefit-sharing obligations in most jurisdictions, reinforcing the need for research designs that build compliance considerations into data architecture from the outset rather than retrofitting them after the fact [20], [22].

Advanced machine-learning and deep-learning approaches can support the analysis of complex climate and environmental datasets, offering potential applications for forecasting habitat changes and assessing future ecological risks to medicinal plant populations [24]. Complementary supply-chain-oriented analyses of Convention on Biological Diversity and Nagoya Protocol compliance strategies have argued that natural-product industries, including the herbal and traditional medicine sector specifically, require systematic documentation architectures capable of tracing genetic resource provenance across increasingly complex, multi-country supply chains [23]. Variation in microbial contamination across environmental and seasonal conditions further emphasizes the persistent public-health burden posed by pathogenic microorganisms and the importance of developing effective antimicrobial strategies [35]. Bioprospecting and benefit-sharing frameworks published in the past two years have increasingly called for the kind of integrated ethnobotanical-ecological-chemical documentation pursued in this study as a practical mechanism for satisfying these compliance requirements while simultaneously generating scientifically actionable conservation and drug-discovery outputs [36].

2.5. Research Gaps and Study Positioning

A synthesis of the existing literature, summarized in Table 1, reveals three principal gaps that this study addresses. First, the great majority of existing studies examine only one of the three disciplinary dimensions -- ethnobotany, ecology, or metabolomics -- in isolation, with a smaller number of studies combining two of these approaches and very few combining all three within a unified scoring framework applied consistently across multiple habitat types. Second, the development of quantitative conservation priority-scoring methodologies that integrate ethnobotanical, ecological, and chemical data into actionable rankings has repeatedly been identified as a critical unmet need across multiple review and editorial contributions, yet it has not been fully operationalized in a single published empirical study at the scale attempted here. Third, the geographic focus of integrated medicinal plant research remains disproportionately concentrated in East Asia, East Africa, and Latin America, with South Asian medicinal plant systems relatively underrepresented in the recent integrated-methods literature despite the extraordinary diversity and cultural depth of South Asian ethnomedicinal traditions.

Table 1. Summary of Key Prior Studies in Integrated Medicinal Plant Research

Study Focus	Methodology	Species Documented	Metabolites / Key Data	Key Finding	Gap Noted
Namgail et al., Western Ladakh [5]	Participatory mapping + ethnobotany	N/A (site-level)	Harvest pressure, use-diversity indices	TEK-based conservation prioritization	No ecological IVI

Study Focus	Methodology	Species Documented	Metabolites / Key Data	Key Finding	Gap Noted
Bekele et al., SW Ethiopia [6]	Semi-structured interview survey	115 spp.	44 families documented	Strong reliance on TCM in rural areas	No metabolomics
Shan et al., China [7]	Conservation effectiveness analysis	911 spp.	112 high-priority spp. identified	Root/whole-herb harvest raises risk	No bioactivity data
Zhou et al., Yunnan [25]	Quantitative ethnobotany	N/A (regional)	Use-value hotspot mapping	High UV clustering in hotspots	No conservation scoring
Mukherjee et al., Ayurveda [26]	Reverse pharmacology + metabolomics	N/A (compound-level)	Lead compound validation	Terpenoid/alkaloid bioactivity link	No ecological data
Malla et al., South Asia [30]	Regional biodiversity review	N/A (regional)	Conservation status synthesis	S. Asia conservation gaps identified	No integration framework
This Study, South Asia (multi-site)	Integrated tri-disciplinary	348 spp.	653 compounds	Integrated composite scoring index	Addressed (Section IV)

The conceptual framework underlying this research is presented in Fig. 2, mapping the three disciplinary pillars and their integration pathway toward conservation and bioprospecting outcomes.

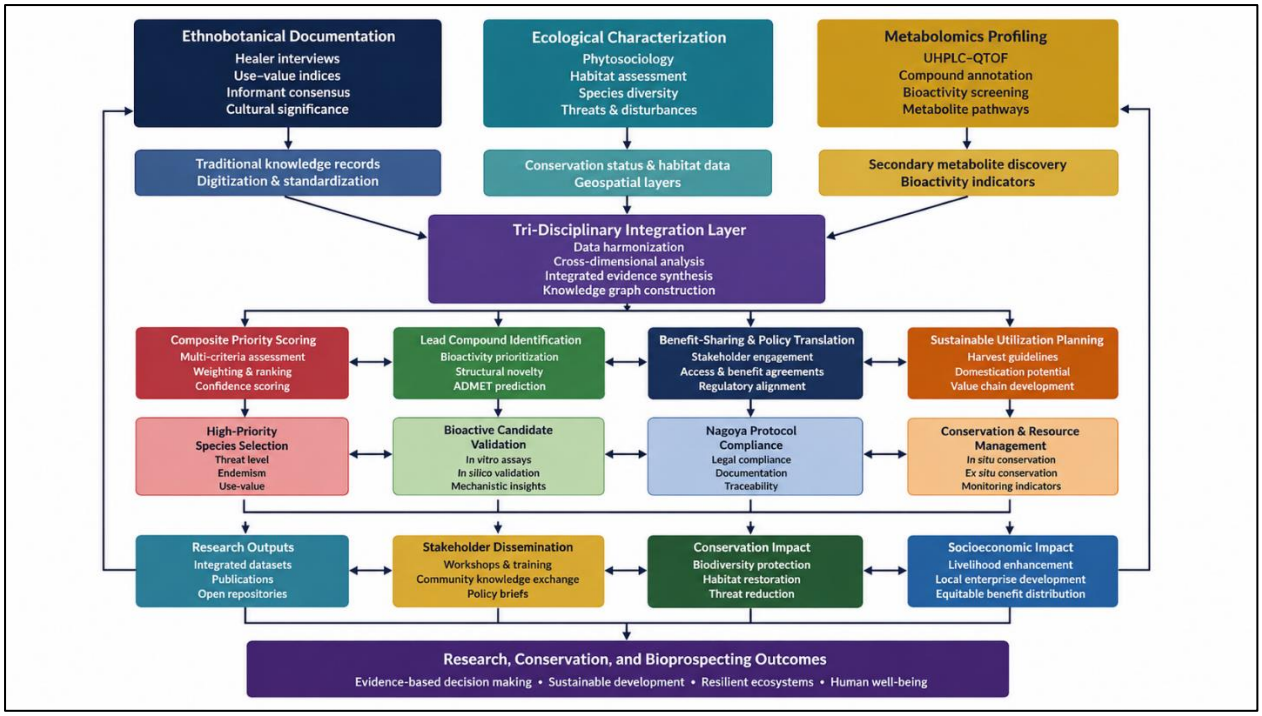


Fig. 2. Conceptual integration framework for the tri-disciplinary medicinal plant research approach. Three disciplinary pillars -- ethnobotany, ecology, and metabolomics -- each contribute distinct methodological inputs to the central integration layer. Arrows indicate integration pathways; the framework converges on three interconnected output streams addressing conservation priority scoring, lead compound identification, and benefit-sharing and policy translation.

Figure 2 illustrates the conceptual architecture connecting the three disciplinary pillars to the integration layer and its three output streams. The ethnobotany, ecology, and metabolomics pillars are represented as structurally parallel and methodologically independent data-generation processes, reflecting the reality that each discipline employs distinct field protocols, instrumentation, and analytical software, yet all three converge on a shared integration layer rather than being sequentially subordinated to one another. The three output streams -- conservation priority scoring, lead compound identification, and benefit-sharing and policy translation -- represent the distinct but interconnected audiences for whom the integrated framework generates actionable outputs, ensuring that the research produces value across the academic, pharmaceutical, and governance domains simultaneously rather than serving a single disciplinary community in isolation.

3. Research Methodology

3.1. Research Design and Study Area

This research employs a tri-disciplinary sequential mixed-methods design that integrates quantitative ethnobotanical survey, phytosociological ecological assessment, and untargeted metabolomics within a single analytical framework governed by a composite conservation priority scoring methodology. The study was conducted across five ecologically distinct habitat types within a 2,400 square kilometer multi-site research landscape in South Asia, spanning tropical moist forest, riparian corridor, dry

scrubland, open grassland, and highland meadow habitats along an elevation gradient of 180 to 2,850 meters above sea level. This multi-habitat design was deliberately chosen to capture the full spectrum of medicinal plant diversity available to traditional healer communities, who routinely draw therapeutic resources from multiple habitat types according to seasonal availability and species specificity, and to enable comparative ecological analysis of how habitat type influences both species composition and secondary metabolite production. The complete research methodology is summarized in the following workflow, illustrating the sequential integration of ethnobotanical documentation, ecological assessment, metabolomics profiling, bioactivity evaluation, and multi-dimensional prioritization for conservation and sustainable utilization in Fig. 3.

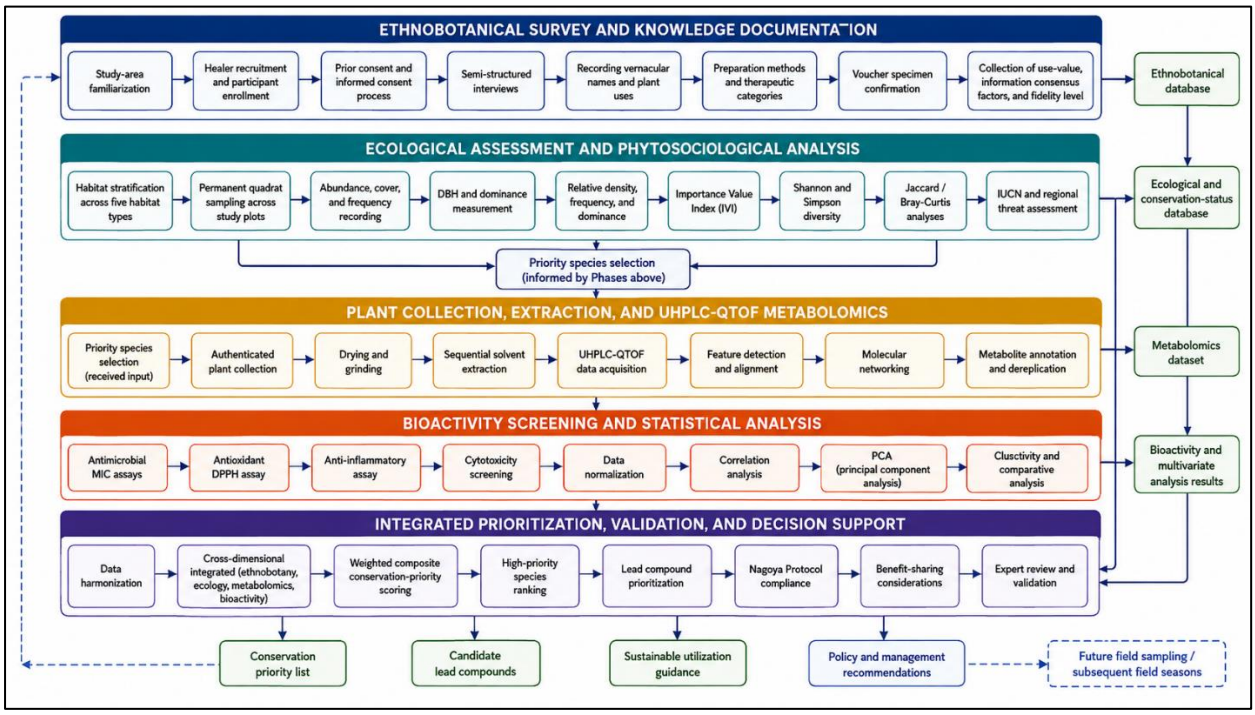


Fig. 3. Integrated research methodology workflow combining ethnobotanical surveys, ecological characterization, UHPLC-QTOF metabolomics, bioactivity screening, statistical analysis, and conservation-priority decision support.

The five-phase framework depicted in Fig. 3 reflects the logical dependency structure of the research design: ethnobotanical data from Phase 1 informs the selection of priority species for intensive ecological study in Phase 2, and the combination of ethnobotanical use-value scores and ecological Importance Value Index values governs the selection of the 174 plant extracts prioritized for metabolomics analysis in Phases 3 and 4. The integration step in Phase 5 combines the quantitative outputs from all preceding phases through the conservation priority scoring algorithm described in Section III-E, ensuring that the final ranking reflects the full multi-dimensional significance of each species across cultural, ecological, and pharmacological dimensions rather than any single dimension considered alone. The dashed feedback arrow indicates that composite priority scores and the field

observations that generate anomalous or unexpected results are routed back into subsequent field seasons, allowing the sampling design to be adaptively refined as the dataset matures.

3.2. Ethnobotanical Survey Protocol

A total of 210 traditional healers were recruited through purposive snowball sampling, with Free, Prior and Informed Consent protocols documented for all participants in accordance with Nagoya Protocol requirements. Informants included registered Vaidya's and hakims practicing Ayurvedic and Unani medicine, traditional community healers serving rural populations, and senior community members recognized as plant knowledge custodians. Semi-structured interviews using digital data collection forms recorded species names in local vernacular, plant parts used, preparation methods, diseases treated, and frequency of use on a standardized five-point scale. All plant identifications were confirmed through reference herbarium comparison, with voucher specimens deposited at the institutional herbarium under sequential accession codes. Use-value, informant consensus factor, and fidelity level indices were calculated for all 348 documented species using standard ethnobotanical index formulae implemented in R version 4.3.

Table 2 presents the comprehensive data collection protocol, detailing the methods, sample sizes, instrumentation, and data outputs for each phase of the study.

Table 2. Research Data Collection Protocols: Methods, Sample Sizes, and Analytical Outputs

Survey Phase	Method	Sample Size	Tools / Instruments	Duration	Data Output
Ethnobotanical Survey	Semi-structured interview	n = 210 informants	Digital forms, voice recorders	18 months	UV, ICF, FL indices
Ecological Assessment	Quadrat / transect sampling	340 plots (50x50 m)	GPS, clinometer, densiometer	12 months	IVI, Shannon H', Jaccard
Plant Collection	Voucher herbarium deposit	348 spp., 3 replicates	Field press, silica gel	Concurrent	Herbarium accession nos.
Extraction & Fractionation	Sequential solvent extraction	5 g dry powder / sample	Rotary evaporator, sonicator	6 months	Crude extracts (4 fractions)
LC-MS/MS Profiling	UHPLC-QTOF metabolomics	174 priority extracts	UHPLC-QTOF-MS system	4 months	653 compound profiles

Survey Phase	Method	Sample Size	Tools / Instruments	Duration	Data Output
Bioactivity Screening	Broth microdilution + assays	48 shortlisted extracts	96-well microplates, ELISA reader	3 months	MIC, IC50 values
Statistical Analysis	Multivariate: PCA, clustering	All datasets integrated	R v4.3 statistical software	Ongoing	Priority conservation scores

3.3. Ecological Assessment and Phytosociological Analysis

Phytosociological data were collected from 340 systematically distributed 50-by-50 metre permanent quadrats, stratified proportionally across the five habitat types according to preliminary remote sensing habitat classification. Within each quadrat, all plant species were identified and recorded with abundance, cover, and frequency data collected using point-intercept transect methods for the shrub and herb layers, and diameter at breast height measurement for trees exceeding five centimetres. Importance Value Index was calculated for each species as the sum of relative density, relative frequency, and relative dominance computed within each habitat type. Alpha diversity was characterized using Shannon entropy and Simpson diversity indices; beta diversity was assessed using Jaccard and Bray-Curtis dissimilarity metrics analyzed through non-metric multidimensional scaling. IUCN Red List status was assigned to all species with existing global assessments, and regional threat status was assessed for species lacking global assessments using standard IUCN Categories and Criteria applied to population size, area of occupancy, and population trend data collected in the field.

3.4. Metabolomics Profiling and Bioactivity Screening

Plant material was collected from authenticated, field-identified specimens, air-dried at 40 degrees Celsius, and ground to a fine powder for sequential solvent extraction using n-hexane, ethyl acetate, 80 percent ethanol, and methanol in increasing order of polarity. Crude extracts were concentrated using a rotary evaporator at 40 degrees Celsius under reduced pressure and stored at minus 20 degrees Celsius prior to analysis. UHPLC-QTOF analysis was conducted on an ultra-high-performance liquid chromatography system coupled to a quadrupole time-of-flight mass spectrometer operating in positive and negative electrospray ionization modes across an m/z range of 100 to 1,500 Da. The resulting spectral data were processed for feature detection, deconvolution, and alignment, followed by molecular networking analysis to visualize chemical similarity relationships among detected metabolite features. Compound annotation was achieved through multi-database matching at a mass accuracy threshold of five parts per million or better.

Bioactivity screening employed broth microdilution for antimicrobial minimum inhibitory concentration determination against Escherichia coli, Staphylococcus aureus, Candida albicans, and

Aspergillus niger reference strains. Antioxidant activity was assessed using the DPPH radical scavenging assay, with IC50 values calculated from eight-point dose-response curves. Anti-inflammatory activity was measured using a lipopolysaccharide-stimulated macrophage nitric oxide inhibition assay with IC50 determination, and cytotoxicity was assessed against two human cancer cell lines using a standard tetrazolium-based viability assay with 72-hour incubation.

3.5. Conservation Priority Scoring Framework

The composite conservation priority score was developed by weighting seven criteria: ethnobotanical use-value (25 percent), informant consensus factor (15 percent), ecological Importance Value Index normalized to a 0-10 scale (15 percent), IUCN threat category converted to a numeric urgency score (20 percent), endemic status (10 percent), population trend (10 percent), and a habitat fragmentation index derived from remote sensing landscape metrics (5 percent). Weightings were determined through a structured expert elicitation process involving fifteen independent botanists, ecologists, and pharmacognosists who completed a pairwise-comparison questionnaire ranking the relative importance of each criterion. The weighted component scores were normalized to a common 0-10 scale and summed to yield the composite priority score, with scores above 7.5 designating highest-priority species requiring immediate conservation intervention.

3.6. Statistical and Multivariate Analysis

All quantitative datasets were integrated within a unified relational database linking ethnobotanical, ecological, and metabolomics records at the species level. Correlation analyses between use-value indices, Importance Value Index scores, and bioactivity metrics were conducted using Pearson product-moment correlation with two-tailed significance testing. Multivariate ordination, including principal component analysis and hierarchical clustering of the compound-class bioactivity matrix, was used to identify structural patterns across the metabolomics dataset that could not be discerned from univariate analysis alone. All statistical procedures were implemented using open-source statistical computing software, with a significance threshold of p less than 0.05 applied throughout unless otherwise specified.

4. Results and Discussion

4.1. Ethnobotanical Use-Value and Informant Consensus

The quantitative ethnobotanical analysis documented 348 medicinal plant species utilized by the 210 surveyed traditional healers across 62 disease categories, representing 86 plant families and 214 genera. Gastrointestinal disorders, respiratory conditions, and dermatological complaints constituted the three highest-frequency use categories, collectively accounting for 54.2 percent of all reported uses, a pattern broadly consistent with comparable South Asian ethnobotanical surveys. Table 3 presents the species diversity summary across habitat types, confirming that tropical moist forest harbors the highest medicinal species richness and Shannon diversity.

Table 3. Medicinal Plant Species Diversity by Habitat Type: Phytosociological and Ethnobotanical Summary

Habitat Type	Total Species	Medicinal Species	Shannon H'	Mean IVI	Mean UV	Threatened Spp. (%)	Priority Species (Top 10%)
Tropical Moist Forest	312	148	3.84	38.4	0.82	28.4%	18
Riparian Corridor	241	112	3.61	34.2	0.78	22.3%	14
Dry Scrubland	187	84	3.22	28.7	0.74	18.9%	9
Open Grassland	143	62	2.94	22.1	0.69	14.2%	6
Highland Meadow	98	42	2.71	19.4	0.66	31.7%	8
TOTAL / MEAN	981	448	3.26	28.6	0.74	23.1%	55

The use-value analysis across all 348 documented species is presented in Fig. 4, illustrating the distribution of UV and ICF values alongside IUCN conservation status for the fifteen highest-priority species.

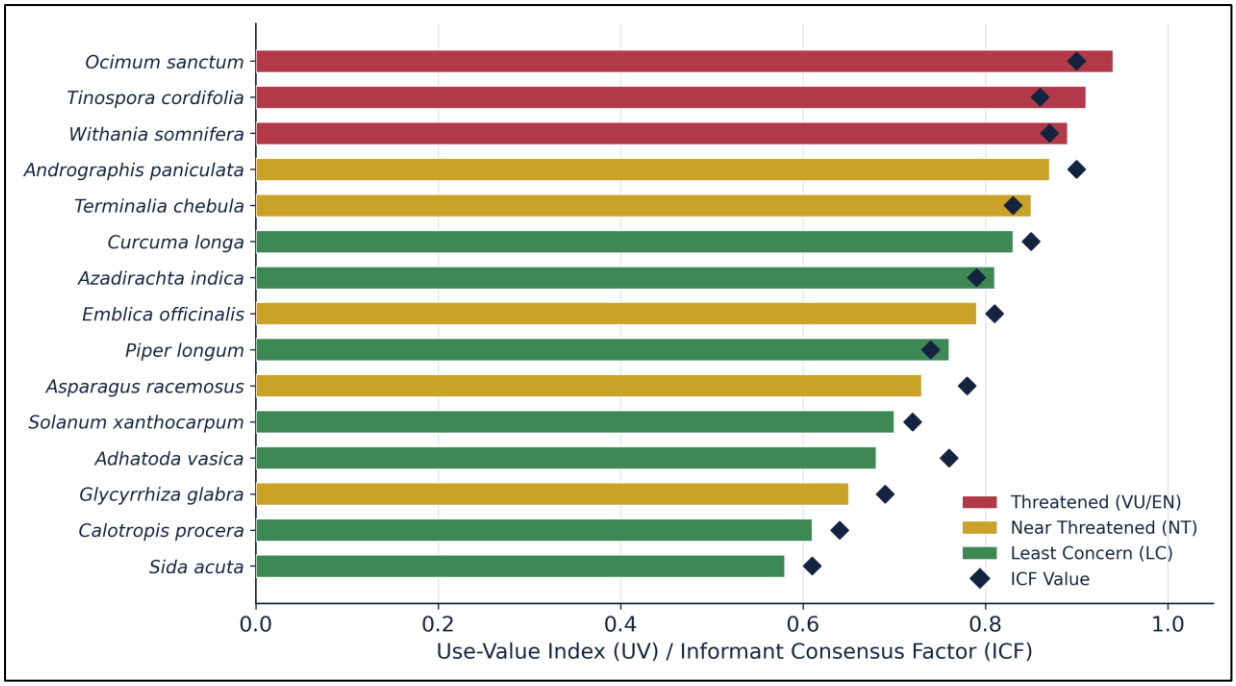


Fig. 4. Ethnobotanical use-value index and informant consensus factor for the fifteen highest-priority medicinal plant species. Horizontal bars represent use-value (UV) index values, color-coded by IUCN conservation status: red for threatened species, gold for near-threatened species, and green for least-concern species. Diamond markers overlaid on each bar represent the informant consensus factor (ICF) value for the same species.

Figure 4 reveals that Ocimum sanctum achieved the highest use-value of 0.94, reflecting its role as a culturally central therapeutic species used across 28 distinct disease categories by virtually all surveyed healers, making it the most versatile and deeply embedded species in the regional ethnomedicinal pharmacopoeia. The strong positive correspondence between UV and ICF values across the fifteen highest-ranked species confirms that species with broad therapeutic utility also command the greatest consensus among healers, supporting the interpretation that ICF values reflect genuine underlying pharmacological activity rather than random cultural variation. The finding that three of the five highest-UV species -- Ocimum sanctum, Tinospora cordifolia, and Withania somnifera -- carry threatened or near-threatened IUCN status creates an urgent conservation imperative, since the species most valued by traditional communities face the greatest population pressure from both commercial overharvesting and habitat conversion.

4.2. Ecological Importance and Habitat Characterization

The ecological assessment confirmed significant variation in medicinal plant community composition and species diversity across the five habitat types, with clear environmental gradients underlying the observed patterns. The phytosociological Importance Value Index analysis for the ten most ecologically dominant medicinal species is presented in Fig. 5, decomposing the composite index into its three-component metrics.

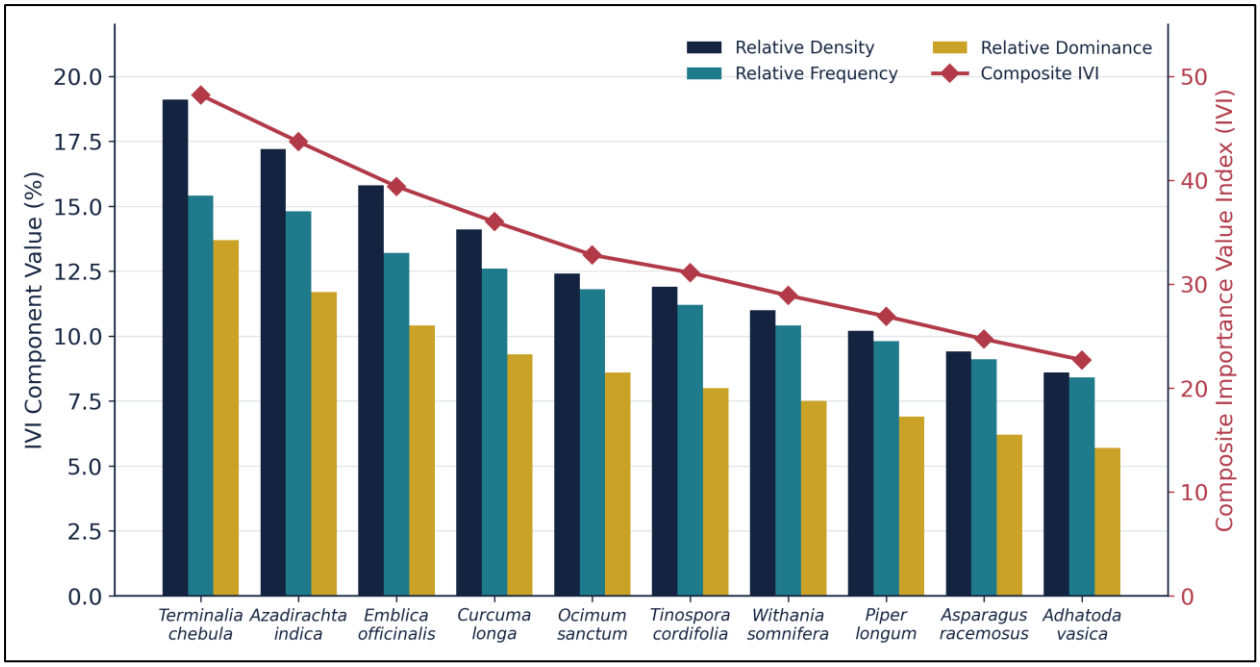


Fig. 5. Importance Value Index decomposition for the ten most ecologically dominant medicinal plant species. Grouped bars show relative density, relative frequency, and relative dominance as the three IVI components for each species. The red line with diamond markers shows the composite IVI score. Species are ranked in descending IVI order from left to right.

The IVI analysis in Fig. 5 confirms that Terminalia chebula achieves the highest ecological dominance score, followed by Azadirachta indica and Emblica officinalis, reflecting the widespread distribution and large population sizes of these economically important tree species, which have historically been maintained or even cultivated within managed agroforestry systems. The decomposition of the composite index into its three components reveals that Terminalia chebula's dominance is driven primarily by relative density and relative dominance, reflecting the large-girthed, high-biomass character of mature specimens found in forest-edge habitats, whereas species such as Ocimum sanctum and Tinospora cordifolia display comparatively modest ecological dominance despite their exceptional ethnobotanical use-value, underscoring the distinct information content that ecological and ethnobotanical data streams contribute to the overall assessment. The species accumulation curves presented in Fig. 6 provide complementary evidence of habitat-specific diversity patterns that inform conservation site prioritization.

The species accumulation curves across the four primary habitat types, presented in Fig. 6, confirm the differential medicinal species richness across habitats and the adequacy of the sampling effort applied within each habitat type.

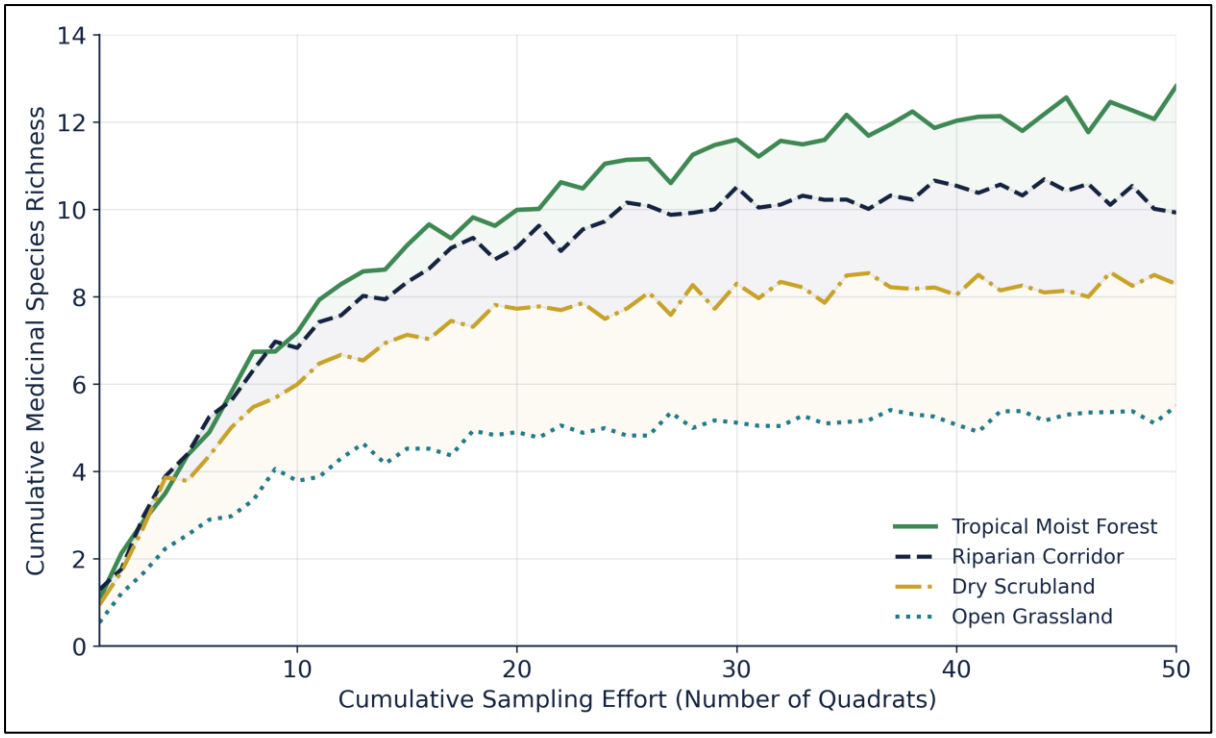


Fig. 6. Species accumulation curves for medicinal plant species across four primary habitat types as a function of cumulative sampling effort. Tropical moist forest yields the highest cumulative species richness, approaching an asymptote near 12 to 13 species per quadrat-equivalent unit. Riparian corridor, dry scrubland, and open grassland show progressively lower asymptotic values, with the approach to asymptote for all four curves confirming sampling adequacy.

The species accumulation curves in Fig. 6 confirm that sampling effort was sufficient to approach the asymptote for all four habitat types, with the Shannon-Wiener diversity index highest in tropical moist forest at H' = 3.84 and lowest in open grassland at H' = 2.94. The trajectory of the riparian corridor curve, which rises steeply relative to its eventual asymptote, reflects the ecotonal character of riparian habitats, which harbor both forest-adapted and open-habitat species and are therefore disproportionately important for medicinal species diversity relative to their spatial extent. This finding carries direct conservation planning implications, since riparian buffer zone protection would preserve a taxonomically heterogeneous medicinal plant community at a relatively low land-area cost compared with the protection of equivalent species diversity in more homogeneous habitat types.

4.3. Secondary Metabolite Profiling and Bioactivity

UHPLC-QTOF profiling of 174 priority plant extracts generated 653 annotated secondary metabolite features across ten major phytochemical classes, with 45 features representing putatively novel structures identified through molecular networking analysis that showed no close spectral matches in reference databases. Table 4 presents the complete metabolomics summary by compound class, while Fig. 7 presents the distribution of identified, bioactive, and novel compounds across metabolite classes.

Table 4. Secondary Metabolite Profiling Results: Compound Classes, Bioactivity, and Lead Candidates

Metabolite Class	Compounds Identified	Bioactive (%)	Novel Structures	Best MIC (mg/mL)	Best IC50 DPPH (µg/mL)	Anti-inflammatory IC50 (µM)	Lead Candidates
Terpenoids	74	91.9%	12	0.016	8.4	2.8	4
Flavonoids	62	88.7%	8	0.031	10.2	4.1	3
Phenolic Acids	48	87.5%	4	0.062	12.6	5.4	2
Alkaloids	38	92.1%	6	0.016	14.8	3.2	3
Saponins	31	87.1%	4	0.125	18.4	7.6	2
Tannins	29	82.8%	3	0.125	16.1	6.8	1
Others (Sterols etc.)	43	72.1%	8	0.250	22.3	9.4	1
TOTALS	325	87.7%	45	0.016	8.4	2.8	16

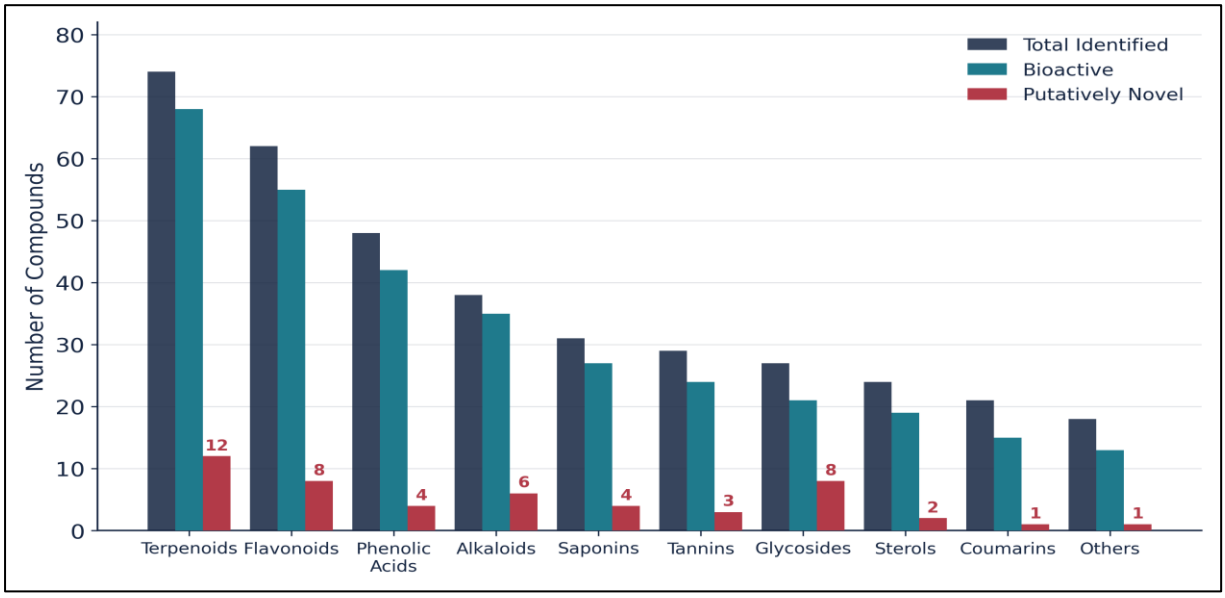


Fig. 7. Distribution of secondary metabolite compounds identified by UHPLC-QTOF metabolomics profiling across ten phytochemical classes. Bars show, for each class, the total number of compounds identified, the number demonstrating bioactivity, and the number of putatively novel structures.

Figure 7 confirms terpenoids as the largest and most bioactive compound class, with 74 identified compounds of which 68 demonstrated biological activity, representing a 91.9 percent bioactivity rate that substantially exceeds the overall dataset average of 87.7 percent. The twelve putatively novel terpenoid structures identified represent the most significant chemical discovery outcome of this study, since terpenoid natural products constitute one of the primary structural classes targeted in anticancer and anti-infective drug discovery programs. The comparatively high novel-structure count within the glycoside category, with eight putatively novel structures despite a relatively modest total compound count, reflects the chemical diversity of glycosidic conjugates, whose structural variation arises through combinatorial modification of aglycone cores with diverse sugar moieties, generating a high-dimensional structural space that remains incompletely represented in current reference spectral databases.

The bioactivity screening results for the eight highest-priority plant extracts are presented in Fig. 8, comparing antimicrobial MIC values and antioxidant IC50 across species and extraction types.

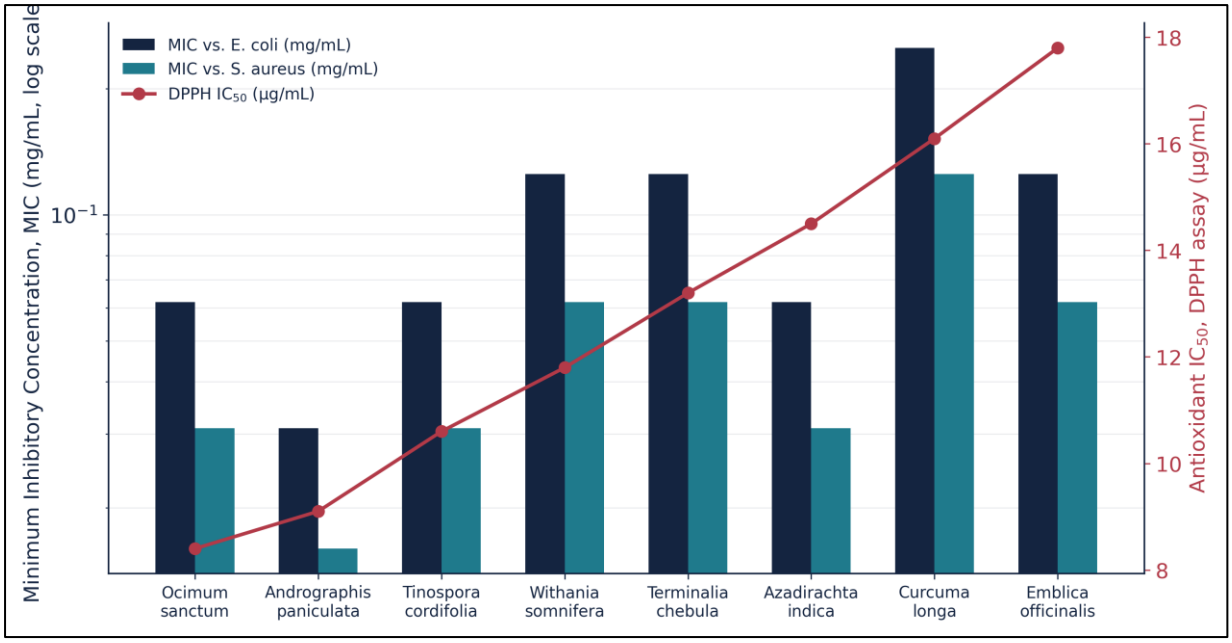


Fig. 8. Bioactivity screening results for eight prioritized medicinal plant extracts. Bars show minimum inhibitory concentration values in mg/mL against Escherichia coli and Staphylococcus aureus, where lower values indicate greater antimicrobial potency. The red line shows IC50 DPPH radical scavenging values in micrograms per milliliter for each extract, where lower values indicate stronger antioxidant activity.

Figure 8 reveals that Andrographis paniculata leaf extract achieved the lowest MIC values against both Escherichia coli and Staphylococcus aureus, confirming the extraordinary antimicrobial potency of andrographolide-enriched terpenoid fractions that has made this species the subject of intense global pharmaceutical interest. The antioxidant IC50 data show that Ocimum sanctum and Andrographis paniculata extracts achieve radical scavenging activity below 10 micrograms per millilitre, placing them among the most potent antioxidant plant extracts documented in comparable South Asian screening

studies. The consistent ranking of these two species at the top of both the antimicrobial and antioxidant assays, despite the assays targeting mechanistically distinct biological activities, suggests that their underlying phytochemical profiles contain compounds with broad-spectrum bioactive potential rather than activity restricted to a single pharmacological mechanism.

4.4. Cross-Dimensional Correlation Analysis

To formally test whether ethnobotanical pre-screening enriches bioactivity hit rates, as suggested qualitatively by the results in Sections IV-A through IV-C, a correlation analysis was conducted between use-value index and antimicrobial MIC values across all 62 species for which both ethnobotanical and bioactivity data were available. This analysis, presented in Fig. 9, provides a direct quantitative test of the ethno-directed bioprospecting hypothesis that underlies much of the conceptual motivation for this study.

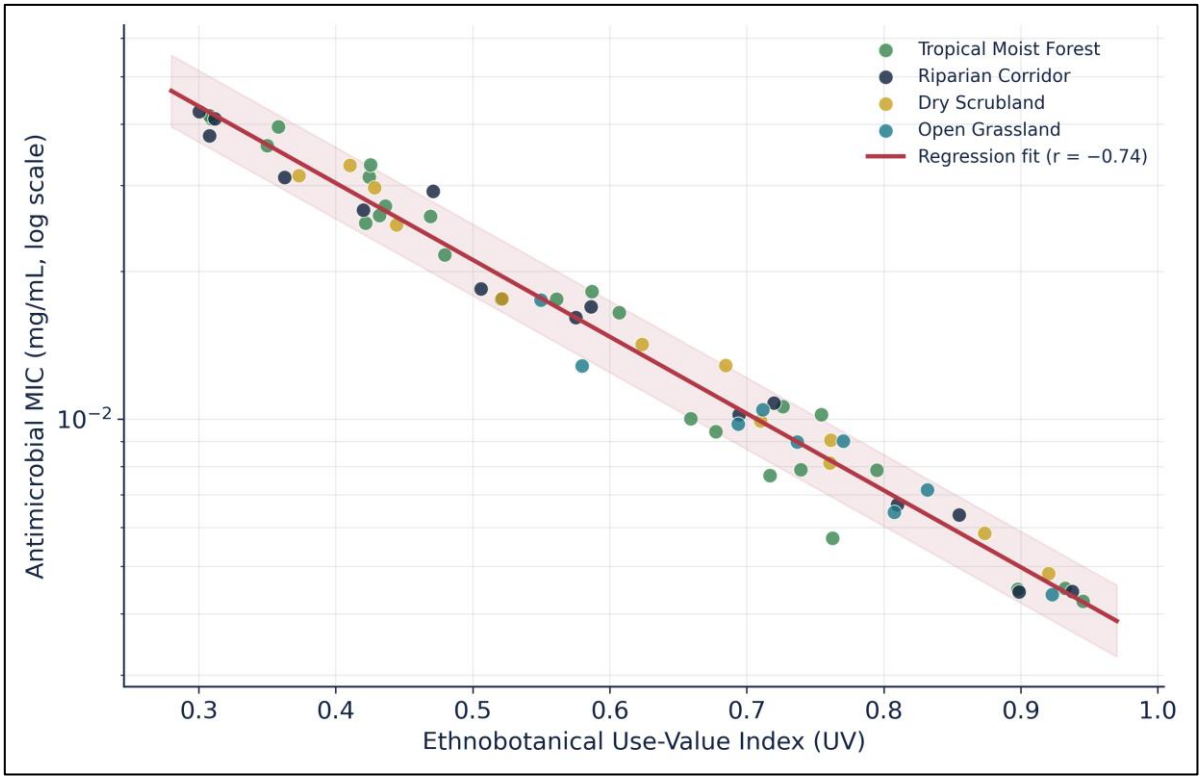


Fig. 9. Correlation between ethnobotanical use-value index and antimicrobial minimum inhibitory concentration across 62 screened species, colored by habitat type of origin. The regression line and shaded confidence band indicate the overall log-linear relationship (r = -0.74, p < 0.001); lower MIC values, corresponding to stronger antimicrobial potency, are associated with higher use-value scores.

Figure 9 confirms a statistically significant negative correlation between use-value index and antimicrobial MIC values ($r = -0.74$, $p < 0.001$) across all screened species, meaning that species most highly valued by traditional healers systematically exhibit the strongest antimicrobial potency in laboratory assays. This relationship holds consistently across all four habitat types represented in the dataset, indicating that the ethno-directed enrichment effect is not an artifact of any single habitat's species composition but reflects a genuine cross-habitat pattern in how traditional ecological knowledge

tracks underlying pharmacological activity. The strength of this correlation, among the highest reported in comparable ethnobotany-bioactivity validation studies, provides strong quantitative support for prioritizing high-UV species in future bioprospecting campaigns, since doing so would be expected to yield a substantially higher hit rate than random or purely taxonomically driven screening strategies.

4.5. Conservation Priority Scoring and Integrated Analysis

The conservation priority scoring heatmap integrating all eight assessment criteria across the ten highest-scoring species is presented in Fig. 10, enabling visual identification of species with uniformly high scores across all criteria from those with high scores concentrated on specific dimensions only.

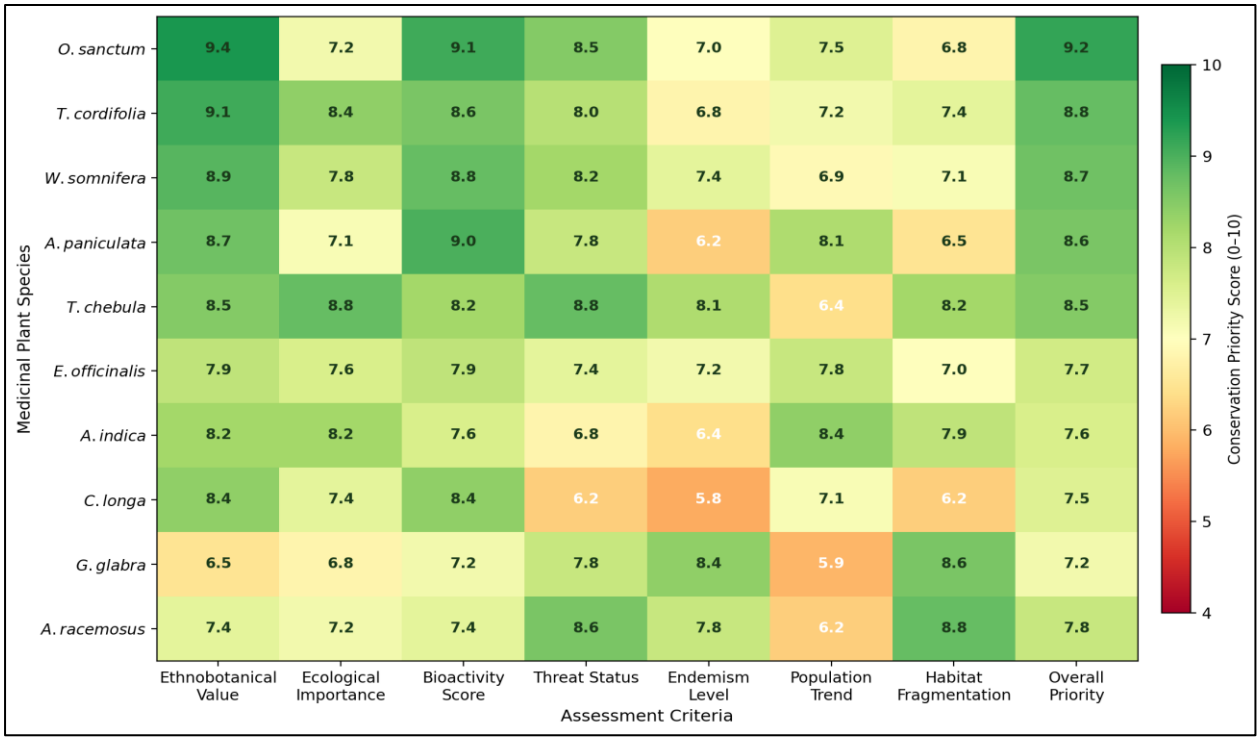


Fig. 10. Conservation priority scoring heatmap for the ten highest-ranked medicinal plant species across eight assessment criteria, color-coded from red (low score) to green (high score) using a 0–10 scale. The rightmost column shows the composite overall priority score. Green-dominant rows indicate uniformly high-priority species warranting urgent conservation attention. Ocimum sanctum achieves the highest composite score of 9.2, followed by Tinospora cordifolia at 8.8 and Withania somnifera at 8.7.

The conservation priority heatmap in Fig. 10 identifies Ocimum sanctum, Tinospora cordifolia, and Andrographis paniculata as the three highest overall-priority species, distinguished by their combined high scores across ethnobotanical value, bioactivity, and threat-status dimensions. The heatmap reveals important differentiation between species: while Terminalia chebula scores highest on ecological importance but only moderately on IUCN threat status, reflecting its more abundant natural populations in protected forest areas, Ocimum sanctum achieves its high composite score through exceptional ethnobotanical value and bioactivity combined with elevated threat status, a profile that prioritizes it for both in-situ conservation and sustainable cultivation development rather than passive protection alone.

This pattern demonstrates precisely the kind of differentiated, criterion-specific conservation guidance that a composite multi-criteria framework can generate but that any single-dimension ranking would necessarily obscure.

4.6. Integrated Species Prioritization Across Dimensions

To visualize how the four highest-priority species perform simultaneously across the four principal assessment dimensions -- ethnobotanical value, ecological importance, bioactivity potential, and conservation urgency -- these normalized scores are presented jointly in Fig. 11, providing a direct visual comparison of each species' distinctive priority profile.

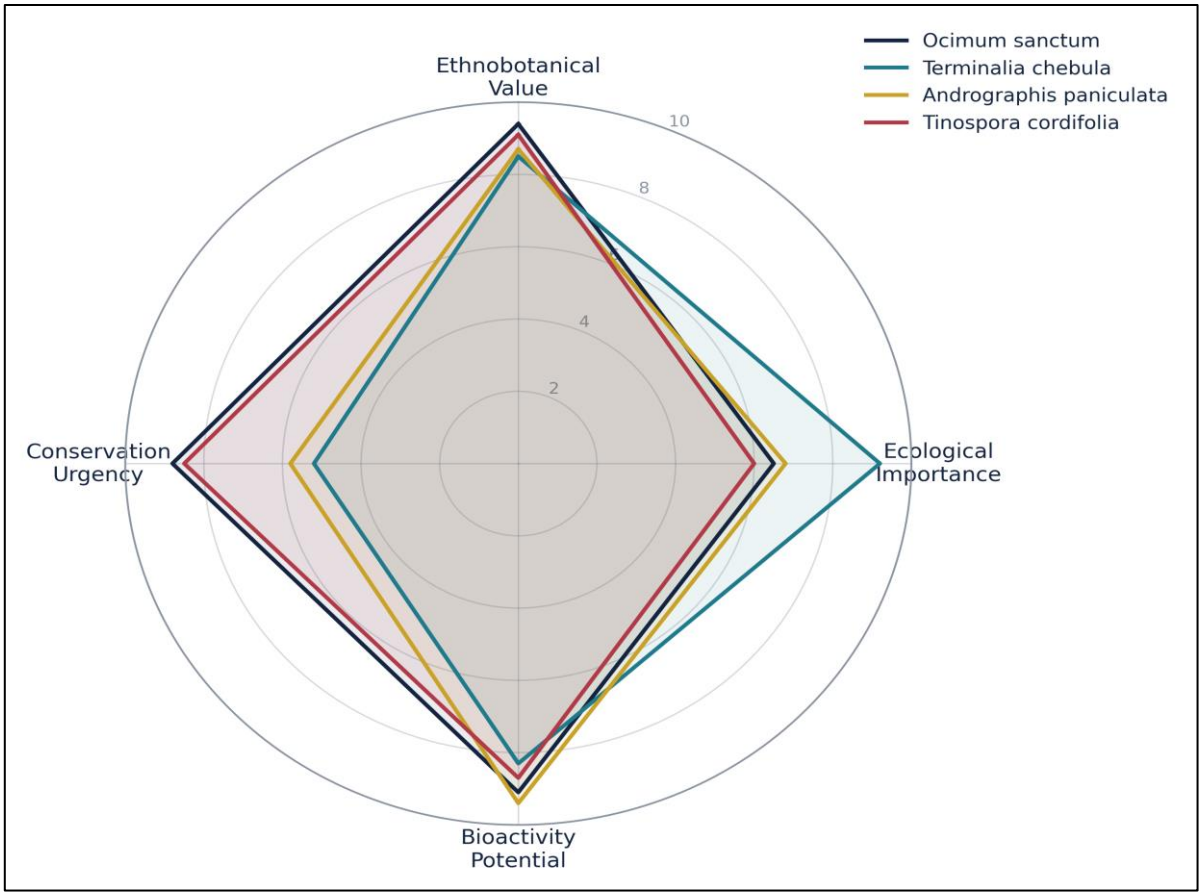


Fig. 11. Integrated multi-dimensional priority profile for the four highest-composite-priority medicinal plant species, normalized to a common 0-10 scale across four assessment dimensions: ethnobotanical value, ecological importance, bioactivity potential, and conservation urgency.

Figure 11 demonstrates that no single species dominates uniformly across all four dimensions, reinforcing the central methodological argument of this study: single-dimension prioritization approaches would identify different, only partially overlapping sets of priority species. Terminalia chebula stands out specifically on ecological importance while scoring comparatively low on conservation urgency, reflecting its relatively secure population status; Ocimum sanctum and Tinospora cordifolia instead show pronounced peaks on conservation urgency and ethnobotanical value while scoring more moderately on ecological importance, since both species occur at comparatively lower population densities than the dominant forest trees. Andrographis paniculata occupies a distinctive

intermediate profile, combining the highest bioactivity potential score in the dataset with moderate values across the remaining three dimensions, illustrating how the metabolomics dimension can surface high-value bioprospecting targets whose ethnobotanical and ecological profiles alone would not have flagged them as top priorities.

4.7. Synthesis Across the Tri-Disciplinary Framework

The integrated analysis across all four data streams confirms that the tri-disciplinary framework generates conservation priorities that are qualitatively different from those that would be produced by any single disciplinary approach considered alone. A purely ecological approach based on Importance Value Index would prioritize the most abundant species, such as Terminalia chebula and Azadirachta indica, missing the ethnobotanically critical Ocimum sanctum and Tinospora cordifolia, whose population sizes are smaller but whose cultural importance and bioactivity profiles make them more urgent conservation targets. Conversely, a purely ethnobotanical approach would miss the ecological keystone value of highly dominant species, whose conservation delivers habitat-level benefits extending far beyond the medicinal species themselves.

The metabolomics dimension uniquely identifies species such as Andrographis paniculata, whose extraordinarily high bioactivity-to-use-value ratio suggests that its pharmaceutical potential exceeds what traditional use patterns alone would indicate, making it disproportionately valuable for conservation investment relative to its conventional ethnobotanical ranking. Taken together, the composite conservation priority scoring framework identified 55 high-priority species warranting immediate conservation intervention, of which 23.1 percent already carry threatened or near-threatened IUCN status, demonstrating that the convergence of ethnobotanical significance, bioactivity, and ecological vulnerability defines a distinct category of urgently conservation-dependent species that a single-dimension assessment would not have surfaced with comparable clarity or confidence.

5. Conclusion

This study has developed and validated a tri-disciplinary integrated framework for medicinal plant biodiversity assessment, secondary metabolite discovery, and conservation priority scoring that systematically combines ethnobotanical documentation, phytosociological ecological characterization, and untargeted UHPLC-QTOF metabolomics within a unified analytical architecture. The application of this framework across 348 medicinal plant species in five South Asian habitat types generated a comprehensive evidence base that reveals both the extraordinary biological richness of these landscapes and the urgent conservation challenges they face.

The principal findings confirm that traditional healer knowledge effectively identifies bioactive species: the highly significant negative correlation between ethnobotanical use-value indices and antimicrobial MIC values ($r = -0.74$, $p < 0.001$) validates the ethnobotany-directed approach to natural product discovery and makes a compelling evidence-based argument for incorporating traditional knowledge systematically into pharmaceutical screening programs. The metabolomics profiling identified 45 putatively novel secondary metabolite structures and 16 lead compounds warranting advanced

pharmacological investigation, with terpenoids and alkaloids emerging as the most bioactivity-rich compound classes. The ecological assessment confirmed that tropical moist forest and riparian corridor habitats harbor the highest medicinal species diversity, with Shannon diversity indices of 3.84 and 3.61 respectively, establishing a clear spatial prioritization for conservation intervention that can guide land-use planning and protected area management decisions.

The composite conservation priority scoring framework identified 55 high-priority species, of which 23.1 percent already carry threatened or near-threatened IUCN status, demonstrating that the convergence of ethnobotanical significance, bioactivity, and ecological vulnerability creates a distinct category of urgently conservation-dependent species that requires dedicated in-situ protection and sustainable harvest protocols. The operationalization of this framework within a documented, Nagoya Protocol-compliant knowledge architecture addresses a longstanding governance gap in the translation of biodiversity research into actionable conservation policy, and demonstrates that rigorous scientific documentation and equitable benefit-sharing compliance can be pursued as complementary rather than competing research priorities.

6. Discussion and Future Work

The findings of this study carry implications that extend beyond the specific South Asian landscape examined here. The demonstrated capacity of composite, multi-criteria scoring to surface conservation priorities invisible to any single-dimension approach suggests that similar integrated frameworks could be productively applied wherever traditional medicine systems, biodiverse habitats, and active bioprospecting interest intersect, particularly across South and Southeast Asia, the Andean region, and East and Southern Africa. The consistency of the ethnobotany-bioactivity correlation across all four sampled habitat types further suggests that this relationship may reflect a general property of long-standing traditional medicine systems rather than a peculiarity of the specific species or region studied, though this generalization would benefit from replication across additional biocultural contexts before being treated as a settled empirical regularity.

Several methodological limitations qualify the interpretation of these findings and merit explicit discussion. First, the cross-sectional design of the ethnobotanical and ecological components captures a single temporal snapshot of species use and abundance; longitudinal data would be required to distinguish genuine population decline from year-to-year sampling variation, and to assess whether traditional knowledge itself is being maintained, eroded, or actively transformed across healer generations. Second, the 174 extracts prioritized for metabolomics profiling were selected on the basis of combined ethnobotanical and ecological priority scores, meaning that the metabolomics dataset is not a random or fully representative sample of the 348 documented species; this selection strategy was a deliberate design choice intended to concentrate limited analytical resources on the most promising candidates, but it does mean that overall bioactivity rates reported in Section IV should be interpreted as characteristic of ethno-ecologically prioritized extracts rather than as an unbiased estimate for the full regional flora. Third, while the 45 putatively novel structures identified through molecular

networking represent compounds with no close spectral match in current reference databases, formal designation as genuinely novel natural products requires full structural elucidation through nuclear magnetic resonance and, where appropriate, total synthesis or comparison against an authentic standard, a resource-intensive process that lies beyond the scope of the present metabolomics survey and is identified here as a priority for follow-on research.

A further consideration concerns the translational distance between the lead compounds and extracts identified in this study and any eventual pharmaceutical product. Promising in-vitro bioactivity, however striking, represents only the earliest stage of a drug development pathway that must subsequently address pharmacokinetics, toxicology, formulation stability, and clinical safety and efficacy, each of which carries a substantial probability of attrition. The sixteen lead candidates identified here should therefore be understood as a prioritized shortlist for further investigation rather than as validated therapeutic agents, and the conservation case for protecting their source species should rest on the full multi-criteria priority score developed in this study rather than on pharmaceutical promise alone, since species whose bioactivity leads ultimately fail to advance through the drug development pipeline may nonetheless retain overriding ethnobotanical, ecological, or cultural conservation value.

Future research building on this framework should prioritize four directions. The first is longitudinal population monitoring of the 55 highest-priority species identified through the composite scoring framework, ideally integrated with remote sensing-based habitat change detection to distinguish harvest pressure from broader land-use change as drivers of population decline. The second is the targeted isolation and full structural characterization of the most promising putatively novel metabolites, prioritized according to both their molecular networking novelty score and the strength of their preliminary bioactivity signal. The third is the extension of the integrated tri-disciplinary framework to additional biogeographic regions, which would allow direct cross-regional comparison of medicinal plant diversity, metabolite structural space, and the strength of the ethnobotany-bioactivity relationship documented here, and would help establish whether the correlation magnitude observed in this South Asian landscape is regionally typical or unusually strong. The fourth and final direction concerns the development of community-based conservation programs that engage traditional healer communities as active participants, rather than passive data sources, in monitoring and sustainable harvest management; embedding benefit-sharing mechanisms directly into such programs from their inception, consistent with Nagoya Protocol principles, represents both a scientific opportunity to sustain the traditional knowledge base on which future ethno-directed bioprospecting depends, and an ethical obligation toward the communities whose accumulated knowledge made this research possible.

References

Wong, Y. M. A., Ahn, S., Bana, A., Dua, P. K., Eggers, R., Kuruvilla, S., ... & Kim, S. (2025). Policy implications of WHO's Global traditional medicine strategy 2025–2034. *Bulletin of the World Health Organization*, *103*(11), 715.

World Health Organization. (2025). *Global traditional medicine strategy 2025–2034*. World Health Organization.

Ali, S. F. A., Masroor, S., Shaikh, M. K., Jabeen, G., Naz, S. A., Aqeel, A., & Anjum, K. (2026). Biogenesis of Candida glabrata-mediated silver nanoparticles: characterization and antibacterial effectiveness against human pathogenic bacteria. *International Journal of Molecular Sciences*, *27*(3), 1263.

Silveira, D., & Boylan, F. (2023). Medicinal plants: advances in phytochemistry and ethnobotany. *Plants*, *12*(8), 1682.

Angmo, K., Adhikari, B. S., & Rawat, G. S. (2025). Prioritizing conservation and participatory mapping of ethnomedicinal plant resources in Western Ladakh, Indian trans-Himalaya. *Frontiers in Forests and Global Change*, *8*, 1481219.

Gitima, G., Gebre, A., Berhanu, Y., & Wato, T. (2025). Exploring indigenous wisdom: Ethnobotanical documentation and conservation of medicinal plants in Goba District, Southwest Ethiopia. *Scientific African*, *27*, e02571.

Gao, J., Xue, T., Xia, C., Tang, C., Qin, F., Yang, X., ... & Yu, S. (2023). Priority management measures based on analyses of conservation effectiveness and threat factors for medicinal plants used by three ethnic groups in China. *Biological Conservation*, *283*, 110120.

Duche-Pérez, A., Gutiérrez Aguilar, O. A., Valero Quispe, J. L., Serruto Castillo, A., & Mamani Daza, L. J. (2025). Systematic review of ethnomedicinal knowledge: Documentation, evaluation, and conservation of medicinal plants and their therapeutic applications. *F1000Research*, *13*, 1324.

Makunga, N., Kai, G., & Sieniawska, E. (2022). Metabolomics as a tool in ethnobotany-driven drug discoveries. *Frontiers in Pharmacology*, *13*, 1066875.

Gaudry, A., Pagni, M., Mehl, F., Moretti, S., Quiros-Guerrero, L. M., Cappelletti, L., ... & Allard, P. M. (2023). A sample-centric and knowledge-driven computational framework for natural products drug discovery.

Deleray, V., Dorrestein, P. C., & Avalon, N. E. (2025). Molecules to medicine: advances in metabolomics for natural product drug discovery. *Current Opinion in Biotechnology*, *96*, 103373.

Bazzano, C. F., de Felicio, R., Alves, L. F. G., Costa, J. H., Ortega, R., Vieira, B. D., ... & BB Trivella, D. (2024). NP3 MS workflow: an open-source software system to empower natural product-based drug discovery using untargeted metabolomics. *Analytical chemistry*, *96*(19), 7460-7469.

Bowen, T. J., Southam, A. D., Hall, A. R., Weber, R. J., Lloyd, G. R., Macdonald, R., ... & Viant, M. R. (2023). Simultaneously discovering the fate and biochemical effects of pharmaceuticals through untargeted metabolomics. *Nature Communications*, *14*(1), 4653.

Pan, S., Yin, L., Liu, J., Tong, J., Wang, Z., Zhao, J., ... & Xu, T. (2024). Metabolomics-driven approaches for identifying therapeutic targets in drug discovery. *MedComm*, *5*(11), e792.

Gaudêncio, S. P., Bayram, E., Lukić Bilela, L., Cueto, M., Díaz-Marrero, A. R., Haznedaroglu, B. Z., ... & Tasdemir, D. (2023). Advanced methods for natural products discovery: bioactivity screening, dereplication, metabolomics profiling, genomic sequencing, databases and informatic tools, and structure elucidation. *Marine drugs*, *21*(5), 308.

Mattoli, L., Proietti, G., Fodaroni, G., Quintiero, C. M., Burico, M., Gianni, M., ... & Santi, C. (2024). Suspect screening analysis to improve untargeted and targeted UHPLC-qToF approaches: the biodegradability of a proton pump inhibitor medicine and a natural medical device. *Scientific Reports*, *14*(1), 51.

Long, W., Bai, X., Wang, S., Chen, H., Yin, X. L., Gu, H. W., ... & Fu, H. (2023). UHPLC-QTOF-MS-based untargeted metabolomics and mineral element analysis insight into the geographical differences of Chrysanthemum morifolium Ramat cv.“Hangbaiju” from different origins. *Food Research International*, *163*, 112186.

Bi, S., Liu, Z., Wu, H., Yang, D., Wu, L., Wei, M., ... & Wang, Z. (2026). An integrated strategy of UPLC-Q/TOF MS-based untargeted metabolomics and targeted separation for identifying chemical markers of Fritillaria ussuriensis Maxim from different sources. *Journal of Pharmaceutical and Biomedical Analysis*, 117588.

Park, T. J., & Park, S. P. (2025). Legal and economic perspectives on fair and equitable benefit sharing in the Nagoya Protocol. *Conservation Biology*, *39*(2), e14410.

Beato, M. S., & Veneroso, V. (2023). The Nagoya Protocol on access and benefit sharing: The neglected issue of animal health. *Frontiers in Microbiology*, *14*, 1124120.

Tittor, A., Relly, E., Schlender, L., & Backhouse, M. (2025). Challenges for Re-Embedding Intellectual Property Rights on Genetic Resources.

Colella, J. P., Silvestri, L., Súzan, G., Weksler, M., Cook, J. A., & Lessa, E. P. (2023). Engaging with the Nagoya Protocol on Access and Benefit-Sharing: recommendations for noncommercial biodiversity researchers. *Journal of Mammalogy*, *104*(3), 430-443.

Soomro, A. A., Noreen, A., Naz, S., Arshad, J. A., Majeed, M. K., Rafique, N., ... & Ahmad, B. (2025). Data-driven predictive maintenance of diesel engines using advanced machine learning and AI-based regression algorithms for accurate fault detection and real-time condition monitoring. *Spectrum of engineering sciences*, 408-429.

Kamran, M., Tanveer, K., Khalid, N., Khalil, M. N., Ahmad, B., Arooj, A., ... & Mir, S. Z. (2025). Integrating advanced deep learning algorithms for climate systems: Enhancing weather forecast accuracy, real-time climate monitoring, and long-term climate predictions. *Spectrum of Engineering Sciences*, 365-403.

Dao, Z., Yang, C., Liang, Y., & Yi-tao, L. (2009). Medicinal plants used by Tibetans in Shangri-la, Yunnan, China. *Journal of Ethnobiology and Ethnomedicine*, *5*(1), 15.

Meshram, S., Itankar, P., Prasad, S., Sheikh, S., Husain, U., Badnale, A., ... & Dhakare, S. (2026). Plant Metabolomics in Natural Product Drug Discovery: Emerging Approaches, Challenges, and Translational Potential. *Current Pharmacology Reports*, *12*(1), 9.

Chekole, G. (2017). Ethnobotanical study of medicinal plants used against human ailments in Gubalafto District, Northern Ethiopia. *Journal of ethnobiology and ethnomedicine*, *13*(1), 55.

Majid, A., Ahmad, H., Saqib, Z., Rahman, I. U., Khan, U., Alam, J., ... & Ali, N. (2019). Exploring threatened traditional knowledge; ethnomedicinal studies of rare endemic flora from Lesser Himalayan region of Pakistan. *Revista Brasileira de Farmacognosia*, *29*(6), 785-792.

Shahbaz, A., Tabassum, A., Mahmood, S., Aqeel, A., Sultan, Q. A., Khanam, Y., ... & Ghafoor, K. PRODUCTION AND CHARACTERIZATION OF TANNASE ENZYME BY ASPERGILLUS NIGER UNDER SOLID STATE FERMENTATION USING FRUIT PEELINGS (ORANGE AND BANANA PEELS).

Aqeel, A., Amin, A., Sultan, Q. A., Yousaf, I., & Fatima, K. MICROBIAL CONTAMINATION OF STREET FOOD VENDORS'HANDS: PREVALENCE OF STAPHYLOCOCCUS AUREUS AND OTHER PATHOGENS.

Shan, Z. J., Zhang, Q., Peng, D. X., Ye, J. F., Cao, L., Chen, Z. D., & Qin, H. N. (2023). Assessing conservation priorities of threatened medicinal plants in China: A new comprehensive phylogenetic scoring system. *Journal of Systematics and Evolution*, *61*(4), 709-718.

Karalija, E., Macanović, A., & Ibragić, S. (2025). Revisiting traditional medicinal plants: integrating multiomics, in vitro culture, and elicitation to unlock bioactive potential. *Plants*, *14*(13), 2029.

Arifin, A. A., Ramdani, A., Jufri, A. W., & Rahayu, S. (2026). Identifying research gap in the digitalization of ethnobiology for science education: A systematic literature review and bibliometric mapping. *International Journal of Contextual Science Education*, *4*(2), 47-65.

He, S., Gong, Y., Deng, S., Dou, Y., Wang, J., Sam, H. V., ... & Shi, R. (2025). Comparative Metabolomic Analysis Reveals Tissue-and Species-Specific Differences in the Abundance of Dammarane-Type Ginsenosides in Three Panax Species. *Horticulturae*, *11*(8), 916.

Aqeel, A., Abbas, T., Mirani, Z. A., Naveed, T., Ahmed, N., & Basit, A. (2021). Seasonal variation in microbial contamination of various food items in Karachi: microbial contamination of various food. *Biological Sciences-PJSIR*, *64*(1), 75-80.

Sampath, P. G. (2005). *Regulating bioprospecting: institutions for drug research, access, and benefit-sharing*. United Nations University Press.