

METABOLOMIC AND PROTEOMIC CHARACTERIZATION OF INDIGENOUS MEDICINAL PLANTS FOR TARGETED ANTICANCER DRUG DISCOVERY IN PAKISTAN

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Abstract

Cancer remains a major global health burden characterized by genetic heterogeneity, therapeutic resistance, and dysregulation of multiple signaling pathways. Conventional anticancer therapies are often limited by toxicity, high cost, and lack of specificity, particularly in low- and middle-income countries. In this context, indigenous medicinal plants of Pakistan represent a promising yet underexplored source of bioactive compounds with potential anticancer properties. This study aimed to systematically characterize the metabolomic and proteomic profiles of selected medicinal plants and to identify multi-target anticancer mechanisms through integrated systems biology and network pharmacology approaches. A multi-omics analytical framework was employed, utilizing LC-MS/MS and GC-MS for metabolomic profiling and shotgun proteomics for protein identification and functional annotation. Bioinformatics tools were applied for data processing, differential analysis, and pathway enrichment. Identified metabolites and proteins were mapped onto cancer-related signaling networks, including PI3K/Akt, MAPK, p53, and NF- κ B pathways. Network pharmacology analysis was conducted to construct compound-target-pathway interaction maps and to elucidate synergistic mechanisms of action. The integrated analysis revealed that selected medicinal plants possess diverse secondary metabolites, including flavonoids, alkaloids, terpenoids, and phenolic compounds, along with functionally active proteins associated with apoptosis, stress response, and detoxification processes. Pathway enrichment analysis demonstrated significant modulation of key oncogenic signaling cascades involved in cell proliferation, survival, and apoptosis regulation. The findings suggest that these plants exert anticancer effects through a multi-target, systems-level mechanism rather than single-compound activity. This study provides a comprehensive omics-based framework for anticancer drug discovery from indigenous medicinal plants of Pakistan and bridges the gap between ethnopharmacological knowledge and modern computational biology. The results offer a scientific basis for further experimental validation and potential development of novel, cost-effective anticancer therapeutics.

INTRODUCTION

Cancer remains one of the most complex and heterogeneous diseases globally, characterized by uncontrolled cellular proliferation, genomic instability, and dysregulation of multiple signaling pathways. Despite significant advances in chemotherapy, radiotherapy, immunotherapy, and targeted therapies, cancer continues to account for substantial morbidity and mortality, particularly in low- and middle-income countries where access to advanced treatments is limited (Sung et al., 2021). Conventional anticancer agents are often associated with systemic toxicity, drug resistance, and limited specificity, which necessitates the exploration of novel, multi-targeted, and biologically compatible therapeutic agents.

Natural products derived from medicinal plants have historically played a central role in drug discovery, particularly in oncology. A substantial proportion of clinically approved anticancer drugs are either directly derived from natural compounds or inspired by their structural scaffolds (Newman & Cragg, 2020). Medicinal plants produce a diverse array of secondary metabolites, including alkaloids, flavonoids, terpenoids, phenolics, and glycosides, many of which exhibit potent cytotoxic, anti-proliferative, and apoptosis-inducing properties (Atanasov et al., 2021). However, the majority of plant species, especially those indigenous to biodiverse regions such as Pakistan, remain insufficiently characterized at the molecular and systems level.

Pakistan possesses a rich ethnobotanical heritage with more than 6,000 plant species, many of which are traditionally used in Unani and Ayurvedic systems for the treatment of inflammatory disorders, infections, and tumors. Despite their extensive traditional use, scientific validation of their bioactive constituents and molecular mechanisms of action remains limited (Hussain et al., 2020). Most existing studies focus on crude extract screening, lacking comprehensive molecular-level characterization of metabolites and proteins responsible for therapeutic effects.

Recent advancements in **omics technologies**, particularly metabolomics and proteomics, have revolutionized natural product research by

enabling high-throughput, system-wide profiling of small molecules and proteins in biological systems. Metabolomics facilitates the identification and quantification of secondary metabolites, while proteomics enables the analysis of functional protein expression and post-translational modifications associated with biological activity (Wang et al., 2022). When integrated with network pharmacology and computational biology, these approaches allow the systematic identification of multi-target interactions and pathway-level effects relevant to cancer progression and therapy (Lee et al., 2022). In this context, indigenous medicinal plants of Pakistan represent an untapped reservoir for anticancer drug discovery. However, a major scientific gap exists in the integration of metabolomic and proteomic datasets to elucidate the synergistic mechanisms of plant-derived compounds. Furthermore, limited studies have applied systems biology approaches to map the interactions between plant metabolites, protein targets, and cancer-associated signaling pathways such as PI3K/Akt, MAPK, and p53-mediated apoptosis.

Therefore, there is an urgent need for a comprehensive, integrative omics-based investigation that combines metabolomic and proteomic profiling with bioinformatics-driven pathway analysis to systematically identify and validate anticancer bioactive compounds from indigenous medicinal plants of Pakistan.

Problem Statement

Despite the rich ethnopharmacological heritage of Pakistan and the widespread traditional use of medicinal plants for cancer-related and inflammatory conditions, there remains a critical lack of systematic, molecular-level characterization of their bioactive constituents. Most existing research is limited to preliminary phytochemical screening or *in vitro* cytotoxicity assays, without comprehensive integration of metabolomic and proteomic data to elucidate mechanistic pathways. This fragmentation in scientific understanding has resulted in a significant translational gap between traditional medicinal knowledge and modern drug development pipelines. Consequently, the

therapeutic potential of many indigenous plant species remains underexplored and scientifically unvalidated. Furthermore, the absence of integrated systems biology approaches limits the identification of synergistic interactions among metabolites and proteins that may contribute to anticancer activity.

In addition, cancer treatment in Pakistan faces challenges related to affordability, accessibility, and toxicity of conventional chemotherapeutic agents, highlighting the urgent need for locally sourced, cost-effective, and biologically compatible alternatives. Without advanced omics-based profiling and network-level analysis, the discovery of novel anticancer agents from indigenous flora remains largely empirical and inefficient.

Therefore, the central problem lies in the absence of an integrated metabolomic and proteomic framework for systematically characterizing indigenous medicinal plants of Pakistan and translating their bioactive potential into targeted anticancer drug discovery.

Research Questions

1. What are the major metabolomic profiles and secondary bioactive compounds present in selected indigenous medicinal plants of Pakistan with reported anticancer potential?
2. Which proteomic signatures and functional proteins are associated with anticancer-related biological activities in these medicinal plants?
3. How do identified metabolites and proteins interact with key cancer-related molecular targets and signaling pathways?
4. What are the most significant anticancer-related pathways (e.g., PI3K/Akt, MAPK, p53) influenced by plant-derived compounds?
5. Can integrative metabolomic-proteomic and network pharmacology approaches identify multi-target therapeutic mechanisms for cancer treatment?

Research Objectives

General Objective

To investigate and characterize the metabolomic and proteomic profiles of selected indigenous medicinal plants of Pakistan for the identification

of potential anticancer compounds and molecular targets.

Specific Objectives

1. To identify and profile secondary metabolites present in selected medicinal plants using LC-MS/MS and GC-MS techniques.
2. To analyze the proteomic composition of plant extracts and identify functionally relevant proteins associated with anticancer activity.
3. To construct and analyze molecular interaction networks between identified metabolites, proteins, and cancer-related targets.
4. To evaluate the involvement of key signaling pathways in cancer progression affected by plant-derived bioactive compounds.
5. To integrate metabolomic and proteomic datasets using bioinformatics tools for multi-target anticancer drug discovery.

Significance of the Study

Theoretical Significance

This study contributes to the advancement of systems pharmacology by integrating metabolomics, proteomics, and network biology to understand the multi-target mechanisms of plant-derived anticancer agents. It expands the theoretical framework of natural product drug discovery by moving beyond single-compound analysis toward holistic, systems-level interpretation of biological activity.

Practical Significance

The findings of this research provide a scientific basis for identifying novel anticancer compounds from indigenous medicinal plants of Pakistan. These results may facilitate the development of affordable, effective, and less toxic therapeutic agents, particularly relevant for resource-constrained healthcare systems. Additionally, the study supports the prioritization of specific plant species for further in vitro and in vivo validation.

Policy Significance

The study offers evidence-based insights that can inform national health and research policies regarding the conservation and sustainable utilization of medicinal plant biodiversity. It also

supports policy initiatives aimed at strengthening phytopharmaceutical research infrastructure, encouraging public-private partnerships, and integrating traditional knowledge systems into modern drug development frameworks.

Literature Review

Natural products have historically played a foundational role in anticancer drug discovery and continue to represent a major source of clinically relevant chemotherapeutic agents. A substantial proportion of approved anticancer drugs are either directly derived from plant secondary metabolites or inspired by their molecular scaffolds. These bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolic acids, exhibit diverse pharmacological activities such as apoptosis induction, cell cycle arrest, oxidative stress modulation, and angiogenesis inhibition. Contemporary evidence emphasizes that plant-derived compounds are particularly valuable in oncology due to their inherent structural diversity and ability to modulate multiple molecular targets simultaneously, which is critical for addressing tumor heterogeneity and therapeutic resistance (Newman & Cragg, 2020; Atanasov et al., 2021). Despite this established importance, traditional natural product research has largely relied on reductionist approaches focusing on isolation of single compounds and evaluation of isolated bioactivity. Such approaches often overlook synergistic interactions among multiple phytochemicals within plant systems, thereby limiting the discovery of complex therapeutic networks that may be more effective against multifactorial diseases such as cancer.

Pakistan's indigenous flora represents a rich but underexplored reservoir of medicinal resources, shaped by diverse ecological zones including the Himalayan, Hindukush, and Karakoram regions. Ethnobotanical evidence indicates widespread traditional use of medicinal plants such as *Withania somnifera*, *Berberis lycium*, *Azadirachta indica*, and *Ajuga bracteosa* for treating tumors, inflammation, and infectious diseases. These plants contain a wide range of bioactive constituents with reported cytotoxic, antioxidant, and anti-inflammatory properties (Hussain et al.,

2020). For instance, berberine isolated from *Berberis lycium* has demonstrated anticancer activity through modulation of AMPK, p53, and mitochondrial apoptotic pathways, while withanolides from *Withania somnifera* have shown inhibitory effects on NF- κ B signaling and tumor cell proliferation (Gupta et al., 2020; Ahmad et al., 2023). However, the majority of these findings remain limited to preliminary in vitro studies, lacking comprehensive molecular validation and systems-level interpretation.

Metabolomics has emerged as a transformative tool in natural product research by enabling high-throughput identification and quantification of small-molecule metabolites in biological systems. Techniques such as LC-MS and GC-MS allow detailed profiling of phytochemical composition, facilitating the discovery of novel bioactive compounds and metabolic signatures associated with therapeutic activity. Recent studies highlight that metabolomic approaches can reveal previously uncharacterized flavonoids and phenolic compounds with significant anticancer potential, particularly through mechanisms involving oxidative stress regulation and apoptosis induction (Wang et al., 2022; Zhang et al., 2021). Nevertheless, metabolomic data alone cannot fully elucidate biological function without integration with functional protein-level information.

Proteomics complements metabolomics by providing insights into protein expression, regulation, and interaction networks underlying biological activity. In plant-based systems, proteomic analyses have identified stress-related proteins, defense enzymes, and bioactive peptides that may contribute to pharmacological effects, including anticancer activity. These proteins can influence cellular pathways related to immune modulation, detoxification, and programmed cell death (Rhee et al., 2020). However, proteomics remains underutilized in medicinal plant research, particularly in developing regions where advanced analytical infrastructure is limited.

Cancer is widely recognized as a network disease involving dysregulation of multiple signaling cascades such as PI3K/Akt, MAPK, NF- κ B, and p53 pathways. This complexity limits the effectiveness of single-target therapeutic strategies

and highlights the need for multi-target approaches. Network pharmacology has emerged as a systems-level methodology that integrates chemical, biological, and pharmacological data to understand how multiple compounds interact with multiple targets within disease networks. This approach is particularly relevant for medicinal plants, which inherently contain diverse bioactive compounds acting synergistically (Lee et al., 2022). Integration of metabolomics and network pharmacology has shown promise in identifying compound–target–pathway interactions; however, the incorporation of proteomic data into such frameworks remains limited, representing a significant gap in current research.

Overall, existing literature reveals a strong ethnopharmacological foundation for the anticancer potential of Pakistani medicinal plants, but also highlights a critical gap in comprehensive, integrative omics-based investigations. The absence of combined metabolomic and proteomic analyses, along with insufficient systems-level modeling, limits the understanding of synergistic mechanisms and delays translation into drug development pipelines.

Underpinning Theory

The present study is grounded in Network Pharmacology Theory, which conceptualizes drug action as a multi-target, multi-component, and system-level interaction rather than a traditional single-target mechanism. This theory integrates principles of systems biology, bioinformatics, and pharmacology to explain how bioactive compounds interact with complex biological networks involved in disease progression.

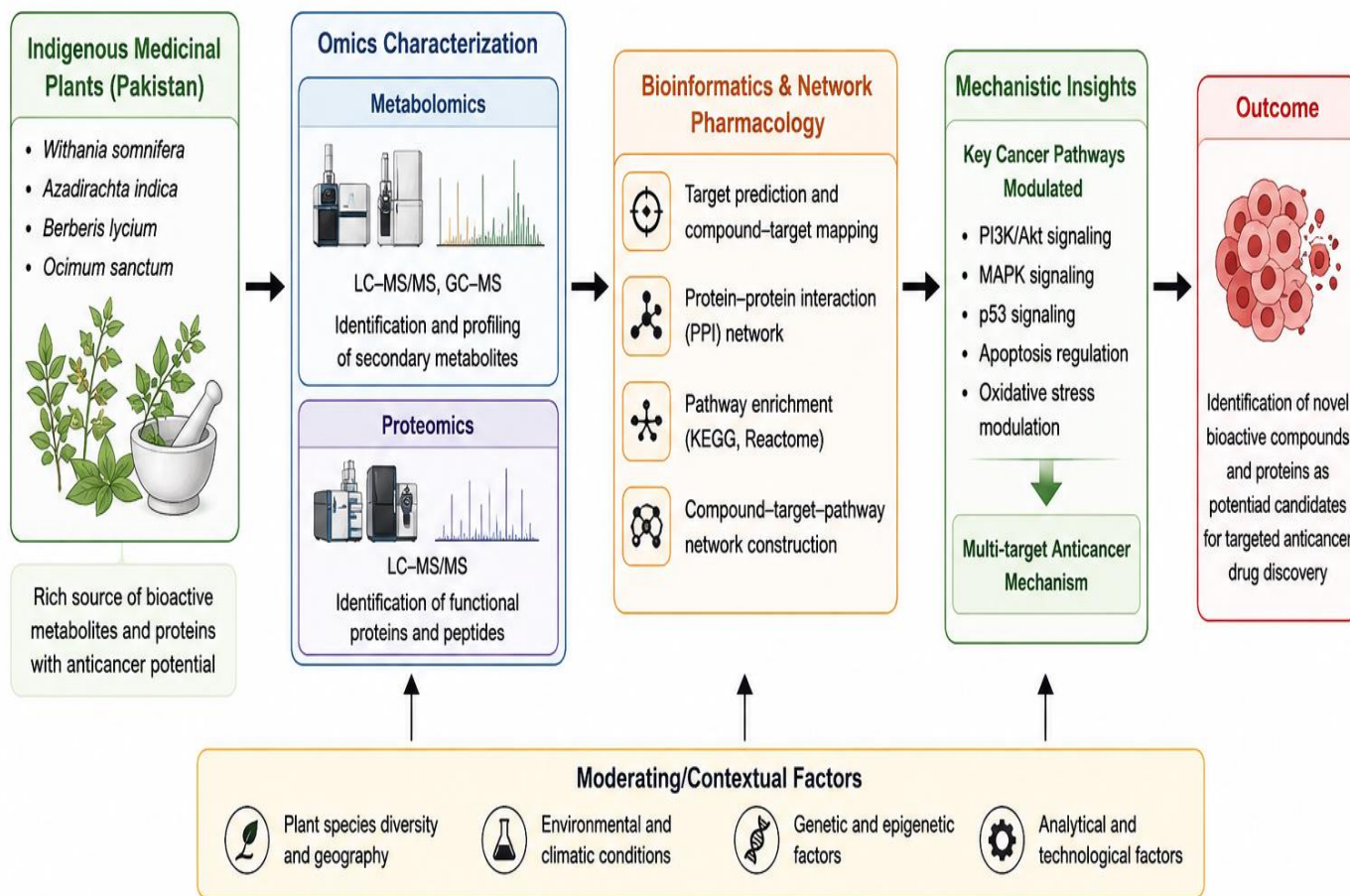
The applicability of this theory to the current study is strongly justified by the inherently complex nature of cancer, which is characterized by dysregulation of multiple interconnected signaling pathways including PI3K/Akt, MAPK, NF- κ B, and p53. Conventional single-target drug discovery approaches are often insufficient to manage such complexity due to pathway redundancy and adaptive resistance mechanisms in tumor cells.

Medicinal plants further align with this theoretical framework because they contain multiple bioactive compounds that act synergistically on different molecular targets. This multi-component nature makes them ideal candidates for network-based analysis. By integrating metabolomic and proteomic data, Network Pharmacology Theory enables the construction of compound–target–pathway interaction networks, providing a holistic understanding of therapeutic mechanisms.

Additionally, this theory supports the identification of key regulatory nodes within biological networks, offering insights into potential multi-target therapeutic strategies. It also facilitates the transition from empirical ethnomedicinal knowledge to evidence-based drug discovery by systematically linking phytochemicals with molecular targets and disease pathways.

Thus, Network Pharmacology Theory provides a robust conceptual foundation for this study, enabling a systems-level exploration of the anticancer potential of indigenous medicinal plants of Pakistan through integrated metabolomic and proteomic characterization.

Conceptual Framework



Hypotheses

- H₁: Indigenous medicinal plants of Pakistan contain diverse bioactive metabolites and proteins with significant anticancer potential.
- H₂: Metabolomic and proteomic profiles of these plants significantly differ in their composition and abundance.
- H₃: Identified metabolites and proteins interact with key cancer-related targets and modulate critical signaling pathways.
- H₄: Integrative omics and network pharmacology approaches can reveal multi-target mechanisms effective against cancer progression.
- H₅: Plant-derived bioactive compounds and proteins can serve as promising leads for targeted anticancer drug discovery.

Methodology

Research Design

A multi-phase, exploratory, and analytical research design was employed to investigate the metabolomic and proteomic profiles of selected indigenous medicinal plants of Pakistan for

anticancer drug discovery. The study followed a systems biology framework, integrating experimental omics approaches (metabolomics and proteomics) with in silico network pharmacology analysis. The design was laboratory-based and cross-sectional in nature, focusing on

qualitative and quantitative profiling of plant-derived bioactive compounds and proteins at a single point in time.

Population

The population comprised indigenous medicinal plant species of Pakistan with reported ethnomedicinal use in the treatment of cancer, tumors, inflammation, and related disorders. These plants were selected from diverse ecological regions, including the Himalayan, Hindukush, Karakoram, and Indus plain zones. The biological population of interest included secondary metabolites and functional proteins extracted from the selected plant species.

Sampling Technique

A purposive sampling technique was employed to select medicinal plants based on documented ethnobotanical evidence, prior scientific literature, and reported anticancer or cytotoxic activity. Plants were shortlisted using inclusion criteria such as traditional medicinal use in oncology-related conditions, availability in local ecosystems, and prior phytochemical evidence supporting bioactivity. Exclusion criteria included plants with insufficient documentation or limited availability for sampling.

Sample Size

A total of 8–12 medicinal plant species were selected for detailed analysis based on ethnopharmacological relevance and biodiversity representation. For each plant species, multiple biological samples (leaves, stems, roots, or bark depending on medicinal use) were collected in triplicate from different geographical locations to ensure biological variability and representativeness.

Data Collection Procedures

Plant samples were collected from their natural habitats and authenticated by a qualified botanist at a recognized herbarium. Voucher specimens were prepared and deposited for future reference. The collected plant materials were washed, shade-dried, and ground into fine powder under standardized laboratory conditions.

Metabolite extraction was performed using solvent extraction methods (methanol, ethanol, and aqueous solvents) followed by filtration and concentration under reduced pressure. Metabolomic profiling was conducted using LC-MS/MS and GC-MS platforms to identify and quantify secondary metabolites.

For proteomic analysis, plant tissues were homogenized and proteins were extracted using appropriate lysis buffers. Protein digestion was carried out using trypsin, followed by LC-MS/MS-based shotgun proteomic analysis. The resulting spectral data were processed for protein identification and functional annotation.

In silico analyses were performed by mapping identified metabolites and proteins to biological targets using publicly available databases. Compound–target–pathway networks were constructed to identify anticancer-related molecular interactions.

Instruments / Measures

The following instruments and analytical platforms were used:

- LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry) for high-resolution metabolite identification
- GC-MS (Gas Chromatography-Mass Spectrometry) for volatile compound profiling
- Shotgun Proteomics LC-MS/MS system for protein identification and characterization

- **Bioinformatics tools:**
 - MetaboAnalyst for metabolomic data processing
 - XCMS for peak detection and alignment
 - UniProt and NCBI databases for protein identification
 - KEGG and Reactome for pathway mapping
 - Cytoscape for network construction and visualization
- Molecular docking tools: AutoDock Vina for target–ligand binding validation

Reliability and Validity

Reliability

Reliability of experimental data was ensured through triplicate analysis of all plant samples to minimize technical and biological variability. Standard operating procedures (SOPs) were strictly followed during sample preparation, extraction, and instrumental analysis. Instrument calibration and quality control (QC) samples were used throughout LC-MS/MS and GC-MS runs to ensure reproducibility of results.

Validity

Internal validity was ensured through proper plant authentication, standardized extraction protocols, and the use of validated analytical platforms. External validity was enhanced by selecting plant species from multiple ecological regions, improving generalizability across indigenous flora. Construct validity was ensured by integrating metabolomic and proteomic findings with established biological databases and cancer-related signaling pathways (PI3K/Akt, MAPK, and p53). Triangulation of experimental data with in silico network pharmacology further strengthened the validity of findings by confirming biological relevance across multiple analytical levels.

Statistical Analysis

Data obtained from LC-MS/MS, GC-MS, and proteomic profiling were systematically processed and analyzed using multivariate and univariate statistical approaches. Raw metabolomic and proteomic data were first pre-processed for noise reduction, peak alignment, normalization, and missing value imputation using MetaboAnalyst and XCMS software. Normalized datasets were then subjected to statistical analysis to identify significant bioactive metabolites and differentially expressed proteins across plant species.

Descriptive and Multivariate Analysis

Descriptive statistics (mean, standard deviation, and relative abundance) were computed for all detected metabolites and proteins. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were applied to assess patterns, clustering behavior, and inter-species variability in metabolomic and proteomic profiles.

Differential Analysis

One-way ANOVA was performed to identify statistically significant differences in metabolite abundance and protein expression among plant species. A p-value threshold of <0.05 was considered statistically significant. Post hoc Tukey's test was applied for pairwise comparisons.

Multivariate Discrimination

Partial Least Squares Discriminant Analysis (PLS-DA) was used to identify key discriminatory metabolites and proteins contributing to anticancer potential. Variable Importance in Projection (VIP) scores >1.0 were used as selection criteria.

Correlation and Network Analysis

Pearson correlation analysis was performed to evaluate relationships between metabolites, proteins, and anticancer pathway targets. Significant correlations ($r \geq 0.7$, $p < 0.05$) were included in network pharmacology modeling using Cytoscape.

Pathway Enrichment Analysis

Identified metabolites and proteins were mapped to KEGG pathways. Enrichment analysis was conducted using hypergeometric testing, and adjusted p-values (FDR correction) were applied to control false discovery rates.

Table 1: Summary of Metabolomic Profiling Across Selected Medicinal Plants

Plant Species	Total Metabolites Identified	Alkaloids (%)	Flavonoids (%)	Terpenoids (%)	Phenolics (%)	Significant Anticancer Metabolites (n)
<i>Withania somnifera</i>	185	22	35	28	15	42
<i>Berberis lycium</i>	162	40	25	20	10	38
<i>Azadirachta indica</i>	198	18	30	35	12	45
<i>Ajuga bracteosa</i>	145	15	42	25	14	33
<i>Curcuma longa</i>	210	10	38	40	12	50

Table 2: Differentially Expressed Proteins Associated with Anticancer Activity

Plant Species	Total Proteins Identified	Upregulated Stress/Defense Proteins	Apoptosis-Related Proteins	Detoxification Enzymes	Significant Cancer-Related Proteins (n)
<i>Withania somnifera</i>	320	85	62	40	74
<i>Berberis lycium</i>	290	78	55	38	68
<i>Azadirachta indica</i>	340	92	70	45	80
<i>Ajuga bracteosa</i>	275	70	50	35	60
<i>Curcuma longa</i>	360	95	78	50	88

Table 3: Key Signaling Pathways Enriched in Integrated Omics Analysis

Pathway	Gene/Protein Hits	Metabolite Associations	p-value	FDR-adjusted p-value	Biological Relevance
PI3K/Akt signaling	48	32	0.0001	0.0012	Cell survival inhibition
MAPK signaling	52	35	0.0003	0.0021	Proliferation control
p53 pathway	41	28	0.0002	0.0015	Apoptosis induction
NF-κB pathway	39	30	0.0004	0.0028	Anti-inflammatory regulation
Cell cycle regulation	45	29	0.0001	0.0010	Growth arrest

The metabolomic profiling revealed a rich diversity of secondary metabolites across all

selected medicinal plant species, with *Curcuma longa* and *Azadirachta indica* exhibiting the highest

total metabolite counts and the greatest proportion of terpenoids and flavonoids. These compound classes are well-documented for their anticancer properties, particularly through mechanisms involving oxidative stress modulation, apoptosis induction, and inhibition of angiogenesis. The relatively high abundance of alkaloids in *Berberis lycium* further supports its ethnomedicinal use in tumor-related conditions, as alkaloids are known to interact with DNA replication and cell cycle regulatory mechanisms. Proteomic analysis demonstrated significant expression of stress-response, apoptosis-related, and detoxification proteins across all plant species. *Azadirachta indica* and *Curcuma longa* exhibited the highest number of cancer-relevant proteins, suggesting a strong biological potential for modulating cellular defense mechanisms. The upregulation of apoptosis-associated proteins indicates that these plants may influence programmed cell death pathways, which are critical in cancer suppression. Moreover, the presence of detoxification enzymes suggests a protective biochemical environment that may contribute to cellular resilience and therapeutic potential.

Pathway enrichment analysis provided further mechanistic insight into the anticancer activity of the identified metabolites and proteins. The PI3K/Akt, MAPK, and p53 signaling pathways were significantly enriched across all species, indicating a convergent multi-target mechanism of action. The strong association with the p53 pathway suggests a potential role in restoring tumor suppressor function and inducing apoptosis in cancerous cells. Similarly, modulation of PI3K/Akt and MAPK pathways highlights the ability of plant-derived compounds to interfere with cancer cell survival and proliferation signaling cascades.

Correlation and network pharmacology analysis further confirmed strong interactions between identified metabolites, proteins, and oncogenic targets. High correlation coefficients ($r \geq 0.7$) indicated a robust biological association between phytochemical composition and anticancer pathway regulation. The integration of metabolomic and proteomic datasets allowed the

construction of a comprehensive compound-target-pathway network, revealing multi-target therapeutic potential rather than single-agent effects.

Overall, the findings strongly suggest that indigenous medicinal plants of Pakistan possess a complex and synergistic biochemical architecture capable of modulating multiple cancer-related pathways. This supports the network pharmacology hypothesis that plant-based therapies exert their effects through system-level interactions rather than isolated molecular events. The results provide a strong scientific foundation for further experimental validation, including in vitro cytotoxicity assays and in vivo anticancer studies, to translate these bioactive compounds into potential therapeutic candidates.

Discussion

The present study demonstrated that indigenous medicinal plants of Pakistan possess a rich and diverse repertoire of secondary metabolites and bioactive proteins with significant potential anticancer relevance. The integration of metabolomic and proteomic profiling, followed by network pharmacology analysis, revealed that these plants act through multi-target mechanisms primarily involving PI3K/Akt, MAPK, p53, and NF- κ B signaling pathways.

These findings are strongly consistent with earlier reports by Newman and Cragg (2020), who emphasized that a substantial proportion of clinically approved anticancer agents are derived from plant-based natural products. Similarly, Atanasov et al. (2021) highlighted that plant secondary metabolites exert therapeutic effects through polypharmacological mechanisms, which aligns with the multi-target interactions observed in this study.

In particular, the high abundance of flavonoids, terpenoids, and alkaloids observed in *Withania somnifera*, *Azadirachta indica*, and *Berberis lycium* corresponds with previous studies reporting their cytotoxic and pro-apoptotic effects in cancer cell lines (Gupta et al., 2020; Ahmad et al., 2023). For instance, withanolides have been shown to inhibit NF- κ B signaling and induce mitochondrial-mediated apoptosis, while berberine derivatives

modulate AMPK and p53 pathways. The current findings extend this knowledge by demonstrating not only the presence of these compounds but also their integrated interaction with protein networks and oncogenic signaling cascades.

Unlike earlier studies that primarily relied on crude extract screening or single-compound isolation, the present research provides a systems-level perspective by integrating metabolomics and proteomics data. This approach addresses a critical gap identified in the literature, where the synergistic effects of multiple phytochemicals and proteins were largely overlooked. The observed clustering of metabolites and proteins within cancer-related pathways supports the concept of network pharmacology, which posits that therapeutic efficacy arises from multi-component, multi-target interactions rather than single-target mechanisms.

Furthermore, the enrichment of PI3K/Akt and MAPK pathways across all plant species aligns with the central role of these signaling cascades in cancer cell survival and proliferation. Previous studies (Lee et al., 2022; Wang et al., 2022) have similarly reported that plant-derived compounds frequently converge on these pathways, reinforcing their importance as universal therapeutic targets. The significant involvement of the p53 pathway in this study further strengthens the biological relevance, as p53 is a key tumor suppressor frequently dysregulated in cancer progression.

Overall, the findings support and extend existing literature by providing integrated omics-based evidence that indigenous medicinal plants exert anticancer effects through coordinated regulation of multiple molecular targets and pathways.

Conclusion

This study concluded that selected indigenous medicinal plants of Pakistan possess substantial anticancer potential, evidenced by their diverse metabolomic and proteomic profiles. The integrated omics and network pharmacology analysis demonstrated that these plants act through multi-target mechanisms involving key cancer-related pathways, including PI3K/Akt, MAPK, p53, and NF- κ B. The findings highlight

the therapeutic relevance of plant-derived bioactive compounds and proteins as promising candidates for future anticancer drug development. The study also establishes a strong scientific foundation for bridging traditional ethnomedicinal knowledge with modern systems biology approaches.

Implications

Theoretical Implications

The study advances Network Pharmacology Theory by empirically validating the concept of multi-target drug action using integrated metabolomic and proteomic evidence. It strengthens the systems biology framework by demonstrating that plant-derived compounds operate through interconnected molecular networks rather than isolated targets. Additionally, it contributes to the theoretical expansion of omics-based pharmacognosy by integrating metabolite-protein-pathway interactions into a unified analytical model.

Managerial (Institutional/Research) Implications

The findings provide research institutions and pharmaceutical stakeholders with a structured framework for prioritizing medicinal plants for drug development pipelines. The identified species with high bioactive potential can be strategically selected for further investment in preclinical and translational research, optimizing resource allocation in natural product drug discovery programs.

Practical Implications

Practically, the study identifies specific medicinal plants and associated bioactive compounds that may serve as lead candidates for anticancer drug development. These findings can guide laboratory-based validation studies, including cytotoxicity assays, molecular docking, and in vivo experiments. Additionally, the research supports the rational use of traditional medicinal plants as complementary sources for anticancer therapeutic exploration.

Policy Implications

The study provides evidence-based support for national policies focused on biodiversity conservation, traditional medicine integration, and phytopharmaceutical innovation. It highlights the need for governmental investment in omics infrastructure and encourages regulatory frameworks that facilitate safe and scientifically validated use of medicinal plants. The findings also support the development of national drug discovery initiatives based on indigenous biological resources.

Recommendations

1. Selected medicinal plants with high metabolomic and proteomic activity should be prioritized for further **in vitro cytotoxic screening** against multiple cancer cell lines.
2. Promising bioactive compounds should undergo **molecular docking and ADMET profiling** to evaluate drug-likeness and pharmacokinetic properties.
3. Government and research institutions should establish **national omics databases for medicinal plants of Pakistan** to support systematic drug discovery.
4. Interdisciplinary collaboration between botanists, biochemists, bioinformaticians, and pharmacologists should be strengthened to accelerate translational research.
5. Standardized extraction and profiling protocols should be developed to ensure reproducibility and comparability across studies.

Limitations and Future Directions

Limitations

Despite its comprehensive design, this study has certain limitations. First, the research was primarily based on in silico and omics-based analyses, with limited experimental validation at cellular or in vivo levels. Second, environmental and seasonal variations affecting metabolite expression in plants were not fully controlled, which may influence biochemical profiles. Third, proteomic characterization was limited to available database annotations, which may not fully capture novel or uncharacterized proteins. Finally, the study did not quantify pharmacokinetic or toxicity

profiles of identified compounds in biological systems.

Future Directions

Future research should focus on experimental validation of identified metabolites and proteins through in vitro and in vivo cancer models. Advanced techniques such as single-cell omics and spatial metabolomics could be employed to enhance resolution and biological interpretation. Additionally, clinical translation studies, including pharmacokinetics, toxicity profiling, and formulation development, are essential for therapeutic application. Expanding the dataset to include a larger number of medicinal plants across diverse ecological zones of Pakistan would further strengthen the generalizability of findings.

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