Medicinal Plants as Sources of Immunomodulatory Drugs: Bioactive Phytochemicals, Mechanistic Insights, and Translational Therapeutic Applications

Jonathan Michael Monroe [1*], Alyssa Nicole Carter [2], Brandon Lee Ramirez [3], Katherine Elise Whitmore [4]
[1] PhD, Department of Pharmaceutical Nanotechnology, Center for Precision Oncology Drug Delivery, Harvard Medical School, Boston, MA, USA
[2] PhD, Division of Nanomedicine and Targeted Therapeutics, University of California San Diego, La Jolla, CA, USA
[3] PhD, Department of Drug Delivery Systems, Johns Hopkins University School of Pharmacy, Baltimore, MD, USA
[4] PhD, Institute for Molecular Pharmaceutics, Purdue University, West Lafayette, IN, USA

* Corresponding Author: **Jonathan Michael Monroe**

Article Info

ISSN (online): 3107-393X
Volume: 01
Issue: 02
March – April 2024
Received: 03-01-2024
Accepted: 05-02-2024
Published: 07-03-2024
Page No: 12-29

Abstract
The discovery and development of novel immunomodulatory therapeutics from medicinal plants represents a critical frontier in pharmaceutical research, yet faces substantial challenges including compound complexity, limited bioavailability, and the difficulty of translating traditional knowledge into evidence-based medicine. This article examines the contemporary landscape of phytochemical-based immunomodulatory drug discovery, with particular emphasis on systematic approaches that bridge traditional botanical medicine and modern pharmaceutical science. The discussion encompasses advanced methodologies in target identification and validation, high-throughput screening platforms adapted for complex plant extracts, and lead optimization strategies that enhance pharmacological properties while maintaining bioactive integrity. Special attention is devoted to preclinical models that accurately predict human immune responses, translational strategies that facilitate clinical progression, and innovative clinical development paradigms including adaptive trial designs and biomarker-guided patient stratification. Technological enhancements, particularly computational modeling and artificial intelligence applications in predicting immunomodulatory activity and optimizing molecular structures, are explored as transformative tools in accelerating discovery timelines. The integration of these modern approaches has yielded promising therapeutic candidates, with several plant-derived immunomodulators advancing through clinical pipelines. However, persistent challenges remain, including standardization of botanical materials, regulatory pathway navigation, and ethical considerations surrounding traditional knowledge. This comprehensive review provides a roadmap for researchers and clinicians seeking to harness the immunomodulatory potential of medicinal plants through rigorous scientific methodology, ultimately contributing to the development of safe, effective, and accessible immune-targeted therapeutics for diverse patient populations.

DOI:

Keywords: Drug Discovery, Pharmaceutical Development, Translational Research, High-Throughput Screening, Lead Optimization, Precision Medicine

1. Introduction

The human immune system, a sophisticated network of cells, tissues, and signaling molecules, maintains homeostasis through carefully orchestrated responses to pathogens, allergens, and malignant cells. Dysregulation of immune function underlies numerous pathological conditions, ranging from autoimmune diseases and chronic inflammatory disorders to immunodeficiency states and cancer. Consequently, immunomodulatory therapeutics have emerged as essential components of modern pharmacotherapy, addressing unmet medical needs across diverse clinical contexts [1]. While synthetic immunomodulators have

achieved notable clinical success, the search for novel agents continues unabated, driven by limitations including adverse effects, treatment resistance, and the need for targeted interventions with improved safety profiles [2].

Medicinal plants have served humanity as sources of therapeutic agents for millennia, with traditional healing systems across cultures documenting extensive use of botanical preparations for immune-related conditions. Archaeological evidence and ancient medical texts reveal sophisticated understanding of plant-based immune modulation, predating modern immunological concepts by centuries [3]. Contemporary ethnopharmacological research has validated many traditional applications, demonstrating that numerous medicinal plants contain bioactive phytochemicals capable of modulating immune responses through multiple mechanisms [4]. These natural products offer remarkable structural diversity, encompassing alkaloids, terpenoids, flavonoids, polysaccharides, and phenolic compounds, each class presenting unique pharmacological profiles and therapeutic potential [5].

The transition from traditional botanical medicine to evidence-based pharmaceutical development requires systematic investigation employing contemporary drug discovery methodologies. Unlike synthetic compound libraries designed according to defined chemical principles, plant-derived materials present unique challenges including chemical complexity, variability in phytochemical composition, and difficulties in isolation and characterization of active constituents [6]. Nevertheless, the pharmaceutical industry recognizes medicinal plants as invaluable sources of lead compounds, with historical precedent demonstrating that plant-derived drugs and their synthetic derivatives constitute a substantial proportion of the modern pharmacopeia [7]. Notable examples of clinically successful plant-derived immunomodulators include paclitaxel, originally isolated from Pacific yew bark and now widely used in cancer immunotherapy combinations, and ingenol mebutate from Euphorbia peplus, approved for treating actinic keratosis through immune activation mechanisms [8].

Modern drug discovery from medicinal plants integrates traditional knowledge with advanced scientific techniques, creating a multidisciplinary framework that encompasses ethnobotany, phytochemistry, pharmacology, and clinical medicine. This approach begins with systematic documentation of traditional uses, followed by biological screening to identify promising candidates, chemical characterization to isolate active compounds, mechanistic studies to elucidate molecular targets and pathways, and ultimately translation to clinical applications [9]. The immunomodulatory properties of plant-derived compounds are particularly intriguing, as they often exhibit pleiotropic effects, simultaneously influencing multiple immune pathways and cell types, potentially offering advantages over highly selective synthetic agents in certain clinical contexts [10].

The current landscape of immunomodulatory drug discovery from medicinal plants is characterized by rapid technological advancement and increasing sophistication in research methodologies. High-throughput screening platforms enable simultaneous evaluation of thousands of plant extracts and purified compounds against relevant immunological targets, dramatically accelerating the identification of bioactive constituents [11]. Target validation strategies employing genomic, proteomic, and systems biology approaches provide mechanistic insights essential for rational drug development, while advanced analytical techniques facilitate detailed characterization of complex phytochemical mixtures [12]. Furthermore, computational tools including molecular docking, pharmacophore modeling, and artificial intelligence algorithms enhance the efficiency of lead identification and optimization processes [13].

Despite these advances, translating plant-derived immunomodulatory compounds from laboratory discovery to clinical therapeutics faces substantial obstacles. Challenges include achieving adequate bioavailability for orally administered phytochemicals, often limited by poor aqueous solubility and extensive first-pass metabolism; ensuring batch-to-batch consistency in botanical materials influenced by genetic, environmental, and processing factors; navigating regulatory frameworks designed primarily for synthetic single-entity drugs; and addressing intellectual property considerations related to traditional knowledge [14]. Additionally, preclinical models must accurately predict human immune responses, a particularly challenging requirement given species-specific differences in immune system organization and function [15].

The strategic importance of developing plant-derived immunomodulatory therapeutics extends beyond purely scientific considerations. Many medicinal plants are accessible in resource-limited settings where conventional pharmaceuticals remain prohibitively expensive or unavailable, suggesting potential for developing affordable treatments addressing global health disparities [16]. Furthermore, growing consumer preference for natural products and complementary medicine creates market opportunities for evidence-based botanical therapeutics, provided they meet rigorous safety and efficacy standards [17]. The sustainable sourcing of medicinal plants also intersects with conservation biology and environmental stewardship, necessitating integrated approaches that balance therapeutic development with ecological preservation [18].

This article provides comprehensive analysis of contemporary strategies for discovering and developing immunomodulatory drugs from medicinal plants, examining the entire continuum from initial target identification through clinical translation. Subsequent sections explore modern approaches in target identification and validation, high-throughput screening methodologies and lead optimization strategies, preclinical and translational research paradigms, clinical development innovations, technological and computational enhancements, and critical examination of challenges, ethical considerations, and regulatory pathways. Through this systematic exploration, we aim to provide researchers, clinicians, and pharmaceutical developers with actionable insights for advancing plant-derived immunomodulatory therapeutics from traditional knowledge to evidence-based medicine.

2. Modern Approaches in Target Identification and Validation

Target identification represents the foundational step in rational drug discovery, establishing the molecular entities whose modulation will produce desired therapeutic effects. In the context of immunomodulatory drug discovery from medicinal plants, target identification faces unique challenges due to the pleiotropic nature of phytochemicals and the complexity of immune signaling networks [19]. Unlike conventional synthetic drug development, which often begins

with a predefined molecular target, plant-based drug discovery frequently employs phenotypic screening approaches, identifying bioactive compounds based on desired functional outcomes before determining their molecular mechanisms of action [20]. This reverse pharmacology paradigm has proven remarkably successful for natural products, as many clinically useful plant-derived drugs were discovered through phenotypic effects long before their molecular targets were elucidated [21].

Contemporary target identification strategies for plant-derived immunomodulators integrate multiple complementary methodologies. Genomic approaches, including transcriptional profiling using microarray and RNA sequencing technologies, reveal global gene expression changes induced by plant extracts or purified phytochemicals in immune cells, identifying pathways and molecular targets potentially responsible for observed biological effects [22]. Proteomic techniques, particularly mass spectrometry-based methods, enable comprehensive analysis of protein expression, post-translational modifications, and protein-protein interactions altered by bioactive compounds, providing functional insights beyond transcriptional regulation [23]. Chemical proteomics, employing immobilized phytochemicals as affinity matrices, allows direct identification of protein binding partners, establishing physical interactions between bioactive compounds and potential therapeutic targets [24].

Systems biology approaches have transformed target identification by enabling holistic analysis of complex biological networks rather than isolated molecular components. Network pharmacology integrates data from genomics, proteomics, metabolomics, and computational modeling to construct comprehensive maps of compound-target-disease relationships, particularly valuable for understanding the multi-target effects characteristic of plant-derived immunomodulators [25]. These network-based analyses reveal that many effective phytochemicals modulate immune function through synergistic effects on multiple targets within interconnected pathways, challenging the traditional one-drug-one-target paradigm and suggesting advantages of multi-target therapeutic strategies [26]. Mathematical modeling of immune signaling networks further enhances target identification by predicting which molecular interventions will produce desired immunomodulatory outcomes, enabling rational selection of targets for experimental validation [27].

Phenotypic screening remains central to discovering plant-derived immunomodulators, particularly when molecular mechanisms underlying traditional therapeutic uses are unknown. Modern phenotypic assays employ physiologically relevant cellular systems, including primary human immune cells and complex co-culture models that recapitulate key aspects of immune microenvironments [28]. Three-dimensional culture systems and organoid technologies provide increasingly sophisticated platforms for phenotypic screening, better approximating *in vi*vo conditions than conventional two-dimensional cell cultures [29]. High-content imaging systems enable simultaneous assessment of multiple phenotypic parameters at single-cell resolution, providing rich datasets that correlate bioactive compound exposure with complex cellular responses including morphological changes, protein localization, and functional marker expression [30].

Once potential immunomodulatory activity is identified through phenotypic screening, target deconvolution strategies determine the molecular mechanisms responsible for observed effects. Genetic approaches, including RNA interference and CRISPR-Cas9-mediated gene editing, validate putative targets by demonstrating that their deletion or knockdown abolishes the phenotypic effects of bioactive phytochemicals [31]. Rescue experiments, where target re-expression restores compound sensitivity, provide additional validation confirming specific target engagement [32]. Chemical genetic approaches complement these molecular techniques, using libraries of well-characterized tool compounds with known mechanisms to identify pathways modulated by plant extracts, then confirming whether purified phytochemical constituents act through similar mechanisms [33].

Biophysical techniques provide direct evidence of molecular interactions between phytochemicals and protein targets. Surface plasmon resonance and biolayer interferometry enable label-free, real-time measurement of binding kinetics, determining association and dissociation rate constants that quantify target engagement [34]. Isothermal titration calorimetry provides thermodynamic parameters of binding interactions, revealing whether compound-target association is enthalpically or entropically driven, information valuable for structure-based optimization [35]. Nuclear magnetic resonance spectroscopy, particularly saturation transfer difference and transferred nuclear Overhauser effect experiments, identifies specific regions of protein targets contacted by bound ligands, guiding medicinal chemistry efforts to enhance binding affinity and selectivity [36].

Structural biology approaches, especially X-ray crystallography and cryo-electron microscopy, reveal atomic-resolution details of phytochemical-target complexes, providing definitive validation of binding modes and enabling structure-based drug design [37]. These structural insights are particularly valuable for optimizing natural product leads, identifying which chemical features are essential for target engagement and which regions tolerate modification to improve pharmacological properties [38]. Computational docking studies complement experimental structural determination, predicting binding poses when experimental structures are unavailable and enabling virtual screening of phytochemical libraries against validated targets [39].

Target validation extends beyond demonstrating that a phytochemical binds to and modulates a molecular target, requiring evidence that such modulation produces therapeutically relevant effects. Genetic validation using disease-relevant cellular and animal models demonstrates that target modulation ameliorates pathological phenotypes, establishing that the identified target represents a viable therapeutic intervention point [40]. Biomarker development, identifying measurable biological signals downstream of target engagement, enables pharmacodynamic monitoring in preclinical and clinical studies, providing evidence of on-target activity in living systems [41]. Translational validation, examining whether targets identified in cellular or animal models are similarly expressed and function in human disease states, is essential given species differences in immune system organization [42].

Pathway analysis and mechanistic studies elucidate how modulation of identified targets influences broader immune

system function. Flow cytometry-based immunophenotyping reveals effects of phytochemicals on immune cell subset distributions, activation states, and effector functions, connecting molecular target engagement to cellular and systemic immunological outcomes [43]. Multiplex cytokine profiling quantifies changes in secreted immune mediators, providing functional readouts of altered immune cell communication and inflammatory responses [44]. Transcriptional analysis of pathway-specific gene signatures confirms predicted downstream consequences of target modulation, validating mechanistic hypotheses and identifying potential biomarkers for clinical development [45].

The concept of polypharmacology, wherein single compounds modulate multiple therapeutic targets, is particularly relevant for plant-derived immunomodulators. Many bioactive phytochemicals exhibit promiscuous binding, interacting with multiple proteins within related families or across different protein classes [46]. While promiscuity is often considered undesirable in synthetic drug development due to toxicity concerns, controlled polypharmacology may offer therapeutic advantages for complex diseases like immune disorders, where modulation of multiple pathway nodes produces synergistic or complementary effects [47]. Network-based approaches help distinguish beneficial polypharmacology from problematic promiscuity, identifying compound-target interaction profiles associated with desired therapeutic outcomes versus those predicting adverse effects [48].

Emerging technologies continue advancing target identification and validation capabilities. Single-cell multi-omics approaches enable simultaneous measurement of genomic, transcriptomic, proteomic, and metabolomic parameters in individual cells, revealing heterogeneous responses to immunomodulatory phytochemicals within seemingly uniform cell populations [49]. Spatial transcriptomics and proteomics maintain tissue architecture during molecular profiling, showing where within complex tissues particular cells respond to bioactive compounds, critical information for understanding drug effects in structured immune environments like lymph nodes or inflammatory lesions [50]. CRISPR screening platforms enable genome-wide interrogation of genes modulating cellular responses to phytochemicals, identifying not only direct targets but also genetic modifiers of compound activity, resistance mechanisms, and pathway dependencies [51].

The integration of traditional knowledge with modern target identification strategies represents a powerful synergy in discovering plant-derived immunomodulators.

Ethnopharmacological research documents traditional therapeutic uses, providing hypotheses about potential immunological effects and disease applications [52]. Reverse pharmacology approaches test these traditional applications using contemporary bioassays, then employ modern target identification techniques to elucidate molecular mechanisms [53]. This bidirectional flow between traditional wisdom and cutting-edge science has proven highly productive, with numerous clinically relevant immunomodulatory targets first suggested by traditional uses of medicinal plants [54].

3. High-Throughput Screening and Lead Optimization

High-throughput screening has revolutionized drug discovery by enabling systematic evaluation of large compound collections against biological targets or disease-relevant phenotypes, dramatically accelerating the identification of bioactive molecules [55]. In the context of discovering immunomodulatory drugs from medicinal plants, high-throughput screening faces distinctive challenges compared to synthetic compound libraries, including the chemical complexity of plant extracts, sample availability limitations, and the need for specialized assay formats compatible with complex mixtures [56]. Nevertheless, adaptation of high-throughput technologies to natural product screening has proven highly successful, with numerous plant-derived lead compounds identified through systematic screening campaigns [57].

The design of screening campaigns for plant-derived immunomodulators requires careful consideration of several factors. Primary screening typically employs relatively simple, robust assays amenable to automation and miniaturization, often using purified recombinant proteins, cell-free enzymatic assays, or reporter gene systems in immortalized cell lines [58]. These assays prioritize reproducibility, dynamic range, and signal-to-noise ratio, enabling confident discrimination between active and inactive samples despite inherent variability in plant extracts [59]. Counterscreening strategies identify false positives arising from assay interference, particularly important for plant extracts containing chromophoric compounds, fluorescent molecules, or aggregation-prone constituents that produce artifactual signals.

Secondary screening employs more physiologically relevant but often lower-throughput assays to confirm and characterize hits from primary screens. These confirmatory assays typically use primary human immune cells, assess functional endpoints rather than surrogate markers, and include concentration-response characterization to determine potency. Orthogonal assays measuring the same biological outcome through different technical approaches provide additional confidence that observed activity reflects genuine immunomodulatory effects rather than assay artifacts. Selectivity profiling against related targets or non-immune cell types helps identify compounds with desired immunological specificity versus those with broader, potentially problematic biological activities.

Plant extract preparation and quality control represent critical considerations for successful high-throughput screening. Standardized extraction protocols ensure reproducibility, typically employing sequential extraction with solvents of increasing polarity to capture diverse phytochemical classes. Extract libraries should be accompanied by detailed documentation of botanical source material, including taxonomic identification, geographic origin, collection season, and plant part utilized, enabling re-collection and scale-up of active samples. Voucher specimens deposited in recognized herbaria provide permanent reference materials for botanical verification, essential for publication and regulatory purposes. Quality control analyses, including thin-layer chromatography, high-performance liquid chromatography fingerprinting, or mass spectrometry profiling, characterize extract composition and detect batch-to-batch variation.

Assay miniaturization and automation enable the throughput necessary for screening large plant extract collections. Microplate-based assays in 384-well or 1536-well formats dramatically reduce reagent consumption and sample requirements while increasing screening capacity. Liquid handling robotics ensure precise, reproducible dispensing of

extracts and reagents, minimizing human error and enabling lights-out operation. Plate readers with advanced detection capabilities, including time-resolved fluorescence, fluorescence polarization, and luminescence, accommodate diverse assay formats suitable for different immunological targets and readouts.

Data management and analysis systems are essential components of high-throughput screening infrastructure. Laboratory information management systems track samples through screening workflows, maintaining chain of custody and associating analytical results with botanical source information. Statistical methods for high-throughput screening data analysis, including Z-score and Z-prime factor calculations, assess assay performance and identify statistically significant hits accounting for plate-to-plate variability and edge effects. Machine learning algorithms can identify patterns in screening data, predicting which plant families or phytochemical classes are most likely to exhibit particular immunomodulatory activities, informing rational library design and prioritization strategies.

Following hit identification through high-throughput screening, bioassay-guided fractionation isolates and identifies the specific phytochemical constituents responsible for observed activity. This iterative process involves chromatographic separation of active extracts, biological testing of resulting fractions to identify those retaining activity, and continued fractionation of active fractions until pure compounds are obtained. Modern approaches employ high-resolution chromatography coupled directly to biological assays, enabling real-time activity profiling across separation runs and more efficient identification of bioactive peaks. Hyphenated analytical techniques, particularly liquid chromatography-mass spectrometry and liquid chromatography-nuclear magnetic resonance, facilitate rapid structure elucidation of isolated compounds without requiring extensive purification.

Lead optimization transforms screening hits into development candidates with improved pharmacological properties suitable for clinical advancement. For plant-derived immunomodulators, optimization typically addresses multiple parameters simultaneously, including potency against the intended target, selectivity over off-targets, physicochemical properties affecting drug-like behavior, and metabolic stability. Structure-activity relationship studies, systematically modifying chemical structures and assessing resulting activity changes, identify which molecular features are essential for biological activity and which regions tolerate modification. These studies may involve semi-synthesis, creating analogs through chemical modification of natural products, or total synthesis, building compounds entirely from simple starting materials, enabling access to analogs unavailable through biosynthetic pathways.

Physicochemical property optimization aims to achieve drug-like characteristics enabling effective delivery and appropriate pharmacokinetic behavior. Lipinski's rule of five and related guidelines, while developed for synthetic orally bioavailable drugs, provide useful benchmarks for assessing natural product leads, though many successful plant-derived drugs violate these rules, suggesting they should guide rather than dictate optimization efforts. Solubility enhancement strategies, including salt formation, prodrug approaches, or formulation technologies like nanotechnology-based delivery systems, address the poor aqueous solubility characteristic of many bioactive phytochemicals. Permeability optimization, modifying structures to enhance membrane penetration while maintaining target affinity, improves oral bioavailability and cellular uptake.

Metabolic stability represents a critical optimization parameter, as many phytochemicals undergo rapid biotransformation limiting their duration of action. *In vi*tro metabolic stability screening using liver microsomes or hepatocytes identifies compounds susceptible to rapid metabolism, while metabolite identification studies determine major biotransformation pathways. Structure-based approaches to improving metabolic stability involve introducing chemical modifications at sites of metabolism, such as replacing metabolically labile groups with bioisosteres or adding steric hindrance to block enzymatic attack. However, optimization must balance improved metabolic stability against maintaining appropriate clearance, as excessively stable compounds may accumulate to toxic levels.

Selectivity optimization reduces off-target interactions that could produce adverse effects or complicate clinical development. Profiling compounds against panels of related targets, including other members of the same protein family or proteins with similar binding pockets, identifies selectivity issues requiring attention. Structure-based design, informed by three-dimensional structural information about on-target and off-target binding sites, guides introduction of modifications enhancing desired selectivity. Cellular selectivity assays assess whether compounds preferentially affect intended immune cell types versus other cell populations, important for maximizing therapeutic efficacy while minimizing systemic toxicity.

Lead optimization increasingly employs computational approaches to enhance efficiency and reduce experimental burden. Molecular modeling and docking studies predict how structural modifications will affect target binding, prioritizing synthesis of promising analogs. Quantitative structure-activity relationship models correlate chemical structures with biological activities, enabling prediction of activity for virtual compounds and guiding design of optimized structures. Pharmacophore modeling identifies essential chemical features required for activity, providing templates for designing novel compounds or searching databases for alternative scaffolds with similar properties.

Multi-parameter optimization, simultaneously improving multiple properties, represents a significant challenge in lead optimization. Pareto optimization approaches identify compound analogs representing optimal trade-offs between competing objectives, such as potency versus selectivity or efficacy versus toxicity. Desirability functions combine multiple parameters into single composite scores, enabling ranking of compounds based on overall drug-like quality rather than individual properties. Machine learning algorithms trained on successful optimization campaigns can predict which combinations of chemical modifications will synergistically improve multiple parameters.

Natural product-inspired design represents an alternative strategy when direct optimization of plant-derived leads proves challenging. This approach uses bioactive phytochemicals as inspiration for designing simplified synthetic compounds retaining key pharmacophoric features while possessing more favorable drug-like properties. Privileged scaffolds derived from natural products, molecular frameworks frequently associated with biological activity, serve as starting points for library synthesis and diversity-

oriented synthesis campaigns. Fragment-based approaches deconstruct complex natural products into smaller molecular pieces, then reconstruct simplified analogs incorporating essential binding elements.

Formulation development proceeds in parallel with chemical optimization, as even ideally optimized compounds require appropriate delivery systems for clinical use. Formulation strategies for plant-derived immunomodulators address challenges including limited aqueous solubility, chemical instability, and targeted delivery to immune tissues or specific immune cell populations. Advanced delivery technologies such as liposomes, nanoparticles, and polymer conjugates enhance solubility, protect labile compounds from degradation, and enable controlled release kinetics. Targeted delivery systems incorporating ligands for immune cell surface markers or exploiting enhanced permeability and retention effects in inflammatory tissues concentrate drugs at sites of desired action while reducing systemic exposure.

4. Preclinical and Translational Strategies

Preclinical development constitutes the essential bridge between *in vi*tro discovery of bioactive phytochemicals and clinical evaluation in human subjects, encompassing diverse studies that characterize pharmacological properties, assess safety, and provide the scientific foundation for human trials. For plant-derived immunomodulators, preclinical research presents unique challenges stemming from the complexity of immune system interactions, species differences in immune function, and the multi-component nature of some botanical therapeutics. Successful translation requires carefully designed preclinical programs integrating multiple experimental approaches and model systems that collectively predict human responses with acceptable accuracy.

*In vi*tro pharmacological characterization provides foundational understanding of compound mechanisms and activities. Primary human immune cell assays, utilizing peripheral blood mononuclear cells, purified lymphocyte subsets, or myeloid cells from healthy donors or patients with relevant diseases, assess effects of candidate immunomodulators on cell proliferation, differentiation, cytokine production, and effector functions. Complex co-culture systems model immune cell interactions, enabling evaluation of compounds affecting antigen presentation, T cell activation, regulatory immune cell function, or innate-adaptive immune crosstalk. Three-dimensional culture models and organotypic systems provide more physiologically relevant environments for assessing immunomodulatory effects, particularly important for compounds whose activities depend on cell-cell or cell-matrix interactions.

Selectivity profiling across immune cell types and activation states determines whether candidates exhibit desired immunological specificity. Differential effects on naive versus memory T cells, regulatory versus effector populations, or pro-inflammatory versus anti-inflammatory macrophage phenotypes may predict therapeutic utility in particular clinical contexts. Multi-parameter flow cytometry enables simultaneous assessment of numerous cellular markers, revealing subtle phenotypic changes induced by immunomodulatory compounds and identifying affected cell populations within heterogeneous mixtures. Functional assays measuring antigen-specific responses, such as mixed lymphocyte reactions or antigen recall assays, assess immunomodulator effects on clinically relevant immune functions.

*In vi*vo pharmacology studies employ animal models to evaluate candidate immunomodulators in living organisms with intact, functioning immune systems. Model selection represents a critical decision, balancing several considerations including relevance to human disease, reproducibility, throughput, and ethical requirements for minimizing animal use. Mouse models predominate in preclinical immunology research due to extensive characterization of murine immune systems, availability of genetically modified strains, and relatively low costs, though species differences in immune function necessitate cautious interpretation when extrapolating to humans. Humanized mouse models, engrafted with human immune cells or tissues, provide systems expressing human immune components, though technical challenges and incomplete immune reconstitution limit their applications.

Disease-specific animal models evaluate therapeutic efficacy in pathophysiological contexts relevant to intended clinical applications. For autoimmune disease research, models include collagen-induced arthritis for rheumatoid arthritis, experimental autoimmune encephalomyelitis for multiple sclerosis, and spontaneous lupus-prone strains for systemic lupus erythematosus. Transplantation models assess immunomodulator effects on allograft rejection and graft-versus-host disease. Infection models evaluate immunostimulatory compounds enhancing antimicrobial immunity. Cancer immunology models, including syngeneic tumor implants and genetically engineered mouse models, assess immune-mediated anti-tumor effects. Each model presents advantages and limitations regarding translatability to human disease, requiring careful interpretation and often validation across multiple models.

Pharmacokinetic studies characterize absorption, distribution, metabolism, and excretion of plant-derived immunomodulators, providing essential information for dose selection and dosing regimen design. Bioanalytical methods, typically liquid chromatography-mass spectrometry, quantify compounds and metabolites in biological matrices including plasma, tissues, and excreta. Pharmacokinetic modeling applies mathematical frameworks to concentration-time data, extracting parameters including bioavailability, half-life, clearance, and volume of distribution that predict *in vi*vo exposure resulting from different dosing strategies. Tissue distribution studies determine whether compounds achieve adequate concentrations in lymphoid organs and inflammatory sites where immune responses occur.

Pharmacodynamic studies correlate drug exposure with biological effects, establishing relationships between pharmacokinetic parameters and immunological outcomes. Biomarker measurements in biological samples from treated animals provide pharmacodynamic readouts, including cytokine levels, immune cell populations, and pathway-specific markers. Integrated pharmacokinetic-pharmacodynamic modeling mathematically relates drug concentrations to observed effects, enabling prediction of dose-response relationships and identification of exposure levels necessary for therapeutic efficacy. These models inform dose selection for toxicology studies and clinical trials, optimizing the probability of achieving effective concentrations while minimizing toxicity risks.

Toxicology studies assess safety of candidate

immunomodulators, identifying potential adverse effects and establishing safe dose ranges for clinical evaluation. Regulatory guidelines specify required toxicology packages for investigational new drug applications, typically including acute toxicity studies establishing maximum tolerated doses, repeat-dose toxicity studies evaluating effects of prolonged exposure, and specialized studies addressing specific safety concerns. For immunomodulatory drugs, particular attention focuses on immunotoxicity, assessing whether compounds produce undesired immunosuppression increasing infection susceptibility, immune activation causing inflammatory toxicity, or hypersensitivity reactions. Comprehensive immunotoxicity assessment includes evaluation of lymphoid organ histopathology, immune cell enumeration, functional immune assays, and host resistance models.

Genotoxicity testing determines whether compounds damage genetic material, critical information given associations between mutagenicity and carcinogenicity. Standard genotoxicity batteries include bacterial reverse mutation assays, chromosomal aberration tests, and *in vi*vo micronucleus assays. Many plant-derived compounds, particularly polyphenols, exhibit pro-oxidant properties under certain conditions that might raise genotoxicity concerns, necessitating careful evaluation while recognizing that *in vi*tro findings may not always predict *in vi*vo effects due to differences in metabolic activation and detoxification.

Reproductive and developmental toxicity studies evaluate effects on fertility, embryo-fetal development, and pre- and postnatal development, required when clinical development plans include dosing individuals of reproductive potential. These studies are particularly important for immunomodulatory drugs given the critical role of immune function in successful pregnancy and the potential for immune-active compounds to affect implantation, placental function, or fetal development.

Translational biomarker development, occurring throughout preclinical development, identifies measurable biological indicators of target engagement, pharmacological activity, and therapeutic effects that can be monitored in clinical trials. Ideal biomarkers exhibit several properties including objective measurement, reproducibility across laboratories, sensitivity to drug effects, and validated correlation with clinical outcomes. For immunomodulatory drugs, potential biomarkers include circulating cytokines and chemokines, immune cell subset frequencies, activation markers, gene expression signatures, and autoantibody levels. Biomarker qualification, establishing that measured changes genuinely reflect meaningful biological processes, requires validation across multiple studies and platforms.

Formulation development for clinical evaluation begins during preclinical stages, with formulations used in pivotal toxicology studies typically matching or closely resembling those intended for early clinical trials. Considerations include chemical stability under relevant storage conditions, compatibility with manufacturing processes, and suitability for intended routes of administration. For plant-derived immunomodulators, additional formulation challenges may include complex mixtures requiring standardization to defined marker compounds, botanical materials with inherent compositional variability, and compounds with limited solubility or stability requiring advanced delivery systems.

Proof-of-concept studies in preclinical models provide critical go or no-go decision points in development programs, demonstrating that candidates exhibit meaningful therapeutic activity in disease-relevant contexts at exposures achievable with acceptable safety margins. These studies should employ rigorous experimental design including randomization, blinding, and appropriate statistical power to minimize bias and ensure reproducibility. Positive and negative control groups, including standard-of-care therapeutics when available, provide benchmarks for interpreting experimental treatment effects.

Translational strategy development synthesizes preclinical findings into comprehensive plans for clinical advancement, identifying optimal patient populations, clinical trial designs, biomarkers for patient selection or pharmacodynamic monitoring, and dose selection rationale. Translational medicine teams integrate expertise across disciplines including preclinical pharmacology, clinical pharmacology, biomarker science, and clinical specialties relevant to intended therapeutic applications. Investigational new drug application-enabling studies generate data packages meeting regulatory requirements, demonstrating sufficient understanding of pharmacology, safety, and manufacturing to support human trials.

Species translation methodologies address the challenge of extrapolating preclinical findings from animal models to humans. Allometric scaling applies mathematical relationships between body size and physiological parameters to predict human pharmacokinetics from animal data, though these approaches require refinement for biological products where scaling relationships may differ from small molecules. Physiologically based pharmacokinetic modeling constructs mechanistic representations of drug disposition incorporating species-specific anatomical and physiological parameters, enabling more accurate human predictions. Cross-species comparisons of target expression, signaling pathways, and disease mechanisms inform interpretation of animal model findings and identify potential translation risks.

5. Clinical Development Innovations

Clinical development represents the ultimate test of therapeutic hypotheses, evaluating plant-derived immunomodulators in human subjects to establish safety, determine optimal dosing, and demonstrate clinical efficacy. This process, traditionally following sequential phases from small early studies to large confirmatory trials, has evolved substantially in recent decades with innovative trial designs, adaptive methodologies, and precision medicine approaches that enhance efficiency and improve success rates. For botanical immunomodulators, clinical development presents distinctive challenges including standardization of complex plant materials, selection of appropriate patient populations, and navigation of regulatory pathways developed primarily for single-entity synthetic drugs.

Phase I clinical trials primarily assess safety and tolerability in healthy volunteers or, for immunomodulatory agents with potential toxicity, in patients with target diseases. First-in-human dose selection employs preclinical toxicology data, pharmacokinetic modeling, and regulatory guidelines to identify starting doses with adequate safety margins. Dose escalation schemes, including traditional three-plus-three designs or model-based continual reassessment methods, identify maximum tolerated doses and characterize dose-limiting toxicities. Pharmacokinetic sampling in Phase I trials establishes human absorption, distribution, metabolism, and excretion characteristics, confirming whether preclinical

predictions accurately forecast human behavior and identifying any unexpected metabolic pathways.

Pharmacodynamic assessments integrated into Phase I trials provide early evidence of target engagement and biological activity in humans. Biomarker measurements in serial blood samples demonstrate whether administered immunomodulators produce predicted effects on immune cell populations, activation states, or cytokine levels. More invasive sampling, such as lymph node or tissue biopsies when ethically justified, enables direct evaluation of drug effects in relevant immune compartments. Establishing pharmacokinetic-pharmacodynamic relationships in Phase I provides critical information for dose selection in efficacy trials, identifying exposure levels necessary to achieve desired biological effects.

Phase IIA proof-of-concept trials evaluate preliminary efficacy in small patient populations, providing critical go or no-go decision points for further development. These exploratory trials typically employ biomarkers or surrogate endpoints that respond more rapidly than definitive clinical outcomes, enabling more efficient evaluation. For immunomodulatory therapeutics, proof-of-concept trials might assess changes in disease activity scores, reductions in inflammatory biomarkers, or modulation of disease-relevant immune responses rather than long-term outcomes like mortality. Patient selection often focuses on populations most likely to respond, enriching trials for success and providing clearer signals of biological activity, though potentially limiting generalizability.

Phase IIB dose-ranging studies characterize relationships between dose and clinical response, identifying optimal doses for Phase III confirmatory trials. Randomized, placebo-controlled designs with multiple dose levels provide robust data for modeling dose-response relationships. Statistical modeling approaches, including Emax models and linear or log-linear regression, quantify these relationships and predict responses at untested doses. Consideration of both efficacy and safety across the dose range identifies therapeutic windows where benefits outweigh risks.

Phase III pivotal trials provide definitive evidence of efficacy and safety in large patient populations representative of intended treatment populations. These confirmatory trials must meet rigorous regulatory standards including randomization, blinding, appropriate control groups, and statistically justified sample sizes ensuring adequate power to detect clinically meaningful treatment effects. Endpoint selection represents a critical decision, with regulatory agencies generally requiring demonstration of effects on validated clinical outcomes rather than surrogate markers, though certain disease contexts accept well-validated biomarkers when clinical endpoints require impractically long trials. For chronic immunological conditions, trial durations often extend to months or years to adequately assess long-term efficacy and safety.

Adaptive trial designs have emerged as powerful approaches enhancing clinical development efficiency. These designs incorporate interim analyses enabling protocol modifications based on accumulating data while maintaining trial integrity and statistical validity. Response-adaptive randomization increases allocation to better-performing treatment arms, potentially improving outcomes for trial participants while maintaining ability to detect treatment differences. Seamless adaptive designs combine traditional trial phases, for example transitioning from dose-finding to confirmatory evaluation within a single protocol, reducing timelines and development costs.

Biomarker-guided patient selection, termed precision medicine or personalized medicine, has transformed clinical development of immunomodulatory therapeutics. This approach identifies patient subgroups most likely to benefit based on molecular, cellular, or genetic characteristics, potentially improving response rates and reducing heterogeneity that obscures treatment effects. Companion diagnostics developed alongside therapeutic products enable prospective identification of appropriate patients in clinical practice. Challenges include biomarker validation requirements, reduced eligible patient populations, and increased trial complexity, but potential benefits of targeted approaches often justify these complications.

Basket trials and umbrella trials represent innovative master protocol designs enabling efficient evaluation of multiple treatments or patient populations. Basket trials evaluate single treatments across multiple diseases or patient subgroups sharing common molecular features, such as particular immune signatures. Umbrella trials assess multiple treatments within single disease populations, often matching therapies to patients based on molecular characteristics. These designs improve efficiency by sharing infrastructure, administrative processes, and control groups across multiple comparisons.

Real-world evidence, derived from electronic health records, insurance claims, patient registries, and wearable devices, increasingly supplements traditional clinical trial data. These pragmatic data sources provide information on treatment effects in broader, more diverse populations than typically enrolled in controlled trials. However, real-world evidence faces challenges including missing data, confounding, and lack of randomization, requiring sophisticated analytical methods like propensity score matching or instrumental variable analysis to draw valid causal inferences. Regulatory agencies increasingly consider real-world evidence in decision-making, though standards for such evidence continue evolving.

Patient-reported outcomes, capturing disease impacts and treatment effects from patient perspectives, have gained recognition as important clinical trial endpoints. These measures assess symptoms, functional status, and quality of life directly from patients rather than relying solely on physician assessments or laboratory values. For chronic immunological conditions, patient-reported outcomes may provide more meaningful assessment of treatment benefits than traditional clinical measures, particularly when diseases primarily affect quality of life rather than survival. Validated instruments with demonstrated reliability, validity, and responsiveness are essential for regulatory acceptance of patient-reported outcome endpoints.

Pediatric development planning, required by regulatory mandates in many jurisdictions, extends clinical evaluation to children when adult indications are relevant to pediatric populations. Pediatric trial designs often employ innovative approaches including modeling and simulation to inform dosing, extrapolation from adult efficacy data when scientifically justified, and opportunistic enrollment strategies making pediatric evaluation more feasible. Special considerations for immunomodulatory drugs in children include developing immune systems that may respond differently than mature adult immune systems and increased concern about long-term effects given life expectancy.

Post-marketing studies continue evaluation after regulatory approval, addressing questions incompletely answered by pre-approval trials. Phase IV commitments may include long-term safety surveillance, evaluation in patient populations excluded from pivotal trials, or assessment of rare adverse events detectable only with large-scale exposure. Risk management programs for immunomodulatory drugs often include enhanced safety monitoring given potential for serious immune-related adverse events. Comparative effectiveness studies evaluate approved therapies against existing standards of care in real-world settings, informing treatment selection and healthcare policy decisions.

Regulatory pathway selection significantly impacts clinical development strategies for plant-derived immunomodulators. Traditional new drug application pathways established for synthetic small molecules generally apply to purified phytochemical constituents. Botanical drug pathways, available in some jurisdictions, accommodate complex botanical preparations with multiple constituents, allowing approval based on well-characterized mixtures when isolation of single active components proves impractical or when activity requires synergistic interactions among multiple constituents. These pathways typically impose rigorous standardization requirements ensuring consistency across batches and production methods.

Orphan drug designations provide incentives for developing treatments for rare diseases, including uncommon immunological conditions. These designations offer benefits including extended market exclusivity, tax credits for clinical trial costs, and regulatory assistance. Many immunological indications, particularly rare autoimmune diseases or primary immunodeficiencies, qualify for orphan designation, making these pathways relevant for plant-derived immunomodulators targeting such conditions.

6. Technological and Computational Enhancements

Contemporary drug discovery increasingly leverages computational technologies and artificial intelligence to enhance efficiency, reduce costs, and enable previously impossible analyses. For discovering immunomodulatory drugs from medicinal plants, these technological enhancements address unique challenges while accelerating translation from botanical materials to clinical therapeutics. Integration of computational approaches with traditional experimental methods creates synergistic research paradigms generating insights unattainable through either approach alone.

Artificial intelligence and machine learning algorithms have transformed multiple aspects of drug discovery from medicinal plants. Supervised learning methods trained on datasets of known bioactive and inactive compounds predict immunomodulatory activities of untested phytochemicals, enabling virtual screening that prioritizes experimental evaluation of molecules most likely to exhibit desired properties. Neural networks with deep architectures, capable of learning complex non-linear relationships between chemical structures and biological activities, have achieved remarkable accuracy in activity prediction when trained on sufficiently large, high-quality datasets. These models enable rapid in silico evaluation of virtual compound libraries, dramatically reducing the number of molecules requiring physical synthesis and experimental testing.

Molecular docking simulations predict binding poses and affinities of phytochemicals with protein targets, facilitating virtual screening and structure-based optimization. These computational approaches model physical interactions between small molecules and macromolecular targets, scoring predicted binding modes based on complementarity of shapes, electrostatic interactions, hydrogen bonding, and hydrophobic effects. Advanced docking algorithms account for protein flexibility, recognizing that binding sites may undergo conformational changes upon ligand binding. Ensemble docking employs multiple protein conformations representing different states sampled during molecular dynamics, improving prediction accuracy particularly for flexible binding sites.

Molecular dynamics simulations provide atomic-level views of time-dependent molecular behaviors, revealing how phytochemical-protein complexes behave under physiological conditions. These simulations model systems containing thousands to millions of atoms, calculating forces and updating positions at femtosecond time intervals to generate trajectories spanning nanoseconds to microseconds. Analysis of molecular dynamics trajectories reveals binding site dynamics, alternative binding modes, and residence times of bound ligands, information valuable for understanding and optimizing drug-target interactions. Free energy calculations based on molecular dynamics enable quantitative prediction of binding affinities, though computational expense currently limits routine application.

Quantitative structure-activity relationship modeling correlates chemical structures with biological activities using statistical or machine learning methods. Traditional quantitative structure-activity relationship approaches employ physicochemical descriptors like molecular weight, lipophilicity, and electronic properties as independent variables predicting activity as dependent variables. Three-dimensional quantitative structure-activity relationship methods consider spatial arrangements of chemical features, identifying regions of molecules contributing positively or negatively to activity. Machine learning quantitative structure-activity relationship models, including random forests, support vector machines, and gradient boosting, often outperform traditional statistical approaches, particularly for modeling complex non-linear structure-activity relationships.

Pharmacophore modeling identifies essential chemical features required for biological activity, creating abstract representations of spatial arrangements of functional groups necessary for target binding. These models guide virtual screening by identifying molecules containing required pharmacophoric features regardless of overall scaffold. Ligand-based pharmacophores derive from known active compounds, identifying common features shared across structurally diverse molecules exhibiting similar activities. Structure-based pharmacophores incorporate target protein information, defining features complementary to binding site characteristics.

Cheminformatics approaches manage, analyze, and extract knowledge from large chemical datasets. Chemical structure databases enable storage and retrieval of millions of compounds, supporting similarity searches that identify molecules structurally related to known bioactive phytochemicals. Substructure searching finds molecules containing specific chemical motifs associated with immunomodulatory activity. Diversity analysis identifies structurally distinct molecules for screening, ensuring compound collections adequately sample chemical space. Chemical space visualization using dimensionality reduction

techniques like principal component analysis or t-distributed stochastic neighbor embedding enables intuitive understanding of structural relationships among large compound sets.

Network pharmacology approaches, integrating systems biology with pharmacology, elucidate complex interactions between drugs, targets, and diseases. These methods construct networks representing relationships among compounds, protein targets, biological pathways, and diseases, enabling systematic analysis of multi-target effects characteristic of plant-derived immunomodulators. Network analysis algorithms identify key nodes, communities, and pathways mediating therapeutic effects, providing mechanistic insights and suggesting combination therapy strategies. Comparison of disease networks with drug target networks predicts therapeutic applications and potential adverse effects.

Proteomics technologies generate comprehensive protein expression and modification data informing target identification and mechanistic studies. Mass spectrometry-based proteomics enables identification and quantification of thousands of proteins in biological samples, revealing changes induced by immunomodulatory phytochemicals. Targeted proteomics approaches like selected reaction monitoring provide precise quantification of specific proteins of interest across many samples. Post-translational modification proteomics identifies changes in protein phosphorylation, acetylation, ubiquitination, and other modifications regulating immune signaling pathways. Metabolomics profiling characterizes small molecule metabolites in biological systems, complementing genomics and proteomics to provide comprehensive molecular phenotypes. Nuclear magnetic resonance spectroscopy and mass spectrometry platforms enable detection of hundreds to thousands of metabolites in biological samples. Metabolomics analysis of immune cells treated with plant-derived immunomodulators reveals metabolic pathway alterations underlying functional changes, while pharmacometabolomics approaches identify metabolic biomarkers predicting treatment responses.

Single-cell technologies enable analysis of individual cells within heterogeneous populations, revealing cell-to-cell variation obscured by bulk measurements. Single-cell RNA sequencing provides transcriptome-wide gene expression profiles for thousands of individual cells, identifying rare cell populations and heterogeneous responses to immunomodulatory compounds. Single-cell mass cytometry simultaneously measures dozens of protein markers in millions of individual cells, enabling comprehensive immunophenotyping. Spatial transcriptomics maintains tissue architecture during molecular profiling, revealing where within tissues particular cell types respond to bioactive compounds.

High-performance computing infrastructure enables computationally intensive analyses essential for modern drug discovery. Cloud computing platforms provide scalable computational resources accessible without substantial capital investments in hardware. Graphics processing units, originally developed for rendering graphics, excel at parallel calculations required for molecular simulations and machine learning, dramatically accelerating these computations. Distributed computing approaches harness networked computers for large-scale virtual screening campaigns. Data integration approaches combine heterogeneous data types from multiple sources to generate comprehensive understanding exceeding insights from individual datasets. Multi-omics integration combines genomics, transcriptomics, proteomics, and metabolomics data, revealing relationships across molecular levels. Knowledge graphs represent biomedical knowledge as networks of entities and relationships, enabling computational reasoning about drug discovery questions. Ontologies provide standardized vocabularies and hierarchical classifications facilitating data integration and knowledge discovery.

Artificial intelligence applications in predicting absorption, distribution, metabolism, excretion, and toxicity properties accelerate identification of compounds with favorable drug-like characteristics. Machine learning models predict oral bioavailability, blood-brain barrier penetration, metabolic stability, and other pharmacokinetic parameters from chemical structures. Toxicity prediction models estimate likelihoods of various adverse effects including hepatotoxicity, cardiotoxicity, and mutagenicity, enabling early elimination of problematic compounds. These in silico predictions, while imperfect, provide useful prioritization criteria for resource-intensive experimental studies. Automated synthesis and purification platforms accelerate analog generation during lead optimization. Robotic systems conduct parallel synthesis reactions, enabling rapid production of compound libraries. High-throughput purification systems combining liquid chromatography with mass-directed fraction collection isolate desired products from complex reaction mixtures. Integration of automated synthesis with rapid biological testing creates feedback loops accelerating optimization cycles.

Electronic laboratory notebooks and data management systems capture experimental data in structured, searchable formats enabling efficient knowledge management and regulatory compliance. These systems track samples, associate results with experimental conditions, and maintain audit trails essential for good laboratory practices. Integration with analytical instruments enables automated data capture reducing transcription errors. Advanced search capabilities facilitate knowledge retrieval from historical experiments informing current work.

7. Challenges, Ethical, and Regulatory Considerations

The development of plant-derived immunomodulatory therapeutics, while promising, confronts substantial challenges spanning scientific, ethical, regulatory, and practical domains. Addressing these multifaceted obstacles requires coordinated efforts among researchers, regulators, healthcare providers, patient communities, and stakeholders in traditional medicine systems. Successful navigation of these challenges determines whether promising botanical immunomodulators successfully translate to accessible, evidence-based therapeutics improving human health. Standardization and quality control of botanical materials represent fundamental challenges for plant-based drug development. Unlike synthetic compounds with defined chemical structures, plants contain complex mixtures of constituents varying based on genetics, environmental conditions, cultivation practices, harvest timing, and post-harvest processing. This inherent variability complicates development of reproducible therapeutics meeting pharmaceutical quality standards. Strategies addressing these challenges include developing standardized cultivation protocols controlling environmental variables, establishing

authenticated plant germplasm with consistent genetic backgrounds, implementing good agricultural and collection practices, and utilizing analytical methods characterizing multiple chemical markers to ensure batch-to-batch consistency.

Authentication of botanical source materials prevents substitution or adulteration with incorrect plant species, which can compromise efficacy and introduce safety risks. Morphological identification by trained botanists provides traditional authentication approaches, while molecular methods including DNA barcoding offer objective, reproducible alternatives less dependent on specialized taxonomic expertise. Establishment of reference standards and voucher specimens in recognized herbaria ensures permanent records enabling verification. Supply chain integrity systems tracking materials from collection through manufacturing prevent introduction of unauthenticated materials.

Sustainable sourcing of medicinal plants raises environmental and conservation concerns, particularly for species with limited wild populations facing harvest pressure. Overharvesting has contributed to population declines for numerous medicinal plants, threatening both species survival and continued availability of therapeutic materials. Sustainable approaches include cultivation replacing wild harvest when feasible, implementation of harvest quotas based on population assessments, development of alternative sources through plant tissue culture or biosynthetic production, and conservation programs protecting wild populations while enabling continued traditional use. Certification schemes verifying sustainable sourcing provide market-based incentives for conservation-friendly practices.

Intellectual property considerations present complex challenges for plant-based drug development, balancing innovation incentives with equitable access to traditional knowledge. Patent systems provide exclusivity motivating investment in expensive development processes, yet concerns arise when patents claim traditional knowledge without appropriate recognition or benefit sharing. The Nagoya Protocol on Access and Benefit Sharing establishes international frameworks for equitable sharing of benefits arising from use of genetic resources and associated traditional knowledge. Implementation challenges include defining prior informed consent requirements, establishing mutually agreed terms for benefit sharing, and monitoring compliance.

Biopiracy, the appropriation of biological resources and traditional knowledge without authorization or compensation, represents serious ethical and legal concerns. High-profile cases involving patents on traditional uses of turmeric, neem, and other medicinal plants have highlighted these issues, spurring development of databases documenting traditional knowledge to establish prior art preventing inappropriate patents. Ethical research practices require engaging with traditional knowledge holders, obtaining appropriate permissions, ensuring fair benefit sharing, and acknowledging contributions of indigenous and local communities.

Regulatory pathways for botanical drugs vary across jurisdictions, creating challenges for global development programs. Some regulatory agencies have established specific guidance for botanical drugs accommodating their complexity, while others apply frameworks developed for single-entity synthetic drugs with minimal adaptation. Key regulatory issues include defining appropriate characterization and standardization requirements, determining acceptable levels of constituent variability, establishing relevant safety testing paradigms, and defining clinical evidence sufficient for approval. Harmonization efforts aim to align requirements across regions, though significant differences remain.

Clinical trial design challenges specific to botanical immunomodulators include selecting appropriate comparators, defining standardized interventions when using complex plant preparations, and addressing blinding difficulties when botanical products have distinctive sensory characteristics. Placebo controls present ethical challenges when effective standard treatments exist, necessitating active comparator designs that require larger sample sizes to demonstrate non-inferiority or superiority. For traditional medicine products used in specific cultural contexts, determining culturally appropriate trial designs and outcome measures requires meaningful engagement with relevant communities.

Safety monitoring for immunomodulatory botanicals requires vigilance for immune-related adverse events potentially emerging only with prolonged exposure or in susceptible individuals. Immunosuppressive effects may increase infection risks, while excessive immune activation could precipitate inflammatory toxicities or autoimmunity. Herb-drug interactions present additional safety concerns, particularly when botanical immunomodulators are used alongside conventional immunosuppressive or immunostimulatory therapies. Post-marketing surveillance systems detect rare adverse events not identifiable in limited pre-approval populations.

Manufacturing challenges for botanical products include establishing consistent processes yielding reproducible products from variable agricultural inputs. Good manufacturing practices adapted for botanical materials address unique considerations including microbial contamination risks, prevention of cross-contamination between different botanical species, and cleaning validation for equipment contacting complex plant materials. Analytical methods must adequately characterize complex mixtures, often requiring multiple orthogonal techniques given that no single method captures complete compositional profiles. Process validation demonstrates that manufacturing procedures consistently produce products meeting predetermined specifications.

Formulation stability issues affect many plant-derived compounds susceptible to degradation through oxidation, hydrolysis, photolysis, or thermal decomposition. Stability testing under stressed conditions identifies degradation pathways and informs formulation strategies incorporating antioxidants, light protection, moisture control, or controlled temperature storage. Real-time stability studies at recommended storage conditions establish product shelf lives supporting expiration dating.

Bioavailability limitations impede clinical development of numerous bioactive phytochemicals exhibiting poor oral absorption, extensive first-pass metabolism, or rapid clearance. Traditional preparations sometimes employ strategies enhancing bioavailability, such as co-administration with absorption enhancers or preparation methods increasing solubility. Modern approaches include

prodrug development, structural modification improving permeability while retaining activity, and advanced delivery systems like nanoformulations.

Translation challenges arise from differences between preclinical models and human diseases. Many immunological conditions lack fully representative animal models, raising uncertainty about how effectively preclinical findings predict clinical responses. Species differences in immune system organization, target protein sequences, and pathway wiring may cause compounds active in animals to fail in humans, or vice versa. Use of human cells, tissues, and in silico models complementing animal studies provides additional confidence, though none perfectly replicate human pathophysiology.

Cost considerations affect accessibility of plant-derived therapeutics. Development costs for bringing new drugs to market, typically hundreds of millions to billions of dollars, must be recovered through product pricing, potentially limiting access in resource-constrained settings. Botanical source materials may themselves be expensive if derived from rare species or requiring labor-intensive processing. Balancing commercial viability enabling sustained development programs with affordability supporting equitable access represents ongoing tension requiring creative solutions including tiered pricing, voluntary licensing, and public-private partnerships.

Cultural sensitivity and respect for traditional knowledge holders require meaningful engagement throughout research and development processes. Appropriate consultation mechanisms vary across communities but generally include early engagement before research initiation, ongoing communication throughout projects, opportunities for community input into research design and interpretation, and acknowledgment of traditional knowledge contributions. Power imbalances between well-resourced research institutions and marginalized traditional knowledge holders necessitate deliberate efforts ensuring genuine partnership rather than extractive research relationships.

Regulatory approval does not guarantee clinical adoption, as healthcare providers and patients must accept new therapeutics. Evidence generation addressing clinician questions about efficacy, safety, and appropriate patient selection facilitates informed prescribing decisions. Patient education regarding proper use, expected benefits, and potential risks supports treatment adherence and appropriate expectations. Integration with clinical practice guidelines and treatment algorithms positions new therapies within broader therapeutic landscapes.

Reimbursement by healthcare payers determines practical accessibility for many patients unable to afford out-of-pocket costs. Health technology assessment processes evaluate clinical effectiveness and cost-effectiveness, informing coverage decisions. Demonstrating value relative to existing treatments through comparative effectiveness studies and health economic analyses supports favorable reimbursement determinations.

8. Conclusion

The discovery and development of immunomodulatory drugs from medicinal plants represents a vibrant scientific frontier integrating traditional botanical knowledge with contemporary pharmaceutical science, systems biology, and advanced technologies. This comprehensive review has examined the multifaceted landscape of plant-based immunomodulatory drug discovery, from fundamental target identification and validation strategies through high-throughput screening and lead optimization, preclinical and translational research paradigms, clinical development innovations, and technological enhancements, while addressing critical challenges, ethical considerations, and regulatory pathways that shape successful therapeutic development.

Several overarching themes emerge from this analysis. First, medicinal plants provide extraordinary chemical diversity, evolved through millions of years of plant-environment interactions, offering molecular scaffolds and bioactive compounds that continue inspiring therapeutic innovation. The structural complexity and multi-target activity profiles characteristic of many phytochemicals present both challenges and opportunities, requiring sophisticated analytical approaches while potentially enabling treatment of complex immunological diseases through synergistic modulation of multiple pathways. Second, successful translation from traditional use to evidence-based therapeutics demands rigorous application of modern drug discovery methodologies, including systematic target validation, careful preclinical development, and well-designed clinical trials generating robust efficacy and safety data. Third, technological innovations, particularly computational approaches and artificial intelligence applications, are transforming the efficiency and scope of natural product drug discovery, enabling previously impossible analyses and accelerating timelines from discovery to clinical application.

The integration of diverse scientific disciplines proves essential for advancing plant-derived immunomodulators. Ethnobotanical research documenting traditional knowledge provides valuable starting points for discovery efforts. Phytochemistry enables isolation, characterization, and structural elucidation of bioactive constituents. Immunology and cell biology illuminate mechanisms through which compounds modulate immune function. Pharmacology characterizes drug actions, while toxicology ensures safety. Clinical medicine translates laboratory discoveries to patient care. Regulatory science navigates approval pathways. This inherently multidisciplinary nature necessitates collaborative research teams and effective communication across traditional disciplinary boundaries.

Looking forward, several promising directions merit emphasis. Continued advancement of precision medicine approaches will enable identification of patient populations most likely to benefit from particular plant-derived immunomodulators, improving clinical trial success rates and therapeutic outcomes. Integration of multi-omics technologies will deepen mechanistic understanding, revealing how compounds influence complex immune networks and identifying biomarkers for patient selection and treatment monitoring. Artificial intelligence and machine learning applications will mature, potentially revolutionizing virtual screening, lead optimization, and clinical trial design. Novel delivery systems will address bioavailability limitations that have hindered clinical development of promising phytochemicals. Combination therapy strategies leveraging complementary mechanisms may achieve superior efficacy compared to monotherapies.

Challenges remain substantial. Standardization of complex botanical materials requires continued methodological innovation. Translation from preclinical models to human

efficacy demands better predictive systems. Regulatory frameworks must evolve to appropriately accommodate botanical products while maintaining rigorous standards. Ethical frameworks ensuring equitable benefit sharing and respect for traditional knowledge require continued refinement and implementation. Sustainable sourcing systems must balance therapeutic development with conservation imperatives. Economic models enabling both commercial viability and affordable access need creative solutions.

The COVID-19 pandemic highlighted both the critical importance of immunomodulatory therapeutics and the potential contributions of plant-based approaches, with several traditional medicinal plants receiving emergency authorization or intensive investigation for managing hyperinflammatory syndromes and supporting immune function. This experience demonstrates how rapidly research can progress when resources, regulatory flexibility, and collaborative approaches align around urgent therapeutic needs, lessons applicable to future drug development efforts.

Patient perspectives deserve central consideration in therapeutic development. Individuals living with chronic immunological conditions seek safe, effective treatments improving quality of life and long-term outcomes. Growing interest in complementary and integrative medicine reflects desires for therapeutic options perceived as more natural or holistic, creating opportunities for evidence-based botanical immunomodulators meeting both scientific standards and patient preferences. However, ensuring that such products deliver genuine therapeutic benefit rather than merely capitalizing on marketing appeal requires unwavering commitment to rigorous evaluation and transparent communication of evidence.

Global health equity considerations demand attention, as immunological diseases affect populations worldwide while access to effective therapeutics remains dramatically unequal. Plant-based approaches may offer pathways to developing affordable treatments accessible in resource-limited settings, particularly when source materials grow locally and production can occur regionally. However, realizing this potential requires intentional efforts including capacity building for research and development in low- and middle-income countries, technology transfer facilitating local manufacturing, and pricing strategies ensuring affordability.

The future of immunomodulatory drug discovery from medicinal plants appears promising, driven by expanding scientific capabilities, growing recognition of natural product value, and increasing sophistication in translating traditional knowledge to modern therapeutics. Success will require sustained commitment to rigorous science, ethical research practices, collaborative partnerships spanning traditional knowledge holders and cutting-edge research institutions, adaptive regulatory frameworks, and patient-centered approaches prioritizing meaningful clinical benefits. The rich botanical heritage cultivated across human cultures over millennia, combined with contemporary scientific methodologies and technologies, positions plant-derived immunomodulators to make substantial contributions to twenty-first-century medicine, addressing unmet therapeutic needs and improving health outcomes for diverse patient populations worldwide.

9. Figures

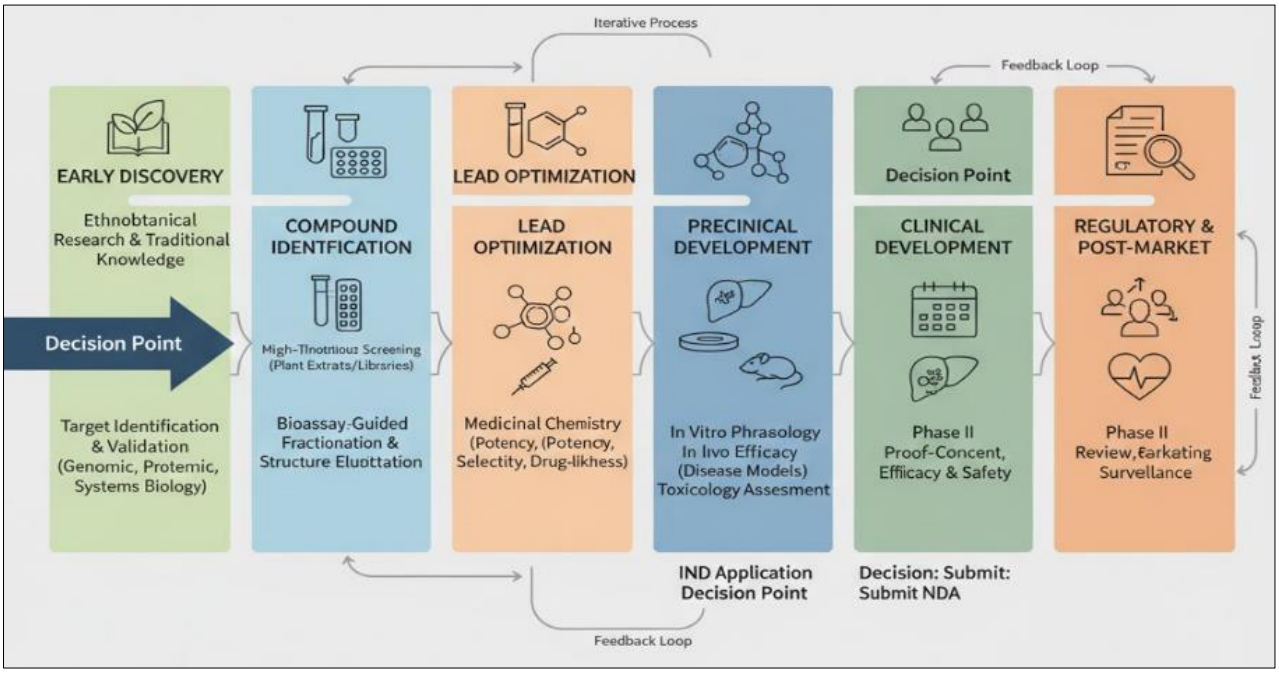

Fig 1: Overview of the drug discovery and development pipeline for plant-derived immunomodulatory therapeutics.

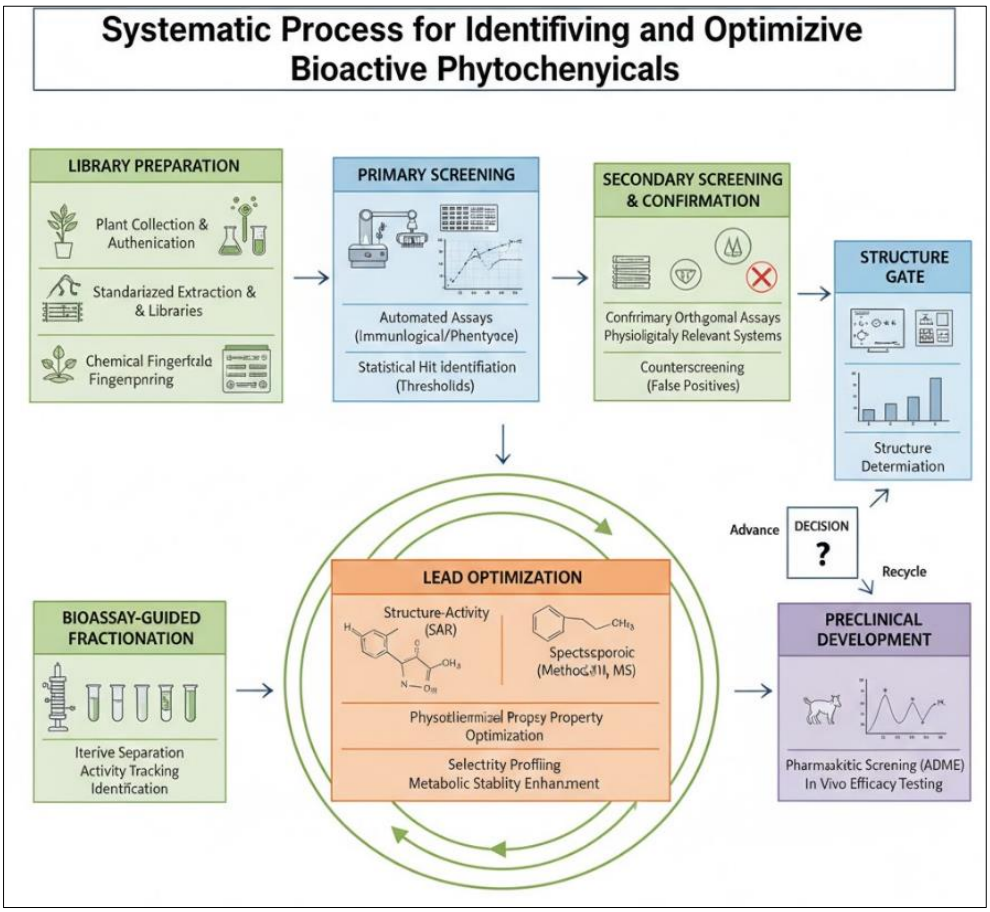

Fig 2: Workflow of high-throughput screening and lead optimization for immunomodulatory compounds from medicinal plants.

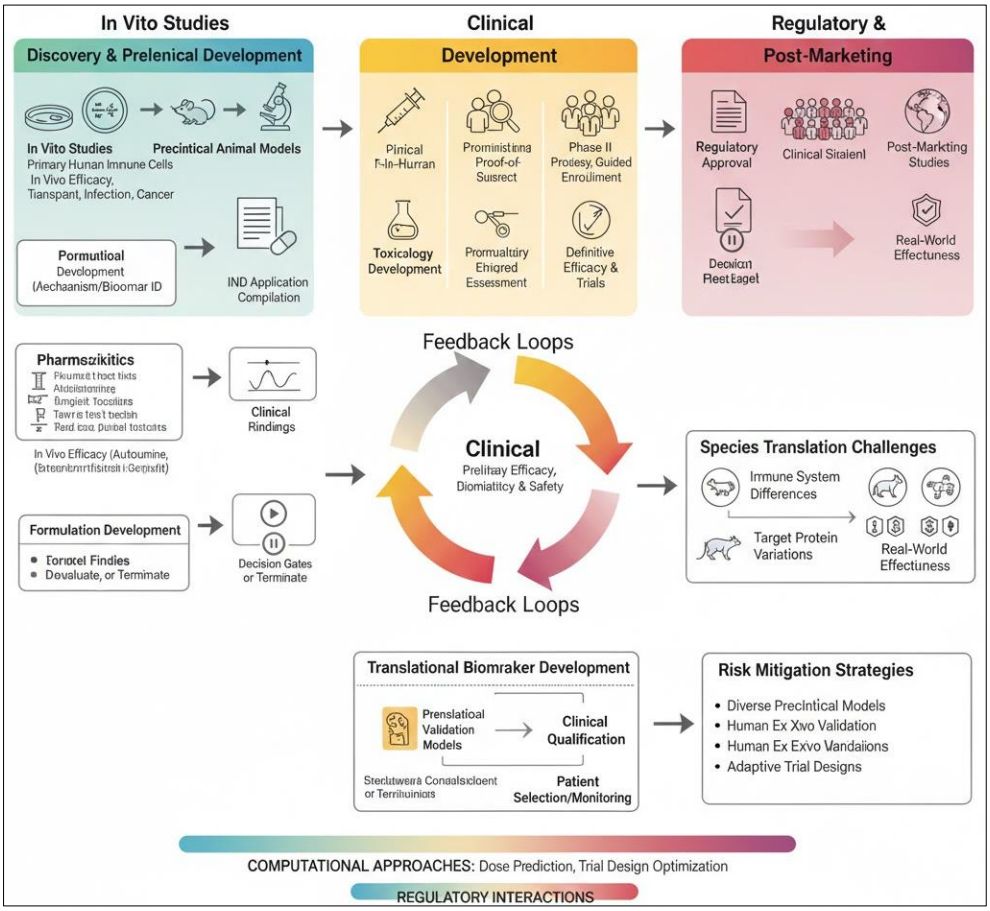

Fig 3: Translational pathway from preclinical models to clinical trials for plant-derived immunomodulators.

10. Tables

Table 1: Comparison of conventional versus modern drug discovery strategies for plant-derived immunomodulators

Aspect	Conventional Approaches	Modern Approaches	Key Advantages of Modern Methods
Target Identification	Phenotypic observation of traditional uses; limited mechanistic understanding; single-target hypothesis	Systems biology integration; multi-omics profiling; network pharmacology; phenotypic screening with target deconvolution	Comprehensive pathway mapping; identification of multi-target mechanisms; data-driven hypothesis generation
Compound Screening	Sequential testing of individual extracts; low throughput; labor-intensive bioassays	High-throughput automated screening; parallel evaluation of thousands of samples; miniaturized assay formats	Dramatic acceleration of discovery timelines; systematic evaluation of large libraries; improved statistical power
Lead Identification	Bioassay-guided fractionation; structure elucidation by classical spectroscopy	Hyphenated analytical techniques; LC-MS-guided isolation; dereplication using databases; chemical proteomics for target identification	Faster compound identification; avoidance of known compounds; direct target binding evidence
Lead Optimization	Limited chemical modification; trial-and-error approach; empirical structure-activity relationships	Computational modeling; structure-based design; predictive QSAR models; automated synthesis; AI-guided optimization	Rational design reducing synthesis burden; improved prediction of drug-like properties; accelerated optimization cycles
Preclinical Models	Simple animal disease models; limited mechanistic readouts; focus on efficacy	Humanized models; transgenic and knockout animals; sophisticated immunophenotyping; mechanistic biomarkers; multi-parameter assessment	Better human translation; mechanistic insights guiding clinical development; identification of responsive patient populations
Clinical Development	Sequential phase progression; fixed designs; limited adaptation	Adaptive trial designs; biomarker-guided enrollment; seamless phase transitions; precision medicine approaches	Improved efficiency; higher success rates; personalized treatment strategies
Data Analysis	Manual analysis; limited statistical sophistication; isolated datasets	Machine learning; integrated multi-omics analysis; network analysis; real-time data monitoring	Discovery of complex patterns; integration of heterogeneous data; predictive modeling

Table 2: Advantages, limitations, and innovations in preclinical and clinical development of plant-derived immunomodulators

Development Stage	Primary Advantages	Key Limitations	Recent Innovations Addressing Limitations
*In vi*tro Pharmacology	Mechanistic control; use of human cells; cost-effectiveness; high throughput capacity	Limited physiological relevance; absence of tissue architecture and immune cell interactions; cell line artifacts	Three-dimensional culture systems; organoid models; complex co-culture systems mimicking immune microenvironments; organ-on-chip technologies
Animal Pharmacology	Intact immune systems; disease pathophysiology modeling; pharmacokinetic assessment	Species differences in immune organization; imperfect disease model fidelity; ethical considerations; interspecies translation challenges	Humanized mouse models; patient-derived xenografts; improved transgenic disease models; advanced imaging enabling longitudinal assessment
Toxicology Assessment	Identification of safety liabilities; dose-response characterization; regulatory requirement fulfillment	Resource intensive; animal use; potential species-specific toxicities not predicting human responses	*In vi*tro toxicology platforms; computational toxicity prediction; microphysiological systems; human-relevant endpoints
Pharmacokinetic Studies	Quantitative exposure characterization; dose optimization; interspecies comparison	Complex analysis requirements; metabolite identification challenges; bioavailability prediction uncertainty	Physiologically based pharmacokinetic modeling; imaging mass spectrometry for tissue distribution; microsampling reducing animal burden
Biomarker Development	Objective efficacy measurement; patient stratification; dose optimization; mechanism confirmation	Validation requirements; assay development complexity; regulatory qualification challenges	Multi-parameter flow cytometry; digital pathology; cell-free DNA and circulating biomarkers; point-of-care devices
Phase I Trials	Human pharmacokinetics; safety assessment; first pharmacodynamic evidence	Limited efficacy data; healthy volunteer use may miss disease-specific effects; small sample sizes	Adaptive dose escalation; integrated pharmacodynamic assessments; early patient enrollment when appropriate; extensive biomarker profiling
Phase II Trials	Proof-of-concept establishment; dose-response characterization; biomarker validation	Heterogeneous patient populations may obscure effects; limited sample sizes; surrogate endpoints may not predict clinical benefit	Biomarker-guided enrichment; basket and umbrella trial designs; adaptive randomization; seamless Phase IIB to III transitions
Phase III Trials	Definitive efficacy evidence; large safety database; regulatory approval basis	Long duration; high costs; recruitment challenges; fixed designs limit adaptation	Pragmatic designs; real-world evidence integration; adaptive features; digital health technologies for remote monitoring
Botanical Standardization	Traditional knowledge preservation; multi-component synergy potential	Compositional variability; characterization complexity; manufacturing challenges	Advanced analytical fingerprinting; quality by design approaches; marker compound standardization; process analytical technology
Formulation Development	Bioavailability enhancement; stability improvement; targeted delivery	Phytochemical physicochemical challenges; complex regulatory requirements	Nanotechnology platforms; prodrug strategies; absorption enhancers; controlled release systems

11. References

1. Abbas AK, Lichtman AH, Pillai S. *Cellular and Molecular Immunology.* 9th ed. Philadelphia: Elsevier; 2017.
2. Schett G, Neurath MF. Resolution of chronic inflammatory disease: universal and tissue-specific concepts. *Nat Commun.* 2018;9(1):3261.
3. Petrovska BB. Historical review of medicinal plants usage. *Pharmacogn Rev.* 2012;6(11):1–5.
4. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. *Molecules.* 2016;21(5):559.
5. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, *et al*. Discovery and resupply of pharmacologically active plant-derived natural products: a review. *Biotechnol Adv.* 2015;33(8):1582–614.
6. Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. *Nat Rev Drug Discov.* 2015;14(4):111–29.
7. Newman DJ, Cragg GM. Natural products as sources of new drugs over nearly four decades from 01/1981 to 09/2019. *J Nat Prod.* 2020;83(3):770–803.
8. Beutler JA. Natural products as a foundation for drug discovery. *Curr Protoc Pharmacol.* 2019;86(1):e67.
9. Heinrich M, Appendino G, Efferth T, *et al*. Best practice in research – overcoming common challenges in phytopharmacological research. *J Ethnopharmacol.* 2020;246:112230.
10. Welch JS. Patterns of intratumor immune heterogeneity. *Sci Transl Med.* 2016;8(334):334fs8.
11. Macarron R, Banks MN, Bojanic D, *et al*. Impact of high-throughput screening in biomedical research. *Nat Rev Drug Discov.* 2011;10(3):188–95.
12. Morgan P, Van Der Graaf PH, Arrowsmith J, *et al*. Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discov Today.* 2012;17(9–10):419–24.
13. Mouchlis VD, Afantitis A, Serra A, *et al*. Advances in de novo drug design: from conventional to machine learning methods. *Int J Mol Sci.* 2021;22(4):1676.
14. Bilia AR, Guccione C, Isacchi B, Righeschi C, Firenzuoli F, Bergonzi MC. Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. *Evid Based Complement Alternat Med.* 2014;2014:651593.
15. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. *J Immunol.* 2004;172(5):2731–8.
16. Pan SY, Litscher G, Gao SH, *et al*. Historical perspective of traditional indigenous medical practices: the current

renaissance and conservation of herbal resources. *Evid Based Complement Alternat Med.* 2014;2014:525340.
17. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.* 2014;4:177.
18. Chen SL, Yu H, Luo HM, *et al*. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. *Chin Med.* 2016;11:37.
19. Schenone M, Dančík V, Wagner BK, Clemons PA. Target identification and mechanism of action in chemical biology and drug discovery. *Nat Chem Biol.* 2013;9(4):232–40.
20. Moffat JG, Vincent F, Lee JA, Eder J, Prunotto M. Opportunities and challenges in phenotypic drug discovery: an industry perspective. *Nat Rev Drug Discov.* 2017;16(8):531–43.
21. Swinney DC, Anthony J. How were new medicines discovered? *Nat Rev Drug Discov.* 2011;10(7):507–19.
22. Lamb J, Crawford ED, Peck D, *et al*. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *Science.* 2006;313(5795):1929–35.
23. Bantscheff M, Lemeer S, Savitski MM, Kuster B. Quantitative mass spectrometry in proteomics: critical review update from 2007 to the present. *Anal Bioanal Chem.* 2012;404(4):939–65.
24. Rix U, Superti-Furga G. Target profiling of small molecules by chemical proteomics. *Nat Chem Biol.* 2009;5(9):616–24.
25. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol.* 2008;4(11):682–90.
26. Anighoro A, Bajorath J, Rastelli G. Polypharmacology: challenges and opportunities in drug discovery. *J Med Chem.* 2014;57(19):7874–87.
27. Aldridge BB, Burke JM, Lauffenburger DA, Sorger PK. Physicochemical modelling of cell signalling pathways. *Nat Cell Biol.* 2006;8(11):1195–203.
28. Kaelin WG Jr. Molecular biology: use and abuse of RNAi to study mammalian gene function. *Science.* 2012;337(6093):421–2.
29. Drost J, Clevers H. Organoids in cancer research. *Nat Rev Cancer.* 2018;18(7):407–18.
30. Boutros M, Heigwer F, Laufer C. Microscopy-based high-content screening. *Cell.* 2015;163(6):1314–25.
31. Shalem O, Sanjana NE, Zhang F. High-throughput functional genomics using CRISPR-Cas9. *Nat Rev Genet.* 2015;16(5):299–311.
32. Nijman SM. Functional genomics to uncover drug mechanism of action. *Nat Chem Biol.* 2015;11(12):942–8.
33. Workman P, Collins I. Probing the probes: fitness factors for small molecule tools. *Chem Biol.* 2010;17(6):561–77.
34. Fang Y. Ligand-receptor interaction platforms and their applications for drug discovery. *Expert Opin Drug Discov.* 2012;7(10):969–88.
35. Ladbury JE, Klebe G, Freire E. Adding calorimetric data to decision making in lead discovery: a hot tip. *Nat Rev Drug Discov.* 2010;9(1):23–27.
36. Pellecchia M, Bertini I, Cowburn D, *et al*. Perspectives on NMR in drug discovery: a technique comes of age. *Nat Rev Drug Discov.* 2008;7(9):738–45.
37. Blundell TL, Jhoti H, Abell C. High-throughput crystallography for lead discovery in drug design. *Nat Rev Drug Discov.* 2002;1(1):45–54.
38. Renaud JP, Chung CW, Danielson UH, *et al*. Biophysics in drug discovery: impact, challenges and opportunities. *Nat Rev Drug Discov.* 2016;15(10):679–98.
39. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. *Nat Rev Drug Discov.* 2004;3(11):935–49.
40. Morgan P, Brown DG, Lennard S, *et al*. Impact of a five-dimensional framework on R&D productivity at AstraZeneca. *Nat Rev Drug Discov.* 2018;17(3):167–81.
41. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther.* 2001;69(3):89–95.
42. Cook D, Brown D, Alexander R, *et al*. Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. *Nat Rev Drug Discov.* 2014;13(6):419–31.
43. Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. *Nat Rev Immunol.* 2012;12(3):191–200.
44. Vignali DA. Multiplexed particle-based flow cytometric assays. *J Immunol Methods.* 2000;243(1–2):243–55.
45. Chaussabel D, Quinn C, Shen J, *et al*. A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus. *Immunity.* 2008;29(1):150–64.
46. Peters JU. Polypharmacology – foe or friend? *J Med Chem.* 2013;56(22):8955–71.
47. Reddy AS, Zhang S. Polypharmacology: drug discovery for the future. *Expert Rev Clin Pharmacol.* 2013;6(1):41–7.
48. Hodos RA, Kidd BA, Shameer K, Readhead BP, Dudley JT. In silico methods for drug repurposing and pharmacology. *Wiley Interdiscip Rev Syst Biol Med.* 2016;8(3):186–210.
49. Stuart T, Satija R. Integrative single-cell analysis. *Nat Rev Genet.* 2019;20(5):257–72.
50. Rao A, Barkley D, França GS, Yanai I. Exploring tissue architecture using spatial transcriptomics. *Nature.* 2021;596(7871):211–20.
51. Wang T, Wei JJ, Sabatini DM, Lander ES. Genetic screens in human cells using the CRISPR-Cas9 system. *Science.* 2014;343(6166):80–4.
52. Heinrich M, Williamson EM, Fretwell N. *Fundamentals of Pharmacognosy and Phytotherapy.* 3rd ed. Edinburgh: Churchill Livingstone; 2017.
53. Patwardhan B, Vaidya AD. Natural products drug discovery: accelerating the clinical candidate development using reverse pharmacology approaches. *Indian J Exp Biol.* 2010;48(3):220–7.
54. Veeresham C. Natural products derived from plants as a source of drugs. *J Adv Pharm Technol Res.* 2012;3(4):200–1.
55. Inglese J, Auld DS, Jadhav A, *et al*. Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries. *Proc Natl Acad Sci U S A.* 2006;103(31):11473–8.

56. Henrich CJ, Beutler JA. Matching the power of high throughput screening to the chemical diversity of natural products. *Nat Prod Rep*. 2013;30(10):1284–98.
57. Lang G, Mayhudin NA, Mitova MI, *et al*. Evolving trends in the dereplication of natural product extracts: new methodology for rapid, small-scale analysis of marine invertebrate extracts. *J Nat Prod.* 2008;71(9):1595–9.
58. Zhang JH, Chung TD, Oldenburg KR. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J Biomol Screen.* 1999;4(2):67–73.
59. Iversen PW, Eastwood BJ, Sittampalam GS, Cox KL. A comparison of assay performance measures in screening assays: signal window, Z' factor, and assay variability ratio. *J Biomol Screen.* 2006;11(3):247–52.