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Modern Approaches to Discovering and Developing Plant-Based Natural Products and Their Analogues as Potential Therapeutic Agents

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Abstract:
Natural products have long been a vital source of novel lead compounds in drug discovery. Many drugs currently used as therapeutic agents have been derived from natural sources, with plants playing a particularly significant role. However, in recent decades, pharmaceutical companies have shown limited interest in natural product-based drug discovery, largely due to the inherent complexity of working with these substances.
Recently, technological advancements have helped overcome many of these challenges, leading to a renewed scientific interest in exploring natural sources for drug development. This review provides a comprehensive overview of modern strategies employed in the selection, authentication, extraction, isolation, biological screening, and analogue development of plant-based natural products, following contemporary drug development principles.
Special emphasis is placed on the bioactivity-guided fractionation approach, including its challenges and recent advancements. The review also presents a brief historical perspective on natural product drug discovery and highlights notable natural drugs developed over the past few decades.
Experts in the field emphasize that the successful development of natural products requires an integrated, interdisciplinary approach that leverages technological innovations. This includes the application of efficient selection methods, well-designed extraction and isolation protocols, advanced structure elucidation techniques, and high-throughput bioassays to establish both the druggability and patentability of phyto-compounds.
Additionally, modern approaches such as molecular modeling, virtual screening, natural product libraries, and database mining are increasingly being used to enhance natural product-based drug discovery.
The resurgence of interest and recent research trends clearly indicate that natural products will continue to play a crucial role in the future development of new therapeutic agents, and the effective application of these new methodologies is expected to further strengthen drug discovery efforts.

Keywords:
Drug discovery (DD), Bioactivity - guided (BG), Extraction, Isolation, Plant based natural products (PBNP), Ethno pharmacological (EP).

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I. Literature review and introduction:

There is a long-standing tradition of using plant materials to treat human diseases. Several plant species, such as opium (Papaver somniferum), myrrh (Commiphora species), and licorice (Glycyrrhiza glabra), were mentioned on clay tablets from Mesopotamia dating back to 2600 BC.

These plants are still in use today, either individually or as components of herbal formulations for treating various ailments. Moreover, organic compounds derived from natural sources have historically been used—and continue to be used—for the treatment of numerous diseases.

These compounds are utilized both in their natural form (as pure drugs or phytomedicines) and as lead molecules for the development of synthetic and semi-synthetic analogues with enhanced drug-like properties. Notable examples of such bioactive constituents that are in clinical use include morphine, codeine, noscapine, papaverine, quinine, artemisinin, and paclitaxel [1–3].

In addition to the discovery of new chemical entities (NCEs) for therapeutic applications, natural products provide a crucial foundation as potential lead compounds for the development of new and more effective drugs through structural modification. Although natural products exhibit diverse and complex chemical structures,

plant secondary metabolites are often considered to possess greater biological compatibility and drug-likeness compared to compounds derived solely from synthetic sources.

As a result, molecules of natural origin are generally regarded as more promising candidates for further drug development [4,5]. For instance, Newman et al. (2003) reported that approximately 28% of all NCEs introduced to the market between 1981 and 2002 were derived from natural sources, with an additional 24% developed through chromophore analysis of natural products [2].

The significance of natural products in modern medicine was further reinforced by a report highlighting that 23 natural products with diverse chemical structures entered the market between 2000 and 2005, alongside many other NCEs undergoing various stages of clinical development [6]. Natural products and their derivatives demonstrate a wide range of pharmacological activities and are employed in the treatment and prevention of many prevalent human diseases. These include infectious diseases, cancer, peptic ulcers, immunomodulatory conditions, as well as cardiovascular, respiratory, digestive, and metabolic disorders such as diabetes [2]. A comprehensive analysis of prescription patterns in the United States by Grifo et al. (1997) revealed that 84 of the 150 most commonly prescribed medicines were natural products or related drugs [7].

Similarly, Patridge et al. (2016) found that over one-third of new molecular entities approved by the United States Food and Drug Administration (USFDA) were natural products or their derivatives [8]. Of the 59 drugs approved by the USFDA in 2018, around 16% were either natural products or inspired by them [9].

Another report showed that in 2000, 2001, and 2002, natural products and their derivatives accounted for 40%, 24%, and 26% respectively of the top 35 highest-grossing ethical drug sales worldwide [10]. Notably, paclitaxel, a plant-derived anticancer agent, is among these successful drugs [11]. Globally, about 35% of the annual pharmaceutical market consists of natural products and related drugs, predominantly sourced from plants (25%), followed by microorganisms (13%), and animals (3%) [12].

Between 1983 and 1994, the USFDA approved 520 new drugs, about 39% of which were natural products or their derivatives. This proportion was even higher—approximately 60–80%—for antibiotics and anticancer agents [13–15].

According to Newman and Cragg (2016), of the 1,562 drugs approved by the USFDA between 1981 and 2014, 64 were pure natural products, 141 were herbal mixtures, 320 were derivatives of natural products, and 61 were synthetic drugs inspired by natural pharmacophores—accounting for 4%, 9.1%, 21%, and 4% respectively of the total approved drugs [16].

Best-selling examples of such medicines include antibiotics and antifungals (e.g., erythromycin, clarithromycin, amoxicillin, amphotericin B), anticancer agents (e.g., paclitaxel, docetaxel, camptothecin), cholesterol-lowering drugs (e.g., atorvastatin, simvastatin, lovastatin), immunosuppressants (e.g., tacrolimus, cyclosporin A), and antihypertensives (e.g., captopril, enalapril) [14, 15, 17]. There is an urgent need to encourage the scientific community to integrate natural products more actively into the drug discovery process.

A major challenge, however, lies in the relatively low success rates during the various stages of drug development, especially when source selection is random. To improve these outcomes, the adoption of well-designed strategies for selecting and prioritizing candidate species is essential.

Detailed knowledge of phytomedicines, such as that found in traditional systems of medicine or ethno pharmacological records, can help address low success rates while also reducing both the cost and time associated with natural product drug development [18, 19]. Historically, traditional therapeutic uses of plant materials have played a significant role in guiding the isolation of single active compounds for modern medicine. Ethno pharmacological knowledge remains a vital source of lead compounds in the early stages of drug discovery. Indeed, Fabricant and Farnsworth (2001) noted that approximately 80% of 122 plant-derived natural products were linked to ethno pharmacological uses [20].

Table 1 summarizes several key natural compounds from plant and microbial sources, along with their chemical structures and therapeutic applications. New chemical entities can originate from four main natural sources: plants, marine organisms, animals, and microorganisms (fungi and bacteria). Besides these, synthetic and combinatorial chemistry can also contribute to new compound development. Among these, plants hold particular importance, and this article focuses on approaches related to botanical sources, emphasizing bioactivity-guided fractionation. Natural compounds from plants may serve as:

(i) Therapeutic agents in their native form,

(ii) Lead compounds with specific biological activities for developing more potent analogues,

(iii) Templates for novel pharmacophores that can be transformed into druggable molecules, or

(iv) Markers for standardizing crude plant extracts. Plant extracts are also widely used to develop herbal formulations.

Typically, compounds isolated from natural sources exhibit distinctive structural features, including a higher number of oxygen atoms, multiple chiral centers, greater satiric complexity and molecular rigidity, increased hydrogen bond acceptors and donors, and a lower ratio of aromatic rings to total heavy atoms. They also present a broader range of molecular properties such as partition coefficient, molecular mass, and ring system diversity [3].

Due to these unique characteristics, developing analogues to enhance potency, improve pharmacokinetics, or reduce toxicity poses significant challenges for medicinal chemists. This article highlights modern strategies for discovering natural products from botanical sources, covering systematic candidate selection, bioactivity-guided extraction and fractionation, biological screening, phytochemical characterization to identify promising lead compounds, and subsequent analogue development using in silico studies and virtual screening techniques (Figure 1).

Plants for Screening: In the drug discovery process from plant sources, one of the first and most crucial steps is the selection of plant candidates for the extraction or isolation of active principles and subsequent screening for biological activities. According to Fabricant and Farnsworth (2001), of the approximately 250,000 known species of higher plants, only about 15% have undergone phytochemical screening, and around 6% have been evaluated for their biological properties [20]. Researchers worldwide generally adopt one or more of the following approaches for this purpose.

Ethno pharmacological Knowledge: This approach relies on empirical knowledge of the traditional use of plants in medicine. The discovery of biologically active compounds through this method is guided by the historical and cultural experiences related to plant use. For instance, andrographolide was isolated from Andrographis paniculata, traditionally used for treating dysentery. Similarly, compounds such as berberine, morphine, and picroside were discovered in Berberis aristata, Papaver somniferum, and Picrorhiza kurroa, respectively, through ethnopharmacological selection. This method involves selecting plants based on observation, detailed descriptions, and sometimes experimental evaluation. It may encompass studies in botany, chemistry, pharmacology, biochemistry, archaeology, anthropology, and the historical context of plant use [18].

Randomized approach: In the random approach, plants are selected without prior knowledge of their medicinal use, often from local or national flora. The selected plants are then screened for target bioactivities. Additionally, specific chemical classes—such as flavonoids, alkaloids, or polysaccharides—may also be targeted. This method can be applied for both focused and broad-spectrum screening and offers a reasonable chance of discovering novel bioactive compounds. Although simple and easy to implement, the random approach has the drawback of offering no preliminary information on the potential biological activity of the chosen species.

Traditional Systems of Medicine: Countries like China and India possess a rich heritage of well-documented traditional and herbal medical systems, which are based on codified medicinal practices using botanical sources. This approach differs from ethnomedicine in three main respects. First, codified systems are built on strong conceptual foundations of pharmacology and human physiology, whereas ethnomedicine relies mainly on empirical experience. Second, the development of pharmaceutical formulations is more advanced in codified systems, while ethnomedicine typically uses crude extracts such as decoctions and juices. The concept of standardization is also more prominent in traditional systems. Third, ethnomedicinal practices are usually localized and controlled by small communities, whereas traditional systems are institutionalized and more widely practiced. Several important natural products have been discovered using this approach, including bacosides from Bacopa monnieri (memory enhancer), artemisinin from Artemisia alba (antimalarial), boswellic acid from Boswellia serrata (anti-inflammatory), and reserpine from Rauwolfia serpentina (antihypertensive) [18]. The comparative features of different approaches for selecting plant candidates are summarized in Table 2.

Authentication of Plant Materials: Authentication of collected raw materials is a fundamental starting point in the development of natural products. This process can be accomplished through one or more methods, including taxonomic, macroscopic, microscopic, chromatographic, spectroscopic, chemometric, immunological, and DNA fingerprinting techniques. The choice of method depends on the type of adulterants present and the similarity of chemical constituents between authentic and adulterated materials. In some cases, simple organoleptic evaluation (assessing properties like color, taste, and odor) may suffice, whereas in others, more sophisticated analytical techniques are required. Therefore, it is the responsibility of researchers to select the most appropriate method for authenticating the plant material of interest. The initial step in authenticating medicinal plants is the determination of their botanical origin, including the identification of the scientific binomial name. Macroscopic identification involves comparing organoleptic characteristics—such as color, odor, taste, size, shape, fracture properties, surface features, and texture—with standard reference materials. Microscopic analysis is commonly employed to distinguish between closely related medicinal plants. This method is rapid, practical, and involves examining the internal structural features at the tissue and cellular levels using light microscopy. While a standard light microscope is usually sufficient, the use of polarized or fluorescence microscopy can enhance the accuracy of identification. Chromatographic techniques, such as thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE), are highly valuable for both qualitative and quantitative analyses of natural products. Herbal medicines containing volatile compounds are typically analyzed using gas chromatography (GC). TLC offers preliminary fingerprinting and is advan istageous due to its simplicity and the ability to analyze multiple samples simultaneously. GC is particularly useful for profiling volatile constituents, providing characteristic fingerprints for plant identification. CE is preferred for its high separation efficiency, minimal sample requirements, and rapid analysis. Since the genetic makeup of each plant species is unique and remains unaffected by environmental

factors, age, or other conditions, DNA barcoding provides reliable information for authentication and quality control. This technique enables species-level identification by analyzing short, standardized DNA regions. DNA barcoding is widely applied in research and industry for molecular identification, resolving taxonomic and population genetic issues, preventing illegal wildlife collection and trade, and ensuring the quality of food and medicinal products. Initially limited to plant identification, DNA barcoding has evolved into an essential tool for quality assurance in the authentication of herbal products. The British Pharmacopoeia (BP) recently introduced the first general DNA-based identification method, using Ocimum tenuiflorum L. (Lamiaceae) as an example. The method outlines procedures for plant sampling, DNA extraction, barcode region selection, purification, amplification, and sequence reference comparison.

Extraction and Isolation of Natural Compounds Using Bioactivity-Guided Fractionation: The growing interest in plants as sources of novel therapeutic agents has led to the development of various techniques for the extraction and isolation of natural products. In recent years, bioactivity-guided fractionation and isolation, combined with chromatographic separation methods, have been extensively employed. In this approach, fractionation is driven by the biological activity of the extract rather than by targeting a particular class of compounds. The process involves sequential separation of the plant extract based on physicochemical properties, followed by screening for biological activity at each step. Fractions demonstrating significant bioactivity are subjected to further fractionation and screening until the pure bioactive compound responsible for the target activity is isolated. Chemical characterization and structural elucidation are carried out after the isolation of the active compound.

Bioactivity-guided isolation has led to the discovery of various plant-derived natural products, including anticancer agents such as camptothecin from Camptotheca acuminata and paclitaxel from Taxus brevifolia. Other notable examples include apomorphine, a dopamine receptor agonist derived from morphine; tiotropium, used to treat chronic obstructive pulmonary disease (COPD), derived from atropine; galantamine, a selective anticholinesterase obtained from Galanthus nivalis; and arteether, an antimalarial agent derived from artemisinin.

Bioactivity-guided fractionation of natural products is a relatively modern technique. In experimental practice, two main strategies are commonly employed to isolate either known or novel compounds that may serve as drugs or as lead structures for developing new analogues with improved drug-like properties. Depending on specific circumstances, other approaches may also be applied. The two primary strategies are as follows:

Parallel Approach: This approach is used when the plant is already known for its biological activities, as indicated by traditional or ethnopharmacological knowledge. In this case, the active compounds responsible for the target activity are isolated from crude plant material following a systematic process of extraction and purification, as illustrated in Figure 2. The extraction and isolation typically proceed in three main stages.

Extraction: Initially, at least three types of extracts—such as 100% aqueous, 100% ethanolic, and a 50% aqueous–50% ethanolic extract—are prepared and evaluated for the desired biological activity in primary screening.

Fractionation: The most active extract(s) identified in the primary screening are further fractionated into sub-fractions using solvents of decreasing polarity, typically in the order of butanol, chloroform, and hexane. These sub-fractions are then tested for biological activity.

Isolation and Purification: The most active sub-fraction(s) obtained from the fractionation stage are subjected to chromatographic techniques for compound isolation. The isolated compounds are purified using appropriate methods such as column chromatography, preparative HPLC, and others. Each purified compound is screened for the target biological activity. The chemical structures of compounds demonstrating significant biological activity are elucidated using advanced analytical techniques such as NMR spectroscopy, mass spectrometry, LC-MS, etc.

Sequential Approach:

This approach is mainly applied to plants selected through random selection strategies, where the biological activity of the plant is initially unknown. The process of extraction, fractionation, isolation, and biological screening is carried out sequentially as summarized in Figure 3. The experimental workflow is typically divided into two main stages.

Extraction and Fractionation: In this stage, the extraction of plant material and the fractionation of extracts occur simultaneously. Extractions are performed using solvent systems of increasing polarity, and fractions are collected sequentially — for example, using petroleum ether, chloroform, ethyl acetate, ethanol, and water. All fractions are subsequently screened for their target biological activity.

Isolation and Purification: Fractions from stage 1 that demonstrate the highest biological activity are selected. The compounds responsible for these activities are isolated using established techniques. Structural elucidation of the isolated compounds is carried out using modern methods such as LC-MS, NMR spectroscopy, FT-IR spectroscopy, and mass spectrometry. As illustrated in Figure 3, primary screening at this stage assesses efficacy, while secondary screening focuses on elucidating mechanisms of action, often involving in vitro molecular-level studies. In both approaches, extraction employs a range of polar and non-polar solvents, though the basic method of extraction and fractionation remains largely consistent. Typically, the chemical classes of constituents present

in a particular extract or fraction can be predicted based on the polarity of the solvent used. Non-polar solvents like n-hexane and ether extract lipophilic compounds (e.g., oils, fatty acids, steroids, hydrocarbons, and low-polarity terpenoids). Medium polarity compounds, such as phenolics and alkaloids, are usually found in ethyl acetate or chloroform extracts. Highly polar compounds — including sugars, flavonoids, glycosidic alkaloids, and small carboxylic acids — are typically obtained from aqueous or methanol/ethanol extracts.

Recent Studies Using Bioactivity-Guided Fractionation:

- **Tu et al. (2019)** applied bioactivity-guided fractionation combined with NMR-based identification to isolate the isoflavone genistein from the methanolic extract of Uraria crinita (L.) roots. Genistein was shown to contribute to the immunomodulatory activity by reducing pro-inflammatory cytokines (IL-6 and TNF-α), aiding in the plant's standardization as a functional food.
- **Nothias et al. (2018)** used a bioactive molecular networking strategy alongside mass spectrometry for dereplication in the bioactivity-guided fractionation of Euphorbia dendroides extract. This approach enabled the isolation of new antiviral compounds that classical bioassay-guided fractionation had missed. Bioactivity scores were predicted based on molecular abundance and bioactivity levels of fractions.
- **Iqbal et al. (2015)** isolated a novel compound, n-octadecanyl-O-α-D-glucopyranosyl (6′→1″)-O-α-D-glucopyranoside, from the methanolic extract of Ficus virens bark. The compound showed antihyperlipidemic and antioxidant activities, supported by in vivo studies, molecular docking, and ADME-T profiling.
- **Abdallah et al. (2021)** performed bioassay-guided fractionation of Sclerocarya birrea stem bark extract. The ethyl acetate fraction showed antibacterial activity against Salmonella typhi, with vidarabine identified as the main bioactive constituent via LC-MS/LC-HRMS.
- **Baldé et al. (2021)** investigated Terminalia albida root extract using bioassay-guided isolation. Among 14 isolated compounds, pentolactone exhibited the strongest antiplasmodial activity. Other active compounds included 3,4,3′-tri-O-methylellagic acid, arjunolic acid, arjungenin, arjunic acid, and calophymembranside-B.

Numerous additional studies have successfully applied bioactivity-guided fractionation to isolate both novel and known bioactive phytochemicals.

Structure Elucidation of Isolated Compounds: Although many pharmaceutical companies show limited interest in natural product research due to complex phytochemistry and sourcing challenges, significant research continues in academia. Collaboration among chemists worldwide and advances in techniques have addressed many technical obstacles related to isolation and structural elucidation. Spectroscopic methods — particularly LC-MS and NMR — are now central to structural determination. Following initial biological screening, bioactive extracts are rapidly fractionated by HPLC, and fractions are characterized using LC-MS and NMR. Mass spectrometry data help differentiate novel compounds from known ones by comparison with databases. Pure compounds are isolated efficiently by automated HPLC and characterized through NMR and MS, allowing processes that once took months to be completed in days. LC-MS/MS offers high chromatographic separation combined with precise mass spectrometry characterization, making it highly effective for profiling active fractions. Techniques like FT-IR, NMR, mass spectrometry, X-ray diffraction, optical rotatory dispersion, and chemical tests provide structural insights. The integration of HPLC with these methods allows identification of both known and unknown compounds directly from crude extracts using minimal sample quantities and time. Advances in fractionation methods (e.g., counter-current chromatography) and analytical techniques now enable natural product screening to align with high-throughput screening timelines. Structural elucidation of bioactive natural compounds is often achievable within 4–5 weeks, sometimes using less than 1 mg of sample. In addition to pure isolates, crude extracts can also be profiled using LC-MS/MS, GC-MS/MS, and preliminary NMR to guide further fractionation strategies. NMR is especially useful for identifying functional groups and major compound classes (e.g., sugars, phenolics, steroids, terpenoids, fatty acid esters), which aids in selecting appropriate chromatographic techniques for separation. The structural elucidation workflow for bioactive natural products is summarized in Figure 4.

Biological Screening of Extracts, Fractions, and Isolates: Natural products are typically screened for biological activity based on their reported ethnopharmacological or traditional uses. For example, a medicinal plant traditionally used to manage diabetes may be tested for hypoglycemic activity, providing scientific validation once a promising 'hit' molecule is identified. However, in many cases, such activity is not replicated during in vitro screening. Additionally, because most natural products are obtained in low yields, biological screening must rely on bioassay methods that offer rapid and sensitive results. These assays are conducted using various animal or human cell lines and microorganisms, supported by numerous accurate and efficient instruments [83–86]. Although animal models are still employed to screen extracts and pure compounds from natural sources, this approach has notable limitations. It requires large quantities of samples, involves lengthy and complex procedures, has low sensitivity, and raises ethical concerns. The practical yield of bioactive pure compounds from natural sources is generally very low, making it difficult to obtain sufficient quantities for animal testing. Moreover, potential hits may be incorrectly deemed unsafe based on toxic effects seen in cell-based assays, even though they

might have an acceptable safety profile in vivo due to detoxification processes (e.g., in the liver) [87]. Advances in life sciences research have uncovered numerous pathophysiological processes and mechanisms of drug action, leading to the development of cellular and molecular bioassays. Many of these assays meet the speed requirements for high-throughput screening (HTS). HTS methods significantly reduce the quantity of test samples required—only micrograms of pure compound are needed—making it possible to screen compounds that are available in very small amounts. Furthermore, automation, computer software, and microfluidic technologies have greatly accelerated the identification of bioactive compounds (hits) as potential lead molecules, enabling thousands of samples to be screened in a short time [88, 89]. Over the past one to two decades, HTS technology has advanced remarkably, increasing the number of compounds screened from around 100,000 per year to approximately 100,000 per day. As a result, the demand for structurally diverse molecules has grown substantially [90]. HTS has also enhanced screening speed by streamlining sample processing, including the rapid extraction and isolation of pure compounds from natural sources, thereby expanding the range and scope of drug bioassays. Importantly, compared to synthetic compounds identified via HTS, natural-source molecules are more likely to exhibit favorable physicochemical properties and drug-like characteristics (i.e., better druggability) [91]. Once the biological profile of a natural product is established, its biological targets, physiological pathways, and mechanism of action should be determined—an essential step requiring modern drug discovery tools. Mechanisms of action and drug-target interactions can be explored using a range of cell-based bioassays. Structure-activity relationship (SAR) studies offer preliminary insights into these interactions and can identify analogues with higher potency than the parent compound. A compound with LC50 or IC50 values in the micro- or nanomolar range is considered potent; such compounds require lower doses, potentially reducing toxicity. When performing biological screening, it is important to consider the effect of solvents on drug dissociation and molecular conformation. Dimethyl sulfoxide (DMSO) is commonly used, as it is an aprotic solvent of intermediate polarity that can dissolve both polar and non-polar compounds. However, DMSO's hygroscopic nature means absorbed water can reduce the solubility of non-polar compounds. Other organic solvents are generally avoided due to their toxicity to test organisms or cells and poor miscibility with assay media. Nonetheless, solvent controls are always used to account for any toxic effects from solvents. Although in vitro screening results do not always translate into in vivo activity—owing to factors such as dosage, solubility in biological media, membrane permeability, and biodegradation—a positive in vitro result typically suggests potential in vivo efficacy. However, the biological profile of a true drug candidate should not be based on a single in vivo screening; multiple screenings are necessary to confirm efficacy [82]. The strengths and limitations of various biological screening models used for natural products are summarized in Table 3.

Since the last few decades, target-based screening has been regarded as the dominant approach in drug discovery. This method begins with the identification of a well-defined molecular target believed to play a crucial role in a particular disease. Before the rise of target-based screening, drug candidates were primarily evaluated using phenotypic screening—where visible traits or characteristics of an organism, such as those seen in animals or cells, were studied. The success of target-based drug screening has been supported by advancements in genomics, chemistry, and molecular biology, enabling the measurement of drug efficacy, safety, dose determination, and patient selection. The expectation was that rational, measurable progress would increase success rates and research productivity, prompting a shift from phenotypic to target-based screening.

However, this shift did not significantly improve success rates in the pharmaceutical industry. Today, with a shortage of new compounds acting on essential targets, there has been a renewed interest in phenotypic screening. Unlike target-based approaches, phenotypic screening does not require prior knowledge of specific drug targets or hypotheses about their role in disease; it relies on measuring biological responses. What was once considered a limitation—its lack of an established mechanism—is now viewed as an opportunity. Moreover, advances in genomics have helped identify new targets using optimized molecules as probes. Through phenotypic screening, many new promising molecules have been discovered, often without a known mode of action.

Both target-based and phenotypic screening approaches have distinct advantages and challenges. While target-based drug discovery offers clear information about drug targets and mechanisms of action, it may not fully capture the complexity of disease. Often, the observed efficacy results from interactions beyond the intended target, as drugs typically bind to multiple targets. Another challenge is the disconnect between in-vitro and in-vivo results, where promising target engagement in vitro does not translate into effective cellular or organism-level activity in vivo. On the other hand, phenotypic screening, though not requiring prior mechanism knowledge, still demands biological understanding—at least of biomarkers relevant to human disease. Moving a compound into development without understanding its mechanism introduces risks in defining dose-response relationships. Additionally, drugs identified via phenotypic screening may act on multiple targets, complicating structure-activity relationship (SAR) studies during optimization. Fortunately, techniques such as affinity purification, biochemical fraction isolation, and modern molecular tools—proteomics, network biology, and chemical biology—have enhanced this approach.

Comparative studies have shown phenotypic screening to be more successful in discovering small molecules and first-in-class drugs. For instance, Swinney and Anthony (2011) analyzed 259 FDA-approved agents (1999–2008) and found that among 75 first-in-class drugs with novel mechanisms, 28 were discovered via phenotypic screening, compared to 17 via target-based approaches. Despite the industry's major focus on target-based screening during that period, phenotypic approaches contributed more substantially to first-in-class discoveries. Both target-based and phenotypic screening are currently applied to natural products, many of which were discovered without prior knowledge of their targets or mechanisms (e.g., quinine and artemisinin). Phenotypic screening has notably accelerated the discovery of new therapeutic agents.

Molecular Modeling and Natural Product Databases: Bioactive natural products discovered through screening can serve as lead compounds for the design of new, more effective analogues. Modern medicinal chemistry—through molecular modeling, combinatorial chemistry, and structure optimization—enables this process. Because natural products often exist alongside structurally related compounds (homologues), a single source can provide SAR-relevant information. Since only a small fraction of plant species has been explored for biological activities, new leads from natural sources will continue to emerge for biological screening and drug discovery. In modern natural product drug discovery, new compounds with acceptable bioactivity undergo SAR studies and molecular modeling to design analogues with improved potency, reduced toxicity, and enhanced pharmacokinetic profiles. These studies can also reveal enzyme interactions influencing biological activity. The most promising analogues can then be synthesized and tested in various in-vitro and in-vivo assays.

In-Silico Ligand Construction and Preparation: Molecular modeling requires optimized 3D ligand structures (e.g., PDB format). Databases like PUBCHEM and ZINC provide reliable structural data in formats such as SDF, MOL, MOL2, and PDB. Retrieved structures should be geometry-optimized for minimum energy, using tools like AutoDock Vina, Discovery Studio, Chimera, Chem3D Ultra, or Avogadro.

Target Preparation: 3D structures of targets (proteins, receptors, enzymes) are obtained from the Protein Data Bank (PDB) and optimized. Examples include human serum albumin, PPAR-α, PPAR-γ, cyclooxygenase, topoisomerase II, and protein kinase. Binding sites are defined, and standard scores for natural ligands are calculated.

Docking: Docking simulations position natural product structures against target sites, ranking interactions based on binding energy. Common docking tools include AutoDock, AutoDock Vina, FlexX, Discovery Studio, and MDock. Docking uses search algorithms (e.g., the Lamarckian genetic algorithm) to identify optimal ligand conformations. Post-docking analyses of intermolecular interactions validate the results.

Hit Identification: Top-scoring interactions are analyzed, and the best candidates (typically the top 10) undergo molecular dynamics (MD) simulations. Both the uncomplexed (apo) target and the target-ligand complex are simulated to assess stability and binding behavior. Hits are ranked by affinity.

Hit Optimization: Promising hits are refined by generating analogues with improved target affinity. Their drug-like properties (stability, pharmacokinetics, pharmacodynamics) are evaluated using QSAR software. An alternative to extracting and testing natural products is virtual screening of large, structurally diverse natural product libraries. This computational approach identifies potential hits and establishes SARs before bioassay testing. While virtual screening can narrow down candidates, physical availability of compounds is essential for experimental validation. These may be synthesized or purchased. Well-known natural product databases include DNP, Phytopure, ChemSpider, Natural Product Alert, and TimTech Natural Products.

Bioactivity-Guided Fractionation Approach – Challenges and Advances

Identification of Bioactive Constituents: In natural product drug discovery, once a bioactive compound is identified, plant extracts can be rationally formulated with enhanced efficacy by adjusting the concentrations of these bioactive constituents. Alternatively, the isolated compound may serve as a lead molecule for developing analogues with improved therapeutic potential. Bioactivity-guided fractionation remains one of the most widely used strategies in natural product research for identifying active molecules. In this approach, as discussed earlier, extraction and biological screening occur simultaneously. Fractions are collected and tested for biological activity, and this process is iteratively continued until the active constituents are isolated and characterized. However, a major challenge in this method is the potential loss of activity during the fractionation process. Furthermore, since fractionation is directed by bioactivity rather than structural data, there is a high likelihood of repeatedly isolating already known compounds. This issue can be addressed by applying the dereplication strategy, which involves preliminary structural characterization to identify and exclude known constituents early in the process. Dereplication is a relatively recent advancement that helps optimize the use of discovery resources by prioritizing samples likely to contain novel bioactive molecules. This is typically achieved by recording mass spectrometric, NMR, and UV-spectroscopic data of mixture constituents and matching the spectral patterns with entries in dereplication databases. One such platform is Global Natural Products Social Molecular Networking (GNPS), which enables spectral annotation and identification of related molecules through MS-MS molecular networking. GNPS also fosters global collaboration by allowing researchers to share raw MS-MS spectral data online.

Identification of Synergists: Natural products usually contain multiple constituents that can act on a variety of biological targets. Among these, some may function as synergists or additives, enhancing the therapeutic effects of other bioactive compounds. Often, synergistic constituents do not show significant biological activity when tested alone, but they increase the potency of active molecules when combined. Such compounds are often overlooked if separated from bioactive compounds during conventional fractionation. Synergy-directed fractionation is a recent development in bioactivity-guided fractionation that integrates chromatographic separation with synergy testing of known active compounds in the original extract. In this method, extracts are tested for synergy, fractionated, and the active fractions are further evaluated for synergistic effects. This iterative process continues until pure synergists are isolated. Using this approach, for instance, three synergists of berberine were successfully identified from Hydrastis canadensis, which would have been missed using traditional methods.

Fruitful Conclusions:

✓ Medicinal plants have long been used in the treatment of both communicable and non-communicable diseases, and they continue to provide a rich source of important therapeutic agents and promising lead compounds.

✓ Many successful drugs have been directly derived from plants or developed from naturally sourced lead molecules. The renewed scientific interest in plant-derived natural products underscores their potential as a source of new therapeutic agents in the future.

✓ In modern plant-based drug discovery and development, plant metabolites are being optimized to develop analogues with desirable safety and efficacy profiles.

✓ The growing interest among medicinal chemists in natural products has led to the emergence of innovative approaches and technological advancements for the selection, identification, isolation, characterization, and biological screening of natural products.

✓ These advances are helping to overcome many of the technical challenges historically associated with natural product development and to address the complexities of natural product behavior.

✓ Technological progress has also facilitated the detailed profiling of complex phytoconstituents, enabling the isolation or synthesis of numerous successful therapeutic agents and novel lead compounds that could serve as core scaffolds for future drug development.

✓ Achieving success in this area will require an interdisciplinary approach that combines traditional and ethnopharmacological knowledge with phytochemistry, botany, analytical chemistry, biological screening strategies, and modern drug development tools.

✓ Looking ahead, these new strategies in natural product drug development will help to minimize challenges and improve success rates.

✓ The use of new molecules from plant sources and chemical libraries based on natural product structures is likely to expand, making a significant contribution to future drug development and helping to address global health challenges.

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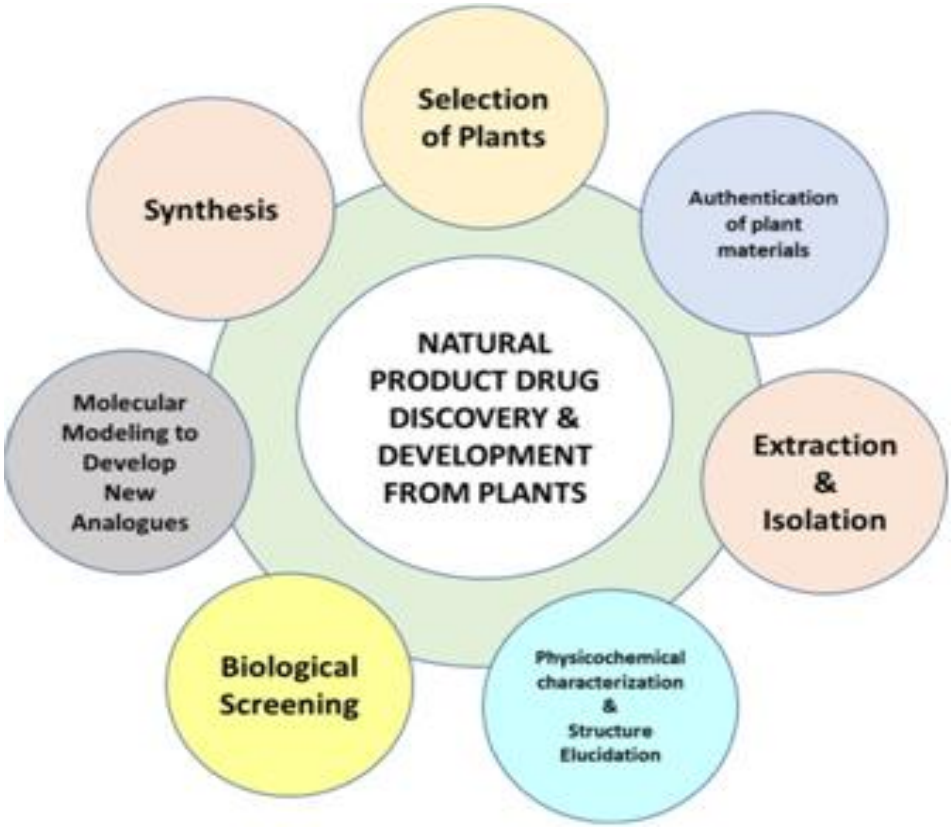

The principal processes involved in the discovery and development of natural products derived from botanical sources Asim Najmi et. al. 2022

	Ethnopharmacological/Traditional Approach	Random Selection Approach
Characteristics	• Traditional or ethnopharmacological knowledge/application is the basis for plant candidate selection and pharmacological assay. It involves the observation, description and experimental analysis of traditionally used plant materials. • The *traditional system of medicine*, such as TCM and Ayurveda, possess well established written knowledge about medicinal plants and regularly revised. • The ethnopharmacological knowledge is easily accessible.	• Plant candidates for natural product discovery are randomly selected, mainly based on their availability.
Strengths	• Comparatively higher success rate. • Based on scientific disciplines including chemistry, botany, pharmacology, biochemistry, history, anthropology et.	• Extremely advantageous, when plant species from a region of high biodiversity has to be screened. • The selected samples has the potential of identification of unexpected biological activities and novel structures. • Can be applied for both general and focused pharmacological screening.

Weaknesses/ Challenges	- Permits are needed for the collection and investigation of plant candidate; even may provoke legal-issues with the ethical groups or the country in which the traditional knowledge was originated, - Traditional systems such as Ayurveda and TCM use multi-component mixtures as formulation and the identification of active constituents out of these mixtures are complicated due to complexity and synergistic effects - The concept of health and disease in traditional medicine widely deviate the modern concepts. For example TCM is highly influenced by Chinese philosophy. This may complicate the correct interpretation of the ethnopharmacological information. - Holistic and personalized approaches of these systems are difficult to access by current bioassay methods.	- Lower rate of success in comparison to ethnophramcological approach. - Flawed in the sense that there is no idea of bioactivity. - The pharmacololgical screening used for randomly selected samples are of small or medium throughput and the test samples (extracts, fractions or pure constituents) availability is low limiting the number of bioassays that can be done.
Examples	- Galegine isolated from *Galega officinallis* L. inspired the synthesis of metformin and other biguanidines antidiabetics; papaverine from *Papaver somniferum* L.; quinine from Peruvian *Cinchona* bark inspired the synthesis of chloroquine and mefloquine [20,48]; artemisinine from TCM herb *A. annua* led to the development of artemether [49]; andrographolide from *Andrographis paniculata*; Berbarine from *Berberis aristata* etc [18].	35,000 plant species screened through random selection between 1960 to 1980 leading to discovery of paclitaxel and camptothecin [18].