Innovations in Bioactive Compound Discovery

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PREFACE

Natural products have been a source of inspiration and discovery in medicine for centuries. Over the past two decades, our journey in natural product-based drug discovery has been filled with both excitement and challenges. While the potential of these compounds is vast, the path to discovering and developing them is rarely straightforward. Many of the difficulties we have faced are not discussed in textbooks or research papers, making it hard for new researchers to anticipate and overcome them.

This realization inspired us to create this book, *"Innovations in Bioactive Compound Discovery."* It is designed to guide students and young researchers who are stepping into the world of natural product research. Our goal is to provide a clear understanding of the steps involved in drug discovery, the limitations one might face, and the innovative solutions that experienced researchers have developed over time.

To make this book truly practical and relatable, we reached out to distinguished faculty members and researchers from this region, inviting them to share their personal experiences, challenges, and successes. Their stories and insights highlight the real-world struggles and innovations that are often missing in academic literature but are crucial for anyone pursuing this field.

This book is not just a manual of techniques but a companion for those navigating the complexities of natural product research. It provides a step-by-step guide to understanding the process and prepares readers for the obstacles they may encounter. We hope it will inspire confidence, spark curiosity, and encourage resilience among the next generation of researchers.

We sincerely thank all the contributors who shared their valuable experiences and knowledge to make this book a reality. Our sincere gratitude is directed to **Prof. Rakesh Maurya**, whose meticulous review of the final draft and insightful comments have greatly enhanced the quality of this book. We believe their inputs will serve as a beacon for

students and early-career scientists, helping them embrace the challenges and unlock the full potential of natural products in drug discovery.

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CHAPTER 1

ANALYTICAL TECHNIQUES AND QUALITY CONTROL FRAMEWORKS IN PLANT DERIVED BIOACTIVE COMPOUND EXTRACTION AND SCALE-UP: ENSURING PURITY, CONSISTENCY, AND EFFICACY

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Abstract

The extraction and utilization of plant derived bioactive compounds have gained immense significance in pharmaceuticals, nutraceuticals, and other products of healthcare industries due to their therapeutic potential. However, ensuring the purity, consistency, and efficacy of these compounds demands robust analytical techniques and quality control frameworks. This chapter provides a comprehensive overview of plant derived bioactive compounds and explores the key extraction techniques, emphasizing the need for sustainable approaches. Different, important aspects of the quality control and standardization of herbal medicines are outlined, alongside the applications of Good Manufacturing Practices in herbal medicines. Challenges in scaling up bioactive compound production are discussed, with focus on maintaining consistency,

addressing batch-to-batch variability, and meeting stringent regulatory requirements along with technological advancements and strategies for overcoming these challenges to facilitate efficient large-scale production.

Keywords: Bioactive Natural Products; Extraction techniques; Analytical Techniques; Scale-up.

1. Introduction

1.1. Overview of bioactive compounds from natural sources

Investigation on natural resources to find bioactive molecules has become a cornerstone of modern scientific research, predominantly in pharmaco therapeutics, pharmacology and toxicology, nutraceuticals, and environmental sustainability (Sen et al., 2011). Bioactive compounds from natural sources are found in a diverse array of sources, including plants, animals, fungi, and microorganisms. They possess different bioactivity and can potentially influence human health positively (Bhadange et al., 2024). Bioactive molecules are essential to the survival and growth of living sources where they are produced, and over centuries, have held immense importance due to their potential to improve human health and contribute to the development of innovative and natural-based therapeutic approaches (Sen et al., 2011; Bhadange et al., 2024).

From ancient times to today, human society has relied on Mother Nature for healthcare. Diverse codified (i.e., Ayurveda, Siddha and Traditional Chinese Medicinal Systems etc.) and non-codified traditional medicine systems (i.e., folk medicine) have long utilized plant-derived, animal-based and minerals substances to treat a wide range of ailments. Modern science is based on traditional information, validating the effectiveness of such medicine/their source in treating diseases, enhancing health, and preventing illness (Sen et al., 2011; Chakraborty et al., 2015). In recent decades, interest in bioactive compounds from natural sources has been resurgent, driven by the growing demand for safer, more sustainable alternatives to synthetic chemicals and pharmaceuticals. Bioactive compounds from nature are generally considered as less toxic, biodegradable, and environmentally friendly. Moreover, bioactive compounds from natural sources exhibit a wide spectrum of biological activities that make them a valuable source of drugs in the prevention and management of aliments (Sen and Chakraborty, 2015; Bhadange et al., 2024).

Natural bioactive compounds have extensive applications in various fields, including nutraceuticals, medicine, agriculture, and industry. In medicine, natural bioactive compounds serve as the basis for drug development, with many modern drugs originating from plants, animals, and microorganisms. Advances in screening technologies, as well as improved separation and isolation methods, have led to the discovery of over a million natural compounds. Among these, approximately 50–60% are plant-derived, including compounds such as alkaloids, flavonoids, terpenoids, steroids, and carbohydrates, while around 5% originate from microorganisms (Sen and Chakraborty, 2015; Cragg and Newman, 2013). Paclitaxel an anticancer agent derived from the bark of the Pacific yew tree, artemisinin from *Artemisia annua* and its analogues are now used to treat malaria, and reserpine isolated from *Rauwolfia serpentina* that is used in Ayurvedic medicine for the treatment of different ailments, quinine isolated from *Cinchona* spp. used to treat malaria, atropine an anticholinergic medication isolated from *Atropa belladonna,* morphine and codeine key opioid analgesic from *Papaver somniferum*, and cardiac glycosides from *Digitalis* spp., are some classical example of bioactive compound from plant sources (Demain and Sanchez, 2009; Sen and Chakraborty, 2017). Animals are also considered as important source of drugs. Teprotide derived from the venom of *Bothrops jaracaca* (pit viper) played key role in the development of ACE inhibitors like captopril and enalapril. Epibatidine found in the skin of *Epipedobates tricolor* (frog) inspired the discovery of pain-relieving drugs. Exendin-4 from the venom of the *Heloderma suspectum* (Gila monster) led to the development of exenatide (Cragg and Newman, 2013). Microorganisms, in particular, have made significant contributions to global health and well-being. In addition to producing essential primary metabolites like amino acids, vitamins, and nucleotides, microbes also synthesize secondary metabolites that account for about half of all pharmaceuticals currently available and provide numerous important in agricultural products. Approximately 20-25% of all known natural products display biological activity, with around 10% of these derived from microorganisms. Of the 22,500 biologically active compounds derived from microbes, 45% come from actinomycetes, 38% from fungi, and 17% from unicellular bacteria (Demain and Sanchez, 2009). Few example for medicine obtained from microbes includes penicillin (from *Penicillium notatum*), actinomycin D (from actinomycetes), bleomycin (from bacterium *Streptomyces verticillus*), streptozotocin (produced by *Streptomyces achromogenes*). Products from microbes such as insecticides (i.e., a mixture of spinosyn A and D), and herbicides (i.e., bialaphos, phosphinothricin tripeptide from *Streptomyces* *viridochromogenes*) also have importance in agriculture (Demain and Sanchez, 2009). Many of the bioactive compounds like ziconotide – a non-steroidal analgesic (cone snail genus *Conu*), ecteinascidin 743 – an antitumor agent (isolated from the colonial tunicate *Ecteinascidia turbinata*), halichondrin B – macrolide (from several sponge sources) has also been developed as drug (Cragg and Newman, 2013). Minerals have been used as therapeutic agents in different traditional medicinal systems since ancient times. Trace amounts of certain minerals are essential for maintaining bodily functions and also used as curing agents. Many of such minerals are used to develop therapeutic agents like ferrous sulfate used to treat iron deficiency anemia, sodium bicarbonate as antacids and zinc oxide ointment for skin protection etc. (Xiaoqing et al., 2022). Bioactive natural components play a pivotal role as nutraceuticals, offering a bridge between nutrition and medicine. As nutraceuticals, bioactive compounds such as polyphenols, flavonoids, carotenoids, and omega-3 fatty acids have been shown to support various bodily functions, including immune modulation, antioxidant defense, cardiovascular health, and anti-inflammatory effects. Unlike conventional pharmaceuticals, nutraceuticals are often derived from food sources and are generally considered safe (Vignesh A, Cheeran AT et al., 2024).

Bioactive molecules from nature or their derivatives constitute a large proportion of drugs that successfully marketed after clinical trials. A study reported that there was a consistent rise in the use of natural products and their derivatives across phase I clinical trial (35%) to phase III clinical trial (45%). In contrast, the proportion of synthetic compounds declined from roughly 65% in phase-I to 55% in phase III clinical trial. Different pharmacological assessments also indicated that natural products were generally less toxic than synthetic alternatives (Domingo-Fernández et al., 2024). Bioactive natural compounds are the foremost source of drugs currently in the market. For example, out of FDA approved 98 small molecule anticancer drugs (in-between 1981 to 2010), 11 entirely natural compounds, 32 derived from natural products, 11 synthesized based on natural product templates, 16 designed as natural product mimics, and another 8 were associated with natural products. It was also estimated that about 83% of 136 anticancer drugs (during 1981-2014) are natural product or based on natural product or analogues of natural products. It is estimated that approximately 60% of all anticancer drugs available on the market today are derived from natural products (Sarker et al., 2020).

With modern scientific knowledge and technology advancement, pharmaceutical companies and researchers have increasingly turned to natural sources as a rich source of new drug candidates, drawing on traditional knowledge and practices. This resurgence in interest has been fueled by breakthroughs in drug discovery, which have been greatly enhanced by state-of-the-art technology and analytical techniques. These innovations have transformed the drug development process, making it more efficient and effective.

1.2. Importance of efficient extraction, analytical methods and quality control

Despite the wide availability and potential of bioactive compounds from natural sources, the successful application of natural products depends heavily on several critical factors: efficient extraction, precise analytical methods, and stringent quality control procedures. These processes ensure that the bioactive components of interest are obtained in high yields, characterized accurately, and maintained consistently for reliable efficacy and safety.

Proper and efficient extraction of bioactive components from natural sources is decisive in gaining their anticipated properties and maximizing their possible applications. Extraction of bioactive components is a critical step in isolating desired natural products. Conventional extraction methods like maceration, digestion, percolation, distillation, and Soxhlet extraction are used traditionally to extract desired natural products from raw materials. Advanced extraction techniques like supercritical fluid extraction, ultra-sound, microwave-assisted, and accelerated solvent extraction are gaining significance in the current time. Choosing the appropriate extraction technique is critical, as it directly influences the reliability and quality of subsequent analytical results. The primary goals of the extraction process are to ensure economic efficiency, environmental sustainability, reduced extraction time, and high yields of bioactive compounds, all while preserving their biological activity (Bhadange et al., 2024; Bitwell et al., 2023)

Utilization of proper analytical methods are crucial to understanding and utilizing the potential of these natural compounds. These methods not only ensure the accurate identification, quantification, and characterization of the compounds of interest, but they also guarantee the reliability and reproducibility of the research outcomes. Bioactive compounds in natural sources are often embedded within intricate matrices, making their discovery, isolation, and characterization particularly challenging. The complexity of these matrices requires the use of advanced purification techniques and state-of-the-art analytical tools. Advances in analytical techniques and bioassay development have significantly accelerated research in natural products. Commonly employed analytical methods include Thin-Layer Chromatography (TLC), High-Performance Thin-Layer Chromatography (HPTLC), High-Performance Liquid Chromatography (HPLC), and Gas Chromatography (GC), along with more recent innovations such as Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) Spectroscopy. These technologies enable precise identification, quantification, and structural characterization of bioactive compounds. One particularly promising approach is targeted metabolomic analysis, which, using Ultra-High Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (UHPLC/Q-TOF-MS), allows researchers to localize compounds from diverse chemical classes within complex natural matrices. This combination of high-resolution chromatography and accurate mass spectrometry enables the detailed profiling of compounds, aiding in the discovery of bioactive molecules and their chemical composition (Nitin, 2021). Beyond identifying and quantifying active compounds, analytical methods are essential for assessing the safety of natural products. For instance, testing for contaminants, toxins, or heavy metals in natural extracts is integral to ensuring their safety for human consumption or medical use. Techniques such as atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS) are used to detect potentially harmful substances in plant materials and other natural sources (Bharti et al., 2024).

The growing market demand for high-quality bioactive compounds is driven by increasing consumer interest in natural health products, pharmaceuticals, and nutraceuticals. As consumers seek safer, more sustainable alternatives to synthetic drugs, the demand for bioactive compounds derived from natural sources continues to rise. Concurrently, regulatory pressures are intensifying, with stricter safety, efficacy, and quality control standards. Regulatory agencies require rigorous testing and certification to ensure that natural products meet stringent safety and purity guidelines. This creates a need for precise, reproducible extraction and analytical methods, ensuring that bioactive compounds are of consistent quality and meet regulatory requirements for market approval.

2. Extraction techniques for retrieving bioactive compounds from natural sources

Extraction techniques play a fundamental role in obtaining valuable bioactive compounds from plants and refining these methods is essential to maximize yield and maintain their functional integrity. And, therefore, effective extraction requires careful consideration of various factors, including the choice of solvent, pre-treatment of raw materials, solvent-to-material ratio, extraction temperature and duration, multiple extraction cycles, and the inclusion of enhancers or co-solvents. Additionally, aspects such as pH adjustments, safety protocols, quality assurance, environmental sustainability, and reproducibility play a vital role in optimizing the process. In this regard, while traditional methods like maceration, infusion, and Soxhlet extraction remain prevalent, modern, eco-friendly technologies are emerging as preferred alternatives. Techniques such as instant controlled pressure drop (DIC), pulsed electric field (PEF), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE) offer improved efficiency, faster processing, and a reduced environmental footprint (Usman et al., 2022). The choice of extraction strategy depends on the plant material, the bioactive compounds of interest, the desired quality of the extract, and the intended production scale. Each method comes with its unique benefits and challenges, necessitating a tailored approach to achieve optimal results. This section categorizes extraction methods into traditional, advanced, and green technologies, offering an in-depth overview of their applications and potential for harnessing plant bioactives sustainably.

2.1. Traditional extraction methods

Traditional extraction methods, often simple and low-cost, have been used for centuries to obtain bioactive compounds. These techniques, while effective, may require longer extraction times and larger solvent volumes.

2.1.1. Maceration:

Maceration, a fundamental extraction method, involves soaking coarse or powdered plant material in solvents like methanol, ethanol, ethyl acetate, acetone, hexane, etc. (Jha et al., 2022). This widely adopted and cost-effective technique is used to extract various bioactive compounds from plant matter. However, maceration has certain limitations, including low extraction yield, inefficiency, and high solvent consumption, which can pose environmental and health risks. Additionally, selecting the appropriate solvent is crucial for optimal extraction of specific compounds from a given plant. To improve the extraction efficiency, plant material is often ground into fine particles to increase the surface area, facilitating better solvent penetration and component extraction. The plant material and solvent mixture is then stored for an extended period, occasionally stirred, and finally filtered to separate the extract. The effectiveness of this technique in extracting bioactive compounds is influenced by factors such as the solvent type, plant material, and solvent polarity (Omeroglu et al., 2019). By varying solvents and time-temperature conditions, optimal extraction can be achieved. Maceration disrupts the plant cell structure, exposing the chemical constituents to the solvent and facilitating their extraction. This technique is widely used in laboratory settings for the extraction of various bioactive compounds (Farooq et al., 2022).

2.1.2. Infusion:

Traditional infusion, a technique dating back centuries, involves soaking plant material in a solvent, often hot water. Infusion involves extracting bioactive compounds from plant material using a solvent, which can be applied either hot or cold. Infusion is particularly suitable for plant materials containing readily soluble bioactive compounds. This traditional method is widely employed to prepare extracts for immediate use. This method is particularly popular in tea-making cultures across Europe, Brazil, Taiwan, China, and various Asian countries. By steeping plant material in boiling water for a specific duration, infusion extracts bioactive compounds. Numerous studies have highlighted the beneficial effects of infusion extracts, particularly their antibacterial and anti-inflammatory properties, which can be attributed to the presence of phytochemicals in the herbal plants. To begin, the plant material is ground into a fine powder. This powdered material is then added to a clean container and covered with the chosen solvent, typically in a 1:4 or 1:16 ratio, depending on the desired application of the extract. The mixture is then allowed to steep for a specific period. This gentler method is suitable for delicate compounds that may degrade at higher temperatures. Infusions typically yield diluted solutions of water-soluble active compounds from the plant material (Ergen et al., 2018; Abubakar and Haque, 2020).

2.1.3. Soxhlet extraction:

Soxhlet extraction is a well-established method for extracting bioactive compounds from solid materials. It involves a specialized apparatus that continuously circulates a heated solvent through the sample, concentrating the extracted compounds. The efficiency of Soxhlet extraction is influenced by various factors, including mass transfer, solubility, and the properties of the solid material. This technique is particularly effective for extracting heat-stable compounds, making it a preferred choice over traditional methods in many applications. The Soxhlet apparatus consists of a thimble containing the powdered sample, a reflux condenser, and a round-bottom flask holding the solvent. The solvent is heated, vaporized, and condensed, dripping onto the sample to extract the desired compounds. Once the solvent reaches a certain level, it siphons back into the flask, carrying the extracted compounds. This process is repeated until the extraction is complete. Selecting the appropriate solvent is crucial for successful Soxhlet extraction. The solvent should be capable of dissolving the target compounds while minimally interacting with the matrix. Factors such as sample type and analyte-matrix interactions should be considered when choosing a solvent. Soxhlet extraction is often employed when the contaminants are insoluble in the solvent and the desired product has a specific solubility. This technique ensures efficient and selective extraction of bioactive compounds (Ergen et al., 2018; Santos et al., 2022).

2.2. Green and Sustainable Extraction Techniques

Traditional extraction methods often involve the use of harmful solvents and high temperatures, which can degrade the bioactive compounds and harm the environment. In recent years; there has been a growing interest in developing green and sustainable extraction methods that are more environmentally friendly and efficient. Green extraction focuses on developing extraction processes that are energy-efficient, use environmentally friendly solvents, and produce high-quality extracts (Chemat et al., 2012). These green methods are designed to improve extraction efficiency, selectivity, and sustainability. Here are some of the most prominent green and sustainable extraction techniques:

2.2.1. Microwave-Assisted Extraction (MAE):

Microwave-Assisted Extraction (MAE) utilizes microwave radiation to heat the solvent and plant material, leading to rapid and efficient extraction. This technique can significantly reduce extraction time and solvent consumption while preserving the thermal integrity of bioactive compounds. MAE is a modern technique that utilizes microwave energy to accelerate the extraction process (Azmir et al., 2013). This method involves mixing the sample material with a suitable solvent and then applying microwave energy. The rapid heating caused by microwave irradiation leads to internal pressure within the sample, rupturing cell walls and facilitating the extraction of target compounds. MAE operates by electromagnetically irradiating the polar solvent and sample, resulting in superheating and disruption of the solid material. This technique offers several advantages, including high extraction yield, reduced process time, low solvent consumption, and a compact extraction unit. However, it is not suitable for thermally sensitive compounds. Factors such as solvent-to-solid ratio, microwave power, irradiation time, extraction time, temperature, and solvent concentration influence the efficiency of the MAE process (Zhang et al., 2023, Nde et al., 2015). To enhance mass transfer, the sample may be stirred during extraction, promoting efficient contact between the solvent and the substrate molecules. Optimization of parameters like microwave power, temperature, and extraction time is crucial for achieving optimal results. It is important to adhere to safety guidelines when performing MAE, as microwave radiation can be hazardous. By following proper procedures, researchers can ensure the safe and effective application of this powerful extraction technique.

2.2.2. Ultrasound-assisted extraction (UAE):

Ultrasound-Assisted Extraction (UAE) is a modern, eco-friendly technique that uses ultrasonic waves to extract bioactive compounds (BCs) from plant materials. UAE employs high-frequency ultrasound waves to disrupt cell walls and enhance mass transfer between the solvent and the plant material. This technique is particularly effective for extracting heat-sensitive compounds. This method requires less solvent and energy compared to traditional methods, while preserving the integrity of the extracted compounds. UAE is particularly useful for extracting BCs as it offers high yields without compromising their quality. By controlling the temperature throughout the process, UAE ensures that heat-sensitive compounds remain intact. Ultrasonic waves, ranging from 20 kHz to 10 MHz, are employed in this technique. Power ultrasound (20-100 kHz) is used for extraction and processing applications due to its high intensity, while signal or diagnostic ultrasound (100 kHz-10 MHz) is used for clinical diagnosis, control, and quality assessment (Carreira-Casais et al., 2021). UAE depends on various factors, including the frequency and intensity of ultrasound waves, the choice of solvent, extraction time, and temperature. These factors must be carefully controlled to maximize extraction efficiency while minimizing degradation of target compounds. UAE encompasses two primary methods: bath extraction and horn extraction. In bath extraction, the sample vessel is either immersed in a liquid medium or directly exposed to ultrasonic waves. In contrast, horn extraction involves directly applying ultrasonic horns to the sample. Both techniques generate cavitation, which disrupts cell walls and facilitates the release of bioactive compounds. While both methods offer advantages such as reduced processing time and improved yields, they differ in the types of cavities generated and the resulting impurities. These techniques are widely used in various industries, including pharmaceuticals and food processing, to extract valuable bioactive components from diverse sources (Bhadange et al., 2024).

2.2.3. Supercritical fluid extraction (SFE):

Supercritical Fluid Extraction (SFE) utilizes supercritical fluids, such as carbon dioxide, as solvents. By adjusting the pressure and temperature, the solvent properties can be fine-tuned to selectively extract specific compounds. SFE is environmentally friendly, as it uses non-toxic solvents and minimizes solvent residues in the final extract. Supercritical fluid extraction is favoured due to its high selectivity, high efficiency, and short extraction time. SFE involves two primary steps: extraction and separation. In the extraction process, solid or liquid samples can be used, although solid samples are more common. Solid samples are typically prepared by drying and milling before being packed into columns. Pressurized supercritical solvent is then passed through the column, dissolving the extractable compounds from the solid matrix. The dissolved compounds are carried by the solvent to the separator. Here, the mixture of extract and solvent is separated by reducing pressure, increasing temperature, or a combination of both methods (Uwineza et al., 2020). Carbon dioxide (CO2) is the most commonly used supercritical fluid in SFE due to its favorable properties such as low toxicity, availability, and relatively low critical point. However, other supercritical fluids like ethane, propane, and water can also be used depending on the application. The extraction vessel, typically made of stainless steel, is a high-pressure chamber where the sample is placed. It is equipped with temperature and pressure controls. Supercritical CO2 is stored in a high-pressure vessel and pumped into the extraction vessel after being heated to its supercritical state. The flow rate and pressure of the fluid are carefully controlled to optimize the extraction process. The supercritical fluid penetrates the sample matrix, dissolving and extracting the target compounds. After extraction, the fluid, now laden with the extracted compounds, is transferred to a separator vessel. Here, reducing the pressure causes the supercritical fluid to revert to its gaseous state, leaving behind the extracted compounds. The recovered supercritical fluid can be recycled or safely vented (Bhadange et al., 2024).

2.2.4. Pressurized liquid extraction (PLE):

Pressurized liquid extraction (PLE) involves extracting compounds using a solvent at elevated temperatures and pressures. This technique enhances the solvent's solvating power, leading to increased extraction efficiency. PLE is a modern approach to extracting valuable compounds from various materials. Unlike traditional methods; PLE utilizes solvents at high temperatures and pressures, but crucially, below their critical point. This ensures the solvent remains a liquid throughout the process. PLE offer several advantages. Higher temperatures, achieved under pressure, improve the efficiency of extracting desired compounds. This efficiency is influenced by three factors: how the material being extracted interacts with the solvent (matrix effect), how quickly the target compounds move into the solvent (mass transfer), and the solubility of the target compounds in the solvent. Optimizing the flow rate, pressure, temperature, and extraction time allows for the best results (Alvarez-Rivera et al., 2020). One key principle of PLE is that liquids boil at higher temperatures when under increased pressure. By pressurizing the system first, the solvent remains liquid even at increased temperatures. PLE typically operates between 50°C and 200°C, with the exact temperature depending on the chosen solvent and the target compounds (e.g., polyphenols). Researchers have found that PLE significantly increases the amount of valuable compounds extracted, particularly due to the higher temperatures. Furthermore, PLE offers an energy-saving benefit. Since liquids require less energy to increase temperature compared to turning them into vapor, PLE uses less energy overall (Usman et al., 2020; de Sousa Sabino et al., 2021).

3. Quality control of herbal products

Quality plays a pivotal role in human life, emphasizing the need for robust quality control measures, especially in products meant for health and wellness. In the case of herbal products, quality control entails a structured process that oversees every stage of their development, from sourcing raw materials to manufacturing and distribution, ensuring consistent product standards (Wang et al., 2023). While synthetic pharmaceuticals are subject to stringent regulatory requirements to safeguard their safety and efficacy, the regulatory framework for herbal products remains relatively lenient (Gatt et al., 2024). This regulatory gap has resulted in challenges such as adulteration, substitution, contamination, and intentional or unintentional compromises in the quality of herbal products (Wang et al., 2023). These issues can undermine the trust in and effectiveness of herbal remedies, despite their growing popularity as alternatives to synthetic medications. With the increasing global demand for herbal therapies, it is imperative to prioritize the standardization and quality assurance of these products (Balekundri & Vinodhkumar, 2020). Rigorous testing protocols, stringent manufacturing practices, and adherence to international quality standards are essential. Such measures not only ensure the safety and therapeutic efficacy of herbal products but also help prevent risks such as contamination, the presence of harmful substances, and variability in active ingredients. Strengthening the quality control of herbal products is vital for safeguarding consumer health and upholding the credibility of herbal medicine in modern healthcare.

3.1. Standardization of herbal products

With the increasing commercialization of formulations derived from medicinal plants, the importance of quality control standards has grown significantly. In traditional practices, local healers directly dispensed remedies, ensuring quality through their expertise. However, the shift to mass production and global trade has introduced challenges such as adulteration and substitution, largely due to the wide geographic distribution of medicinal plants and the confusion caused by diverse vernacular names (Kunle et al., 2012). These issues make it imperative for manufacturing facilities to establish replicable and robust quality standards to ensure consistency across products. Although some medicinal plants are listed in pharmacopoeias, comprehensive criteria, especially those related to chemical markers and chromatographic profiles, are often lacking (Yang et al., 2017). Proper quality control measures are essential to maintain the integrity of medicinal plants, as raw materials collected from wild sources exhibit significant variations due to natural diversity (Gatt et al., 2024). To address these inconsistencies, there has been a growing emphasis on the cultivation of key medicinal plants to achieve uniformity in raw materials (Wang et al., 2023).

Standardization remains central to ensuring that herbal products maintain consistent levels of active compounds or markers. The process faces numerous challenges, including variations in climatic conditions, soil properties, cultivation methods, and geographic origin, all of which impact the phytochemical composition of plant materials (Alum, 2024). Coupled with rising incidences of adulteration and substitution, these factors complicate efforts to produce high-quality herbal products (Balekundri & Vinodhkumar, 2020). Moreover, the scarcity of skilled personnel further limits the availability of authentic plant materials. To mitigate these challenges, advanced quality control strategies and stringent regulatory standards are crucial. Methods such as macroscopic and microscopic analyses for physical identification, chromatographic techniques like HPTLC and HPLC for chemical profiling, and spectroscopic approaches for compound characterization have become integral to ensuring product authenticity and quality (Balekundri & Vinodhkumar, 2020). The advent of molecular tools such as DNA barcoding has also added precision to species identification, reducing the risk of misidentification (Ganie et al., 2015).

Guidelines established by organizations like the World Health Organization emphasize the evaluation of parameters such as organoleptic properties, ash values, moisture content, microbial contamination, and chemical profiles (WHO, 1998). These evaluations, alongside innovations in analytical technologies such as LC-MS and GC-MS, provide a robust framework for ensuring the safety, efficacy, and reliability of herbal products. As the herbal medicine industry continues to evolve, the adoption of such comprehensive quality control measures will remain essential to meeting the expectations of both consumers and regulatory bodies. The choice of a specific method, however, should be guided by the intended analytical objectives, whether they involve confirming the presence of active compounds, detecting adulterants, or ensuring compliance with regulatory standards (Pore et al., 2023). By implementing these practices, the herbal industry can achieve a higher level of quality assurance, thereby ensuring the safety and effectiveness of its products for consumers worldwide.

3.2. Quality Control Parameters

3.2.1. Pharmacognostic evaluation

3.2.1.1. Organoleptic or macroscopic evaluation

Organoleptic and macroscopic evaluations are vital initial steps in assessing the quality and authenticity of herbal drugs. These methods rely on sensory and visual examination to identify plant materials and ensure their purity. Organoleptic evaluation focuses on sensory attributes like appearance, odor, taste, and texture, offering clues about the botanical source and detecting contamination. For instance, licorice's sweetness and the aroma of umbelliferous fruits illustrate the importance of sensory cues (Ramadan et al., 2020). Macroscopic evaluation involves the detailed visual inspection of morphological features such as leaf venation, bark texture, and seed structure, enabling differentiation between genuine materials and adulterants (Anjum et al., 2023). Unique traits like the fractured surfaces of quassia wood or the thickness of cascara bark serve as key identifiers. These methods are cost-effective and accessible, making them indispensable for verifying plant authenticity and quality, particularly in resource-limited settings. However, their effectiveness depends on evaluator expertise and is less suitable for processed materials. To address these limitations, integrating organoleptic and macroscopic methods with advanced techniques like microscopy, chromatography, and molecular analysis enhances the overall reliability and comprehensiveness of quality control.

3.2.1.2. Microscopic evaluation

Microscopic analysis is essential for verifying the authenticity and quality of herbal medicines by examining their structural and cellular details (Wang et al., 2023). This technique is particularly effective in identifying species-specific features, such as anomocytic stomata in *Cinchona* bark, rosette-shaped calcium oxalate crystals in Rhubarb, and lignified fibers in *Cassia* pods. Diagnostic elements like trichomes, stomata types, starch grains, calcium oxalate crystals, and aleurone grains are key indicators used to differentiate plant materials (Prakash et al., 2019). Microscopy is indispensable for detecting adulteration and impurities, especially in powdered or processed herbal products (Ichim et al., 2020). For example, the absence of characteristic starch granules and cork cells in powdered ginger can indicate adulteration. Similarly, the detection of silica crystals may suggest substitution with lower-quality ingredients. This method also aids in distinguishing closely related species (Ichim et al., 2020). For instance, the presence of multicellular branched trichomes helps identify *Datura* leaves, whereas *Senna* leaves are characterized by paracytic stomata and unique crystal patterns. Furthermore, microscopic examination can uncover microbial contamination, such as fungal spores or bacterial colonies, highlighting issues with storage or handling conditions (Ichim et al., 2020). While it requires expertise and access to reference standards, microscopic evaluation is a cost-effective and reliable technique. Its integration with advanced methods like chromatography or molecular analysis enhances its utility in ensuring the safety, efficacy, and consistency of herbal products.

3.2.1.3. DNA barcoding

DNA barcoding is a powerful molecular technique used to authenticate and verify herbal drugs by analyzing specific DNA sequences, known as barcodes, to distinguish plant species (Ganieet al., 2015). It is particularly useful for identifying closely related species or plant parts that may look similar but have different therapeutic properties. This method overcomes the limitations of traditional identification methods, especially for powdered or processed materials where visible features are absent. By targeting specific genetic markers, such as rbcL, matK, and the ITS region, DNA barcoding ensures that the correct species is used in herbal products, and it helps detect adulteration or substitution with inferior or harmful species (Chen et al., 2023). It is also valuable in verifying complex herbal formulations where traditional methods may fail. However, the technique requires skilled personnel, specialized equipment, and access to comprehensive reference databases (Dev et al., 2021).

3.2.2. Physicochemical evaluation

Physicochemical evaluation is essential for ensuring the quality, safety, and efficacy of herbal drugs and it involves assessing various parameters such as foreign matter, ash content, pesticide residues, heavy metals, extractive values, swelling and foaming index, viscosity, pH, etc. (Mollah et al., 2021). The presence of foreign matter, like dust or other plant parts, should be minimized to maintain the authenticity and effectiveness of the herbal drug. Visual inspection or sieving methods are commonly used to identify and remove foreign material (Payne et al., 2023). Ash content, including total and acid-insoluble ash, is measured to detect inorganic impurities such as sand or soil particles (Liu, 2022). The analysis of pesticide residues and heavy metals is essential to assess safety. Pesticide residues, which can remain on plant materials after cultivation, pose health risks. Techniques like gas or liquid chromatography are used to ensure pesticide residues fall within permissible limits (Wahab et al., 2022). Similarly, the detection of heavy metals like lead, arsenic, or cadmium, either from cultivation or handling is performed using methods such as atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS), ensuring levels are below harmful thresholds (He et al., 2024). Swelling and foaming indices measure the water absorption and foaming characteristics of substances, which are particularly important for certain herbal formulations, such as those containing plant gums or mucilages used in shampoos or creams (Tosif et al., 2021). Extractive values indicate the amount of soluble compounds in a plant material, providing insight into its potency. A higher extractive value typically reflects a higher concentration of active ingredients (Sasidharan, 2011). Additionally, moisture content is tested to prevent microbial growth and preserve the stability of active compounds, as excess moisture can degrade the herbal drug (Wang et al., 2023). Viscosity and pH are other key properties that influence the quality of herbal formulations. Viscosity impacts the texture, ease of use, and absorption of liquid products like syrups and tinctures, while pH affects solubility, stability, and bioavailability. These physicochemical parameters ensure that herbal drugs meet quality standards, are safe for consumption, and maintain their efficacy throughout their shelf life.

3.2.3. Phytochemical characterization

Phytochemical characterization is an essential step in standardizing herbal medicines, as it enables the identification and quantification of bioactive compounds that determine their therapeutic efficacy. By systematically analyzing the chemical structures and concentration levels of these compounds, researchers ensure the consistency, safety, and quality of plant-based products used in medicine, nutraceuticals, and cosmetics (Chaachouay et al., 2024). This comprehensive profiling not only facilitates the discovery of new bioactive or potentially harmful constituents but also supports the reproducibility of their health effects, which is essential for establishing standardized formulations. In standardization, chromatographic fingerprints serve as valuable tools, providing a reference for comparing specific fractions with desired biological activities. This reduces the risk of false-positive results and enhances the reliability of herbal medicines. Traditional isolation techniques, while effective for compound identification, often lead to a reduction or loss of bioactivity due to repeated processing. Modern analytical methods, such as HPTLC, HPLC and other hyphenated techniques, address these limitations by enabling rapid and accurate profiling of multiple compounds in complex herbal matrices (Patel et al., 2010). The adoption of advanced techniques is critical in standardization, as it ensures that herbal medicines meet stringent quality control criteria. By delivering consistent and reliable therapeutic outcomes, such approaches not only build trust in herbal medicine but also drive innovation in the development of safe and effective plant-based therapies.

To develop pharmacopeial standards for herbal formulations, HPTLC fingerprinting has emerged as a major quality control technique. With advancements in scientific methods, HPTLC is increasingly recognized as a reliable approach for ensuring the authenticity and quality of herbal plants (Noviana et al., 2022). This technique allows for simultaneous analysis by comparing reference standards with sample formulations, serving not only as a tool for identification but also as a comprehensive quality control method. Guided by monograph recommendations, HPTLC facilitates various tests to verify the identity and quality of herbal plants and their formulations. By analyzing peak profiles and intensities from fingerprint images, it provides both qualitative and quantitative data relative to reference standards (Shivatare et al., 2013). The technique also supports the identification of marker compounds, determination of purity levels, and verification of minimum content requirements (Klein-Junior et al., 2021). These capabilities make HPTLC a vital tool for maintaining the standardization and consistency of herbal medicines.

HPLC is an efficient analytical tool widely employed for separating complex liquid mixtures and analyzing various plant metabolites (Queiroz et al., 2024). Its versatility is enhanced by coupling with advanced detectors like UV photodiode arrays (HPLC-DAD) and mass spectrometry (HPLC-MS). The integration of mass spectrometry with HPLC has been a significant advancement, especially for analyzing intricate plant extracts and facilitating drug discovery. Among liquid chromatography (LC) techniques, reverse-phase (RP) chromatography is the most common for characterizing plant extracts, utilizing polar mobile phases and hydrophobic stationary phases like C18 columns (Rusli et al., 2022). The advent of ultra-high-pressure liquid chromatography (UHPLC), which uses smaller particle-sized columns (<2 μm), has further improved chromatographic resolution for analyzing complex samples(Queiroz et al., 2024). LC-MS techniques offer unparalleled advantages for characterizing non-volatile, high-polarity compounds, such as glycosylated flavonoids with multiple hydroxyl groups. While standalone HPLC has limitations in handling complex matrices and requires extensive preprocessing, coupling with mass spectrometry overcomes these challenges, improving sensitivity and enabling precise identification of chemical structures. Techniques such as ion trap LC-MS, quadrupole-time-of-flight mass spectrometry (Q-TOF MS), and triple quadrupole LC-MS allow for detailed structural elucidation of herbal extracts, identification of chromatographic peaks, and real-time comparison of complex samples. In addition to these, new advanced techniques like LC-NMR are being used that combines the high-resolution separation capabilities of liquid chromatography with the structural elucidation power of NMR spectroscopy (Gathungu et al., 2020). This hybrid technique is particularly useful in the analysis of complex mixtures, enabling researchers to separate individual components of a sample and then directly characterize them based on their NMR spectra, without the need for further isolation or purification.

On the other hand, Gas chromatography (GC) complements LC-based techniques by separating volatile compounds based on their volatility and interactions with the stationary column. GC-MS combines the separation capabilities of GC with the identification power of mass spectrometry, relying on spectral libraries for analyte characterization. It is particularly effective for analyzing low-molecular-weight compounds such as essential oils and terpenoids (Wang et al., 2023). Derivatization techniques, like forming trimethylsilyl derivatives, are often employed to increase the volatility of non-volatile analytes for GC analysis (Piergiovanni et al., 2022). GC-MS configurations such as GC-TOF offer enhanced sensitivity and accuracy, while the robustness and cost-effectiveness of GC-EI-Q-MS make it the preferred method for routine analysis.

Despite the success of these methods in identifying therapeutic compounds, they are labor-intensive and primarily detect abundant secondary metabolites rather than the most bioactive ones. Continuous advancements in mass spectrometry, including high-resolution analyzers like TOF and orbitrap, along with other hyphenated techniques like LC-NMR have expanded the ability to quantify metabolites with high precision and identify novel compounds through molecular formula generation.

3.2.4. Microbiological evaluation

It involves the detection and quantification of microbial contamination, which can compromise the safety, efficacy, and shelf-life of herbal products. Contamination by microorganisms, such as bacteria, fungi, yeasts, and molds, can occur during the harvesting, processing, storage, or handling of herbal materials (Ovuru et al., 2024). The presence of harmful pathogens such as *Salmonella, Escherichia coli*, *Aspergillus*, or *Penicillium* species can lead to serious health risks for consumers (Opuni et al., 2023). One of the key aspects of microbiological evaluation is the determination of the total microbial load, which includes both beneficial and harmful microorganisms. This is typically done through plate count methods, where herbal samples are cultured on specific media to encourage microbial growth, followed by counting the colonies formed. Specific pathogens are also tested in herbal drugs to ensure consumer safety. *Salmonella* and *E. coli* testing is particularly important for herbs used in food or medicine, as these bacteria are associated with foodborne illnesses. Similarly, molds like *Aspergillus flavus*, which produces aflatoxins, can contaminate herbs such as ginseng, licorice, or other medicinal roots and pose serious health risks, including carcinogenic effects (Benkerroum, 2020). Testing for mold contamination, particularly through mycotoxin screening, is therefore a crucial part of microbiological evaluation. Fungal contamination can also affect herbal drugs in terms of efficacy and consumer safety. Similarly, mold and yeast growth, especially in moist conditions, can spoil herbal preparations, lower their efficacy, and introduce allergens, making microbiological testing essential for quality assurance.

3.2.5. Bioassay based standardization:

Bioassays can also be used an important component in the standardization of herbal medicines, offering valuable information about their biological properties and ensuring their therapeutic reliability (Wang et al., 2023). Unlike conventional chemical analysis, which focuses on identifying and quantifying individual components, bioassays assess the functional activity of herbal formulations (Wei et al., 2020). This approach is particularly beneficial for correlating the chemical profile of a product with its pharmacological effects, thereby strengthening the link between its composition and intended health benefits. Additionally, they facilitate the identification and verification of bioactive markers, ensuring the presence of critical compounds in consistent and adequate concentrations. By detecting unexpected biological responses or missing expected effects, bioassays are also effective in identifying contamination or adulteration in herbal products, enhancing their safety and quality.

Beyond quality assurance, bioassays complement analytical techniques by confirming that the detected chemical constituents deliver the intended biological activity. For instance, they can identify issues such as compound degradation or poor bioavailability, even when the concentration of a compound appears sufficient. They are equally vital for safety evaluations, using toxicological assays to uncover potential risks like cytotoxicity or organ-specific toxicity, thereby ensuring compliance with safety standards (Jităreanu et al., 2022).

Bioassays are also a valuable tool for innovation, aiding in the discovery of novel bioactive compounds by isolating fractions with significant activity and linking these findings to their phytochemical makeup. They provide insights into synergistic interactions in multi-component systems, which may not be apparent from chemical analysis alone. Emerging technologies such as high-throughput screening, omics approaches, and artificial intelligence are set to revolutionize bioassay applications by enhancing their accuracy, scalability, and reproducibility (Wei et al., 2020). By integrating these advances with existing bioassay methodologies and state-of-the-art analytical tools, the process of standardizing herbal medicines can become more robust and comprehensive.

3.3. Good manufacturing practices for herbal products

Good Manufacturing Practices (GMP) is very much vital to the herbal industry to maintain product quality and protect consumer health (Wang et al., 2023). These practices provide a structured framework for maintaining consistency throughout the entire production cycle, from sourcing raw materials to the final packaging and labelling (Gouveia et al., 2015). Given the variability inherent in herbal materials, GMP ensures that stringent controls are in place at every stage to guarantee that products meet regulatory requirements. The quality of raw materials is a cornerstone of GMP for herbal products. It involves confirming the identity, purity, and potency of the herbal components. Essential to this process is traceability, which ensures that every raw material can be traced back to its origin, with full documentation of cultivation, harvesting, and storage conditions (Melethil, 2006). Variations in these factors can significantly affect the final product’s quality, making standardization of raw materials essential. Facilities and equipment used in herbal product manufacturing must meet specific standards to prevent contamination and ensure cleanliness. Regular validation and calibration of equipment are vital for maintaining consistency and accuracy during production (Williams et al., 2012). Documentation and maintenance of equipment play a key role in safeguarding the quality of the finished product. Furthermore, process validation ensures that every manufacturing step, such as extraction or purification, consistently meets quality standards. Training and hygiene practices for personnel involved in the production are also integral to GMP compliance (Wang et al., 2023). Staff must be adequately trained in handling procedures, equipment operation, and hygiene protocols. This ensures that proper cleanliness is maintained, minimizing the risk of contamination throughout the production process. The GMP framework also emphasizes the development of standard operating procedures (SOPs) for all production stages (Williams et al., 2012). This helps to standardize manufacturing practices and ensures uniformity across batches. Additionally, detailed batch records and thorough documentation of every manufacturing step, including raw materials, quality control tests, and corrective actions, are necessary to maintain transparency and accountability. Regular quality control testing of raw materials, in-process samples, and finished products is a fundamental aspect of GMP. The validation of processes, equipment, and analytical methods used in production further ensures the reliability and effectiveness of the manufacturing process. Herbal products are also subjected to stability testing to establish their shelf life and appropriate storage conditions, ensuring that they retain their potency and quality over time. Adhering to GMP principles requires continuous improvement through regular audits, inspections, and process reviews. This ongoing compliance ensures that herbal products consistently meet the required standards, providing consumers with safe and effective products. By following GMP guidelines, manufacturers can build consumer trust and ensure the widespread acceptance of herbal products in both traditional and modern healthcare systems.

4. Challenges and Solutions in Scaling-Up

The extraction and scale-up of bioactive compounds from natural sources present considerable challenges in maintaining quality, consistency, and efficacy. Effective analytical techniques and reliable quality control frameworks are crucial for addressing these challenges and ensuring the provision of high-quality, safe, and effective products.

4.1. Maintaining Consistency in Quality and Bioactivity

Raw material selection and quality have an important effect on the quality of extraction of bioactive compounds. Good quality raw material yields good herbs and medicinal plants, which in turn ensure the desired bioactivity in the extracted compounds (Oubannin et al., 2024). The chemical composition of plant material is greatly affected by factors such as species, time of harvesting, location, and mode of cultivation. Normally, those plants that reach maturity in terms of harvesting times tend to have higher amounts of bioactive compounds. This is in addition to ensuring that raw materials are held under standardized sourcing conditions that otherwise ensures consistency in the extraction results (Koraqi et al., 2023).

Optimization of extraction technology has a significant impact on the quality and bioactivity of extracted compounds. Different extraction