Chapter 1

Introduction, Literature Review and Objectives

1.1 Introduction

Human history and ancient civilizations have been using medicinal plants for their primary healthcare. With advancement in the civilization, the knowledge of botanicals has been more profound and so as the application of these natural remedies (Perveen & Al-Taweel, 2019). This knowledge of medicinal plants was primarily passed down orally, from one generation to the next. Later on, introduction of writing transformed this process, allowing for the precise and widespread distribution of information. While oral and written traditions often influence and inform one another, these complied documentations offer enhanced access of knowledge (Leonti, 2011; Totelin, 2009). Some of the earliest and most well-documented traditional medicine systems include Ayurveda and Traditional Chinese Medicine (TCM). Ayurveda, which originated in India around 3,000 years ago, is one of the oldest systems that have been systematically documented (Pandey et al., 2013). Ayurveda is known through Sanskrit texts like “Charaka Samhita” and “Sushruta Samhita,” which describe the medicinal properties of various plants and herbs, as well as surgical techniques and general healthcare wisdom (Leonti & Casu, 2013). Similarly, TCM was a 2,000 years old compilation known as the "Yellow Emperor's Classic of Internal Medicine" (Matos et al., 2021). In ancient Egypt, Greece, and Rome, herbalism was a key component of medical practice, with famous physicians and botanists like Hippocrates, Galen, and Dioscorides compiling extensive records of medicinal plants and their uses (Petrovska, 2012). The collective wisdom of these civilizations passed down through generations, has contributed immensely to both traditional and modern medical practices (Salmerón-Manzano et al., 2020).

1.2 Integration of Medicinal Plants into Modern Medicine

Medicinal plants play a significant role in modern medicine, serving as the source of approximately 40% of current pharmaceutical drugs. Notable examples of this long-standing practice include the use of willow bark for alleviating pain, Madagascar periwinkle for treating childhood diseases, and hawthorn and foxglove for heart health. Modern medicine has expanded on this traditional knowledge, leading to the

development of drugs like aspirin from willow bark and the discovery of important compounds in plants such as Madagascar periwinkle, which has provided treatments for childhood cancers. Ongoing scientific research has also uncovered the potential of star anise for its antiviral effects and wild Mexican yam as a source of contraceptive compounds.

The combination of ancient wisdom and contemporary scientific research has not only confirmed numerous traditional healing methods but also facilitated the creation of synthetic medications that replicate the benefits of herbal remedies. Acknowledging the significance of this connection, the World Health Organization (WHO) encourages studies on traditional medicines and their integration into current healthcare frameworks (WHO, 2013).

The WHO defines "Traditional *medicine as a sum of the knowledge, skills and practices based on the theories, beliefs and experiences Indigenous to different cultures, whether explicable or not, used in the maintenance of health and the prevention, diagnosis, improvement or treatment of physical and mental illness*" (WHO, 2002). This definition emphasizes the holistic approach taken by ancient cultures, where the natural world was intricately tied to health and healing.

1.3 Complementary and Alternative Medicine (CAM)

The traditional practices of using medicinal plants for healthcare, fall under the categorization of Complementary and alternative medicine (CAM) (Gurib-Fakim, 2006). CAM includes many health and wellness practices and products not considered part of conventional Western medicine. These diverse approaches can be categorized CAM into several categories: natural products, mind and body practices, and other complementary health approaches (WHO, 2013). Natural products include herbal medicines, vitamins, minerals, and probiotics, which are often marketed as dietary supplements to improve health and well-being. Mind and body practices such as yoga, meditation, acupuncture, and massage therapy aim to enhance the mind-body connection and promote relaxation and healing (Fischer et al., 2014). Other complementary health approaches encompass a range of traditional and culturally specific practices, including traditional Chinese medicine, Ayurvedic medicine, and homeopathy (Sanadhya et al., 2015).

1.3.1 Herbal Medicines: A Key Component of CAM

Herbal medicines, a key component of CAM, are defined as *" herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants, other plant materials or combinations thereof as active ingredients. In some countries, herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g. animal and mineral materials)*" (WHO, 2013). Herbal products are sold under different labels, including over-the-counter (OTC) products, dietary supplements, functional foods, medicines, and cosmetics and personal care products. OTC products are available without a prescription and are typically used for self-care, including herbal teas, topical ointments, and capsules containing herbal extracts (Fischer et al., 2014). Dietary supplements include vitamins, minerals, and herbal extracts designed to supplement the diet and support general health. Functional foods are enhanced with additional ingredients, such as herbs, to provide health benefits beyond basic nutrition. In some regions, herbal products are regulated as medicines and can be prescribed by healthcare practitioners, meeting specific safety and efficacy standards (Ekor, 2014).

1.4 Global Expansion and Key Drivers of the Herbal Medicine Market

The global herbal medicine market is experiencing rapid expansion, driven by the increasing consumer demand for natural and plant-based healthcare. A 2023 report by BCC Publishing projects the market to reach $279.8 billion by 2028, with a significant CAGR of 10.6% from its 2023 value of $169.1 billion (BCC Publishing, 2023). India and China dominate the export market for medicinal and aromatic plants. Between 2017 and 2021, India experienced a 6.14% annual growth rate in exports, while China's exports declined. Their trade patterns are having differences, as China primarily selling to neighboring countries and India focusing on long-distance trade with the US and Germany. Other countries, such as Luxembourg, Iceland, and Tanzania, have also seen significant export growth. Strong demand from developed markets, especially the US and EU, contributes to the industry's resilience. India's exports of Ayurvedic and herbal products reached $628.25 million in the 2022-23 fiscal year (Raju & Das, 2024; RIS, 2023).

This trend reflects a significant shift towards natural and less invasive options for managing various health conditions (Saggar et al., 2022). In developing nations,

traditional medicine, including herbal remedies, remains a cornerstone of primary healthcare, serving 80% of the population (WHO, 2013). Traditional medicine use is rising in developed countries, with notable rates in Germany (40-50%), the USA (42%), Australia (48%), and France (49%) (Sen & Chakraborty, 2017). The perceived nontoxic nature, reduced side effects, affordability, and accessibility of herbal products are key drivers of their use, particularly in developing countries (Oyebode et al., 2016).

Several factors contribute to this rising demand. Consumers are increasingly drawn to herbal products due to a perception of natural thus safer ideology, the proven effectiveness of plant-based treatments, a general preference for natural therapies, and a belief in their superiority over synthesized pharmaceutical products (Gunjan et al., 2015; Robinson & Zhang, 2011). The lower incidence of side effects in plant-based medicines compared to allopathic medicines contributes to their increasing demand. This demand is further amplified by concerns about pharmaceutical costs and side effects, along with growing trust in the safety and quality of herbal products. Herbal remedies also present an alternative for those seeking options beyond conventional medicine, resulting in a rise in self-medication (Byard et al., 2017; Eichhorn et al., 2011). These factors, combined with key industry trends such as the development of new plant-based medicines, the integration of traditional healing into modern healthcare, the incorporation of medicinal plants into food and beverages, and the prioritization of sustainable sourcing, are contributing to the rapid expansion of the herbal medicine market (Raju & Das, 2024).

1.5 Quality and Safety Concerns

Quality and safety are crucial for herbal products, given their growing global demand and popularity. These products are derived from plants and their processed parts are having natural health and wellness solutions. However, ensuring their effectiveness and safety is complex due to various factors influencing their composition and potency. These factors can include the plant source, growing conditions, harvesting and processing methods, and storage conditions. Variations in these factors can lead to inconsistencies in the final product, affecting their quality and safety. Therefore, rigorous quality control measures are essential throughout the entire production process, from sourcing raw materials to the final product, to guarantee consumer safety and maintain the integrity of herbal medicine.

1.5.1 The Risks of "Natural" Remedies: Toxicity and Long-Term Effects

The concurrent use of herbal medicines with conventional drugs and dietary substances presents complex challenges to patient safety. Herbal products can interact with pharmaceuticals and food, leading to unpredictable and potentially serious adverse effects. For example, combining herbal medicines containing *Radix Bupleuri*, *Fructus Gardenia*, *Fructus Schisandrae Chinensis*, *Radix Rehmanniae*, *Akebia Caulis*, and *Semen Plantaginis* with antipsychotics like quetiapine, clozapine, or olanzapine increases the risk of adverse events by nearly 60% (Hazra & Singh, 2024; Zhang et al., 2015). Several specific herbal remedies and their constituents pose significant risks. Aristolochic acid, found in some weight-loss supplements and traditional Chinese medicines, is a potent nephrotoxin and carcinogen linked to kidney failure and urothelial cancer (Beebe, 2023; Liu, 2024; Yang et al., 2014). Pyrrolizidine alkaloids present in various plants can cause veno-occlusive disease, a severe liver condition, and are potentially carcinogenic. Contamination of herbal products with these alkaloids is a significant safety concern. The Nigerian herbal formula Yoyo "Cleanser" Bitters has been linked to liver enzyme elevation and potassium loss, while prolonged use of tonics containing *Entandrophragma utile* and *Anacardium occidentale* has caused adverse effects in mice. Ephedra, specifically its active component ephedrine, carries risks of cardiovascular issues, CNS toxicity, hepatotoxicity, and even rare cases of blindness and death, particularly when used for weight loss. Beyond immediate toxicity, long-term herbal medicine use can also pose risks, including liver fibrosis (Oyedepo & Palai, 2021). Some herbal compounds, especially in Chinese herbal medicines, can interact with DNA, raising concerns about cellular toxicity and genotoxicity. The misconception that "natural" equates to "safe" is dangerous, as evidenced by the numerous reported adverse effects of herbal substances. Therefore, rigorous safety assessments and regulations are essential for the herbal medicine industry (Ekor, 2014; Liu, 2024; Oyedepo & Palai, 2021).

1.5.2 Adulteration and Substitution: A Growing Concern for Herbal Quality and Safety

The quality and safety of herbal products are the growing concern due to the increasing demand for these products, henceforth increasing the risk of adulteration (Wang et al., 2023). They are herbal constituents, materials, or other substances added, either unintentionally or intentionally, to herbal preparations during processing or to the

finished product. The complex system of plant nomenclature, which includes scientific names, binomials, synonyms, and vernacular (common) terms, can create confusion and lead to unintentional adulteration. This lack of uniformity, along with taxonomic similarities between species, plays a significant role in non-intentional adulteration. On the other hand, intentional adulterants are often economically motivated, with increased demand leading to the substitution of non-certified, cheaper plant material, look-alike species, or different parts of the plant during herbal preparation (Keshari, 2021). These adulteration practices compromise the safety and therapeutic effectiveness of herbal medicines, underscoring the need for rigorous quality control measures and regulatory oversight in the herbal industry (Alyas et al., 2024; Agarwal & Goyal, 2021) (Figure 1.2).

1.5.2.1 Factor Influencing Unintentional Adulteration

Unintentional adulteration of herbal products can occur due to various factors, leading to significant impacts on their quality and efficacy. The primary causes include confusion in vernacular names, lack of knowledge about the authentic plant, non-availability of the authentic plant, similarity in morphology and color, careless collection practices, improper storage, and imperfect preparation. Each cause of unintentional adulteration is described in detail below.

a) **Confusion in Vernacular Names:** The confusion in vernacular names between different indigenous systems of medicine and local dialects often leads to the unintentional adulteration of herbal products. For example, 'Parpatta' refers to *Fumaria parviflora* in Ayurveda, but 'Parpadagam' refers to *Mollugo pentaphylla* in Siddha medicine, causing these herbs to be interchanged (Sahoo et al., 2010).
b) **Lack of Knowledge About the Authentic Source:** A significant cause of adulteration is the lack of knowledge about the authentic plant species among collectors and suppliers. For example, The Ayurvedic drug 'Nagkesar' is authentically derived from *Mesua ferrea*, but the market often contains the flowers of *Calophyllum inophyllum* instead, due to unawareness and restrictions on collecting *Mesua ferrea* (Agarwal & Goyal, 2021).
c) **Similarity in Morphology and Color:** Morphological and color similarities between plant species can lead to their misidentification and adulteration. For example, *Mucuna pruriens* seeds are frequently adulterated with similar *Mucuna*

seeds like *M. utilis* and *M. deeringiana*, which differ in size, color, and pattern from the authentic *M. pruriens* seeds, easily confusing untrained collectors and suppliers (Shetty & Harsha, 2021; Sarin, 1996).

d) **Careless Collection Practices**: Careless collection practices by unskilled collectors often lead to adulteration. Collectors, often from local communities without formal training in species identification, rely on indigenous names and traditional knowledge, which increases the chances of misidentification. For example, *Parmelia perlata* is often mixed with similar species due to collectors' lack of taxonomic skills to recognize its distinctive thallus. Additionally, ignorance of proper collection methods, such as gathering specific plant parts during the right seasons and locations, further contributes to the collection of incorrect species, causing adulteration (Roy et al., 2013; Poornima, 2010).

e) **Improper Storage:** Improper storage conditions can also lead to adulteration. Physical factors such as air (oxygen), humidity, light, and temperature can cause the deterioration of herbal products, directly or indirectly. For example, exposure to air and light can oxidize the active constituents of herbs, while high humidity can promote the growth of mold and other microorganisms. Using degraded herbs as drugs can act as adulterants, compromising the quality and efficacy of the final product (Preethi et al. 2014; Shetty & Harsha, 2021).

f) **Imperfect Preparation:** Some crude drugs require processing before marketing, and improper techniques during this stage can destroy active constituents, leading to adulteration. For example, over-drying crude drugs can degrade their active components, while removing the cork from ginger can lead to the loss of essential oils and other beneficial compounds. Such improper preparation methods can significantly impact the therapeutic properties of herbal products (Agarwal & Goyal, 2021).

1.5.2.2 Factor Influencing Intentional Adulteration

Intentional adulteration of herbal products is a deliberate act aimed at increasing profit margins by substituting, diluting, or altering genuine products with inferior, synthetic, or harmful substances. This practice compromises the quality, efficacy, and safety of herbal drugs, and it is driven by economic motives and market demand. Each cause of intentional adulteration is described in detail below.

a) **Substitution with Substandard Commercial Varieties:** Substitution with substandard commercial varieties is one of the most prevalent types of adulteration. In this method, low-standard drugs that resemble the original in terms of morphology, chemical composition, and therapeutic properties are mixed with or substituted for the genuine product. *Arabian Senna* (*Cassia angustifolia*) is often used instead of the more expensive *Indian Senna* (*Cassia acutifolia*). Despite their similarities in appearance and laxative properties, Arabian Senna is considered inferior in quality (Ahmed & Hasan, 2015).

b) **Using Superficially Similar Inferior Drugs:** This type of adulteration involves substituting similar-looking but inferior products that lack the same chemical or therapeutic value as the genuine article, such as using *Carica papaya* seeds to adulterate *Piper nigrum*, which can deceive consumers and manufacturers due to the visual resemblance, despite the lack of pungent flavor and medicinal properties in the adulterant (Ahmad et al., 2012).

c) **Using Artificially Manufactured Substances:** Adulteration through the use of artificially manufactured substances that resemble original crude drugs, often to reduce costs, is a prevalent issue. For example, artificially produced calcium carbonate is sold as "Vansha Lochan," mimicking the traditional bamboo silica used in Ayurvedic medicine (Agarwal & Goyal, 2021; Keshari, 2021).

d) **Using Exhausted Drugs:** Exhausted drugs are those from which the primary active constituents have been extracted. These exhausted materials are then sold as if they were still potent. For instance, exhausted cloves: After the volatile oil is extracted from clove buds (*Syzygium aromaticum*), the remaining buds are often sold as whole cloves. Sometimes, additives are mixed in to make the exhausted product appear fresh and attractive (Agarwal & Goyal, 2021).

e) **Using Synthetic Chemicals to Enhance Natural Characteristics:** Synthetic chemicals are added to natural products to enhance their perceived quality or characteristics. An example is Citral in Citrus Oil: Citral, a synthetic compound with a strong lemon fragrance, is added to citrus oils (like lemon or orange oil) to enhance their scent, misleading consumers about the purity and quality of the oil (Prager & Miskiewicz, 1982).

f) **Presence of Vegetative Matter of the Same Plant:** Instead of using the specific parts of a plant that are known for their therapeutic properties, other parts of the

same plant or smaller plants growing around it are mixed with the genuine product. For example, the root (Moola) of Ashwagandha (*Withania somnifera*) is the desired part, but sometimes stems or whole plants are used instead (Mundkinajeddu et al., 2014).

g) **Harmful Adulterants:** To increase the weight and bulk of crude drugs for higher profit, harmful substances are added. An example is Stone Pieces and Sand in Guggulu: In *Commiphora mukul* (Guggulu), stone pieces and sand particles are mixed to increase weight. This not only reduces the therapeutic value but also poses health risks to consumers (Keshari, 2021).

h) **Adulteration of Powders:** Powdered drugs are particularly vulnerable to adulteration with substances that resemble the genuine product in texture and color. For instance, Dextrin in Ipecacuanha: Dextrin, a polysaccharide, is added to *Cephaelis ipecacuanha* powder, which dilutes the concentration of the active emetic alkaloids. Another example is Kampillak Powder with Annatto Dye: *Mallotus philippensis* (Kampillak) powder is adulterated with *Bixa orellana* (Annatto) dye, which affects the color and possibly the therapeutic properties (Keshari, 2021; Patil-Patankar, 2024).

1.6 Regulatory Framework and Guidelines

The global herbal medicine market grapples with a complex and often fragmented regulatory landscape. While many countries recognize the cultural and economic significance of traditional medicine, ensuring the quality, safety, and efficacy of herbal products remains a significant challenge (Fan et al., 2012: Knoess & Wiesner, 2019). Addressing adulteration issues requires urgent implementation of stringent quality control measures and universal regulatory framework and guidelines, to ensure the authenticity, purity, and safety of herbal products. This includes implementing standardized testing methods, enforcing clear labeling requirements, and conducting regular inspections of manufacturing facilities (Picking, 2024: Thakkar et al., 2020) (Figure 1.2; 1.3).

The WHO plays a vital role in promoting the safe and effective use of traditional medicine around the globe, by facilitating international cooperation and sharing information of herbal medicine regulation through initiatives like the International Regulatory Cooperation for Herbal Medicines (IRCH, 2024). To ensure quality, safety,

and efficacy throughout the supply chain, the WHO has developed guidelines for good agricultural and collection practices for medicinal plants, as well as good manufacturing practices for herbal medicine. These guidelines provide a framework for standardized cultivation, harvesting, processing, and manufacturing of herbal products, contributing to a more robust and reliable global herbal medicine market (WHO, 2013).

In India, the Ministry of AYUSH is responsible for overseeing the development, regulation, and promotion of these traditional medicine systems (RIS, 2023). While India has established frameworks for classifying and licensing AYUSH medicines, challenges remain in ensuring quality control and regulatory enforcement, particularly within the vast informal market (Dias & Joshi, 2024). The increasing global interest in AYUSH systems necessitates stronger regulatory frameworks and international collaboration to ensure the safety and efficacy of these traditional practices (Katiyar at el., 2023). China has a comprehensive regulatory system for traditional Chinese medicine herbal products, managed by Chinese State Food and Drug Administration (Fan et al., 2012). China has improved standardization of TCM formulas and manufacturing. However, ensuring consistent quality of herbs from different regions and addressing use of endangered species in some TCM products are ongoing challenges (Picking, 2024; Zhang at el., 2012).

The European Union (EU) has implemented a relatively stringent regulatory framework for herbal medicinal products, supervised by the European Medicines Agency (EU, 2002: 2004; Knöss, 2018). The EU system emphasizes quality, safety, and efficacy, requiring pre-market authorization for most herbal medicinal products. However, challenges remain in regulating traditional herbal products used for centuries but lacking extensive scientific documentation to meet the EU's standards (EU, 2018). The US adopts a less stringent approach, regulating herbal products as dietary supplements under the Dietary Supplement Health and Education Act of 1994 (Wu et al., 2020). The Food and Drug Administration (FDA) is responsible for overseeing the safety of dietary supplements but does not require pre-market approval. This approach allows for greater market access but raises concerns about potential adulteration, misleading health claims, and the lack of pre-market efficacy evaluation (FDA, 2019; Oketch-Rabah et al., 2020; Ross, 2000).

1.7 The Global Landscape of Pharmacopoeias

Herbal pharmacopeias are instrumental in maintaining the quality, safety, and efficacy of herbal medicines across the globe, serving as authoritative guides that set standards and ensure consistency in their production and use. According to the Index of Pharmacopoeias by the WHO (2006), standards for pharmacopeias provide essential guidance on the quality and safety of herbal products. The American Herbal Pharmacopoeia is renowned for its comprehensive monographs on botanicals used in the United States, covering a wide array of herbs from Ayurvedic, Chinese, and Western traditions. These meticulously researched monographs serve as critical references for academics, healthcare providers, manufacturers, and regulators, providing detailed specifications on the identity, purity, and quality of herbal substances to ensure standardized practices in herbal medicine production. The British Herbal Pharmacopoeia offers standards for 169 herbal raw materials, focusing on herbs not covered extensively in other pharmacopeias. It plays a vital role in quality assurance by establishing clear guidelines for botanical identification, purity testing, and quality control measures, ensuring the integrity of herbal products in the UK and beyond (Kumar, 2015).

The European Pharmacopoeia includes monographs on herbal drugs and extracts, setting quality standards that are recognized across European countries. It provides detailed specifications for the identity, purity, and potency of herbal medicines, facilitating harmonization in quality control practices and supporting regulatory compliance within the European Union. The Chinese Pharmacopoeia, a cornerstone of Traditional Chinese Medicine, sets rigorous standards for the quality and safety of Chinese herbal medicines. It includes extensive monographs that define the characteristics, processing methods, and quality specifications of numerous TCM herbs, ensuring the authenticity and therapeutic efficacy of TCM formulations both in China and globally. The Korean Herbal Pharmacopoeia features official monographs for 384 herbal substances, providing comprehensive standards for herbal medicines in Korea. It covers a diverse range of herbs used in traditional Korean medicine, contributing to quality assurance and regulatory compliance within the country's healthcare system (Kumar, 2015).

The Ayurvedic Pharmacopoeia of India is having standards for over 600 plant, animal, and mineral-derived drugs used in Ayurvedic medicine. This document is divided into two parts: Part I focuses on standardized formulations, while Part II details monographs of single drugs derived from traditional Ayurvedic texts. Each entry in the API provides comprehensive information about the drug, including its Sanskrit name, scientific identification, source, synonyms, and safety precautions. First published in 1978, the API continues to serve as the authoritative guide for ensuring the quality and authenticity of Ayurvedic medicines in India (Table 1.1) (Joshi et al., 2017).

1.8 Quality Control Methods in Herbal Medicine

Pharmacopoeias set quality and safety standards for herbal products, but traditional quality control methods have limitations. Current methods, including microscopic, macroscopic, chromatographic, and spectroscopic techniques, face challenges like time consumption, subjectivity, and limited specificity. Advanced methods like DNA barcoding, metabolomics, and next-generation sequencing offer more precise and comprehensive quality control to ensure the safety and efficacy of herbal medicines globally (Figure 1.2).

1.8.1 Microscopic Authentication

Microscopic analysis is a pioneering technique for verifying the identity and purity of herbal ingredients. It involves examining the unique cellular and anatomical features of plant materials and comparing them to established reference standards. This method can identify specific plant species, differentiate between closely related species, and detect adulteration or contamination (Upton et al., 2020). For example, using features like star-shaped sclereids in cinnamon bark or the absence of glandular trichomes in peppermint leaves can confirm their authenticity (Khan et al., 2024). Additionally, light microscopy enhanced with histochemical staining, helps distinguish authentic herbs from adulterants by revealing their unique microscopic characteristics (Osman et al., 2019). For instance, microscopic examination can differentiate genuine ginseng roots from adulterants based on their characteristic wood fiber arrangements. Furthermore, microscopy excels in detecting contamination by foreign materials, often missed by DNA-based methods (Michetti et al., 2019). This could include identifying starch grains from a cheaper source used to adulterate a powdered herbal product. However, challenges remain, including the need for reliable reference materials, specialized

equipment for advanced techniques, and difficulties analyzing heavily processed products. Despite these limitations, microscopic authentication remains a cornerstone of quality control in the herbal industry. Continuous efforts to expand reference databases, standardize protocols, and provide adequate training are essential to maintaining its accuracy and effectiveness (Ichim et al., 2020).

1.8.2 Analytical Authentication

Analytical authentication of herbal products employs various laboratory techniques to verify their identity, purity, and quality. These methods analyze the chemical and biological composition of herbal materials, providing crucial information for quality control, adulteration detection, and standardization.

Thin Layer Chromatography (TLC) serves as a valuable preliminary screening tool, providing insights into the chemical complexity of herbal extracts. Its simplicity and cost-effectiveness make it a valuable asset for rapid comparisons and preliminary identification of characteristic compounds. For instance, TLC can readily differentiate between genuine *Ginkgo biloba* extracts and potential adulterants by visualizing their distinct flavonoid profiles. However, its limitations in sensitivity and resolving power become apparent when dealing with complex mixtures or trace adulterants, necessitating the deployment of more sophisticated analytical weaponry (Ichim & Booker, 2021).

High-performance liquid chromatography (HPLC) is a powerful analytical technique that can separate and quantify the chemical compounds in herbal products (Sontag et al., 2019). This allows for the precise identification and measurement of marker compounds and active ingredients, ensuring consistent quality in herbal medications (Malherbe et al., 2012). It's ability to enhance separation and quantification makes it valuable for analyzing complex herbal extracts, detecting even subtle adulterations, and ensuring batch-to-batch consistency (Luo et al., 2024). For instance, HPLC analysis has revealed the substitution of authentic North American black cohosh with cheaper Asian *Actaea* species in some commercial products (Ichim & Booker, 2021). Advanced MS techniques like tandem MS (MS/MS) further elevate selectivity and sensitivity, enabling the detection of multiple contaminants simultaneously. LC-MS/MS effectively detects and quantifies multiple mycotoxins in herbal products, ensuring consumer safety and protecting public health (Steiner et al., 2021).

Gas chromatography (GC) plays a key role in authenticating herbal products by analyzing volatile and semi-volatile compounds. It provides a characteristic fingerprint to verify the authenticity of essential oils, detect adulteration, and assess quality (Silva et al., 2022). GC is often coupled with mass spectrometry to enhance identification and quantification, enabling the detection of subtle adulterants and unknown compounds. This combined approach is valuable for quality control, ensuring the consistency and purity of essential oils and volatile herbal products (Attrey, 2017). GC analysis can also differentiate between chemotypes or varieties of the same species based on their volatile profiles (Stashenko & Martínez, 2012) .

Regulatory agencies use methods like microscopy, chromatography, and spectroscopy for herbal product quality control. These methods face limitations, including high costs, limited reference standards, and variations in chemical composition due to location, storage, and processing. Chemical markers may not differentiate species effectively. DNA-based methods offer a more accurate approach to species identification because DNA is stable and readily available (Parveen et al., 2016; Mishra, 2016) (Table 1.2).

1.8.3 DNA-based Authentication

DNA-based methods provide a more accurate, universal and robust, species identification due to its stability and universal nature of DNA. Unlike morphological markers, DNA remains unaffected by tissue characteristics, age, environmental conditions, or processing and storage variations (Raclariu et al., 2023). Major pharmacopeias globally, including the Chinese Pharmacopoeia, United States Pharmacopeia, British Pharmacopoeia, Japanese Pharmacopoeia, and Hong Kong Chinese Materia Medica, advocate the use of DNA-based authentication methods (Wu & Shaw, 2022).

1.8.3.1 PCR-Based Authentication: Conventional, Second, and Third-Generation PCR

Polymerase Chain Reaction (PCR) is a fundamental technique in molecular biology that has revolutionized DNA authentication. It amplifies specific DNA sequences, enabling precise identification and authentication of biological samples. PCR-based authentication can be divided into three generations: conventional PCR, quantitative PCR (qPCR), and digital PCR (dPCR), each offering unique advantages and disadvantages (Table 1.3).

a) First generation PCR (Conventional PCR) was developed by Kary Mullis in 1983. In that, the exponential amplification of a target DNA sequence was followed by repeated cycles of denaturation, annealing, and extension. PCR is a valuable tool for authenticating herbal products due to its ability to detect specific DNA markers. This allows for the verification of the presence of desired species and the identification of potential adulterants. Various PCR-based methods like RAPD, RFLP, microsatellites, ISSRs, SNPs, and ARMS have been developed for plant identification, with SCAR markers developed from RAPD, ISSR, and other regions being frequently mentioned (Kumar et al., 2018; Vural & Dağeri, 2009; Yadav et al., 2012). Conventional PCR is simple and cost-effective, suitable for basic identification and low-cost applications, but limited in quantification and relies on end-point detection (Shah et al., 2023)

(**b) Second-generation PCR (Quantitative PCR or qPCR)** is a DNA amplification technique that offers real-time monitoring and quantification of DNA by utilizing fluorescent probes or dyes (Bustin & Nolan, 2004). This technique is particularly valuable for authenticating herbal products because it accurately measures target DNA specific to a particular herb. By quantifying DNA, qPCR confirms both the presence and correct quantity of the desired species, ensuring product purity and accurate labelling. This precision makes qPCR a powerful tool for detecting adulteration and ensuring the quality and authenticity of herbal products (Shanmughanandhan et al., 2021; Sousa et al., 2019). Developed qPCR methods can differentiate between accidental contamination and intentional adulteration in six common spices and herbs (paprika/chili, turmeric, saffron, cumin, oregano and black pepper), further enhancing product authenticity (Behr et al., 2024).

c) Third-generation PCR (digital PCR or dPCR) divided and diluted the sample into thousands or millions of individual reactions, while enabling absolute quantification of DNA without the need for standard curves (Hindson et al., 2011). This enhances sensitivity and precision, making it suitable for challenging samples and resistant to PCR inhibitors. In herbal product authentication, dPCR can detect and quantify GMOs or contaminants with high precision, ensuring product purity and regulatory compliance (Singh et al., 2016). The development of specific primers for the detection of *Carica papaya* adulteration in *Piper nigrum* products and for *Ocimum sanctum* and *Ocimum basilicum* in Tulsi products has significantly enhanced the authentication of botanicals (Travadi, Shah, et al., 2022; Travadi, Sharma, et al., 2022).

The choice of PCR method depends on the specific requirements of the authentication task, including the need for quantification, sensitivity, and available resources. Thus, understanding the capabilities and limitations of each PCR generation is crucial for selecting the appropriate method for DNA authentication in various applications (Shah et al., 2023; Travadi, Shah, et al., 2022).

1.8.3.2 Sequence-based authentication of herbal products

Sequence-based authentication of herbal products uses DNA analysis to verify their identity and purity. This method relies on unique DNA barcodes within plant species to distinguish between authentic herbs and potential adulterants or contaminants. Techniques such as DNA barcoding and metabarcoding have been widely utilized for this purpose (Table 1.3) (Mahima et al., 2022; Raclariu et al., 2018; Wu & Shaw, 2022).

a) DNA Barcoding is a technique that uses a short, standardized DNA sequence to identify species (Hebert et al., 2003). DNA barcoding is measured as the finest technique for species-level resolution and identification for taxonomists. Global analysis using DNA barcoding found significant adulteration in herbal products, with the highest rates in Australia, South America, Europe, North America, and Africa. Asia had the lowest rates, with Brazil exhibiting the most adulteration (Ichim, 2019). A comprehensive review analyzed 17 potential barcode regions (*matK, rbcL, ITS1, ITS2,* psbA-*trn*H, *atpF-atpH, ycf5, psbKI, ad1, trnL-F, rpoB, rpoC1 atpF-atpH, and rps16*) extensively used in authenticating and identifying medicinal plants (Techen et al., 2014). These DNA barcodes are revolutionizing the identification and authentication of medicinal plants. Key barcodes include chloroplast markers like *rbcL*, *matK*, and *trnH*-psbA, each offering varying levels of variability and resolution for species discrimination. While *rbcL* boasts universality, *matK* provides higher variability, and *trnH*-psbA demonstrates good discriminatory power (Mishra et al., 2016). Nuclear markers like *ITS* are valuable for distinguishing closely related species. Depending on a single DNA region for species differentiation, single-locus barcoding provides a straightforward and cost-effective approach. The use of the *ITS2* region has been demonstrated as an effective DNA barcoding method for medicinal plants. Characterized by high genetic variability, the *ITS2* region can accurately identify over 90% of 4,800 species across 753 genera, establishing it as a reliable tool for species discrimination. Combining multiple DNA regions enhances species discrimination and overcomes the limitations of single-locus approaches. To overcome the limitations of

single-locus barcoding, researchers use multi-locus barcoding, combining two or more DNA regions for enhanced accuracy in identifying herbal products. This method is particularly useful for closely related species or highly processed products. Commonly used combinations for multi-locus barcoding, including *rbcL* + *matK* and *ITS2* + psbA-*trnH*, have demonstrated promising results in various studies, effectively enhancing species discrimination for specific plant groups (Table 1.2; 1.3; 1.4).

b) Metabarcoding, which combines Next Generation Sequencing and DNA barcoding, can identify multiple taxa within a sample (Coghlan et al., 2012). This offers advantages over traditional methods in terms of efficiency and sensitivity, including the detection of trace contamination (Bohmann et al., 2022). The workflow involves DNA isolation, PCR amplification, adaptor ligation, library preparation, emulsion PCR, NGS, and a bioinformatics pipeline including sequence trimming, clustering, and BLAST analysis (Wu & Shaw, 2022). However, challenges include selecting appropriate DNA barcodes for diverse species, analyzing massive datasets with specialized software and potentially unreliable databases, and addressing technical limitations like PCR bias and sequencing errors. Ongoing research focuses on identifying and validating new DNA barcodes and exploring informative regions within existing ones to improve accuracy in herbal product authentication (Table 1.2) (Raclariu et al., 2018).

1.9 Research gap for the quality control methods

Ensuring the quality and safety of herbal products, particularly in key export markets like India, faces significant challenges to sustain the consumer trust. Traditional quality control methods often lack the precision needed for accurate species identification, especially in complex polyphenol and polysaccharide formulations. DNA-based authentication offers a more robust approach, but its widespread adoption is hindered by several factors. These include difficulties in DNA extraction from processed products, incomplete reference libraries, the inability to differentiate plant tissues that contains different metabolite profiles, and the lack of bioactive compound quantification. Varying regulatory guidelines and frameworks across different countries add further complexity to the issue. To overcome these challenges, India needs to develop optimized, cost-effective DNA-based protocols, expand reference libraries for indigenous species, and integrate DNA-based authentication in The Ayurvedic Pharmacopeia of India. Standardized regulatory protocols aligned with global standards are also crucial. By addressing these gaps, India can strengthen its

position in the global herbal product market, build consumer trust, and enhance the credibility of traditional medicine.

1.10 Significant of *Phyllanthus emblica, Terminalia bellirica* and *Terminalia chebula*

***Terminalia bellirica* (Gaertn.) Roxb (TB)**, a large deciduous tree of the Combretaceae family, is native to tropical and subtropical South and Southeast Asia (Zhang et al., 2019). It is characterized by a buttressed trunk and clustered leaves at branch ends. Its hairy, light-yellow fruits have a long history of medicinal use, treating ailments like digestive disorders, respiratory problems, skin conditions, and eye diseases (Deb et al., 2016). Phytochemical studies have identified a variety of bioactive compounds in TB, including tannins, ellagic acid, gallic acid, and others like bellericanin, termilignan, thannilignan, phyllemblin, and triterpenoids. These compounds demonstrate antioxidant, anti-inflammatory, antimicrobial, and pain-relieving properties, supporting the tree's diverse medicinal uses (Kumari et al., 2017).

Taxonomic Classification:

- **Kingdom:** Plantae
- **Clade:** Tracheophytes
- **Clade:** Angiosperms
- **Clade:** Eudicots
- **Clade:** Rosids
- **Order:** Myrtales
- **Family:** Combretaceae
- **Genus:** *Terminalia*
- **Species:** *T. bellirica*

***Terminalia chebula* Retz. (TC)**, a respected member of the Combretaceae family, is known for its healing abilities. This medium-sized deciduous tree, up to 25 meters tall, has distinctive dark brown, fissured bark and spreading branches with lush green leaves. Native to South and Southeast Asia, Terminalia chebula has been used in traditional medicine for millennia (Chattopadhyay & Bhattacharyya, 2007) Its ellipsoidal drupes,

known as "Haritaki" or "Chebulic Myrobalan," contain a wealth of bioactive compounds, including tannins, gallic acid, ellagic acid, and flavonoids. This phytochemical combination having potent antioxidant, anti-inflammatory, antimicrobial, and astringent properties. Traditional healers have long valued TC for its versatility in treating a wide range of ailments, from digestive issues to respiratory problems and skin conditions. Modern research continues to validate its therapeutic potential, solidifying its reputation as a cornerstone of traditional medicine and inspiring further exploration for contemporary healthcare applications (Cock, 2015; Sultan et al., 2023).

Taxonomic Classification:

- **Kingdom:** Plantae
- **Clade:** Tracheophytes
- **Clade:** Angiosperms
- **Clade:** Eudicots
- **Clade:** Rosids
- **Order:** Myrtales
- **Family:** Combretaceae
- **Genus:** *Terminalia*
- **Species:** *T. chebula*

***Phyllanthus emblica* L. (PE)**, is a deciduous tree valued in traditional medicine, especially in Ayurveda. Native to India and surrounding regions, it's known for its feathery leaves and nutrient-rich fruits, used for various health benefits. Its pale green, gooseberry-like fruits are a powerhouse of Vitamin C and contain tannins, ellagic acid, gallic acid, and flavonoids (Khopde et al., 2001). In Ayurveda, PE is considered a Rasayana, a rejuvenating and life-prolonging substance. It's believed to enhance overall health and longevity (Mirunalini & Krishnaveni, 2010). Its traditional uses span boosting immunity, promoting digestion, managing diabetes, supporting liver health, and enhancing skin and hair vitality (Gaire & Subedi, 2014). Modern research is indeed exploring and validating the traditional uses of PE, focusing on its antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties.

Taxonomic Classification:

- **Kingdom:** Plantae
- **Clade:** Tracheophytes
- **Clade:** Angiosperms
- **Clade:** Eudicots
- **Clade:** Rosids
- **Order:** Malpighiales
- **Family:** Phyllanthaceae
- **Genus:** *Phyllanthus*
- **Species:** *P. emblica*

1.11 Rationale for DNA-Based Authentication of *Phyllanthus emblica, Terminalia bellirica,* and *Terminalia chebula*

Traditional medicine uses both single herbs and polyherbal formulas. Single herbs offer targeted therapy. Triphala is prepared form dried fruits of TB, TC and PE. For over 1,000 years, Triphala has been praised in ancient texts like the *Charaka Samhita* and *Sushruta Samhita* for promoting longevity and healing. Triphala supports digestion, absorption, and elimination, aligning with the principle that health begins in the gut. It is offering antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory benefits. Each fruit contributes specific properties: PE for immunity, TB for respiratory health, and TC for digestion (Peterson et al., 2017).

In 2023, the global market for Triphala extracts was valued at USD 1.18 billion and is anticipated to grow to USD 1.89 billion by 2031, experiencing a compound annual growth rate (CAGR) of 6.1% from 2024 to 2031. The Asia-Pacific region is set to lead the Triphala extracts market, driven by the high demand for Triphala powder and its accessibility. Countries like India and China, with strong herbal traditions, are key contributors to this growth, reflecting a regional shift toward natural health products and traditional medicine. Meanwhile, North America is expected to see substantial growth due to rising consumer demand for organic and certified natural health products, aligning with a broader trend toward wellness and natural ingredients. (Global Triphala Extracts Market Size, Share, and Trends Analysis Report, 2024).

Ongoing research continues to reinforce its status as a valuable natural health supplement, indicating strong potential for market expansion. The widespread use and therapeutic significance of these plants highlight the importance of accurate identification, which can be difficult due to their similar morphological traits and phytochemical profiles, particularly among the *Terminalia* species (Table 1.6). The Ayurvedic Pharmacopoeia of India specifies quality control methods such as macroscopic, microscopic, HPTLC, and HPLC techniques, which primarily depend on chemical and morphological characteristics rather than DNA-based authentication (Table 1.7). The presence of gallic acid, a key component in all three plants, adds to the complexity of traditional identification. More straightforward analytical techniques like UV spectrophotometry and TLC often fall short in providing a thorough quantification of bioactive compounds, while advanced methods like HPLC and LC-MS can be expensive and intricate. These challenges highlight the necessity for accurate authentication methods, especially in light of the growing demand and risk of adulteration. Current methods, along with chemical reference standards, are vital for ensuring the quality, consistency, and safety of Ayurvedic medicines, effectively linking traditional practices with modern pharmaceutical standards (Table 1.1).

1.12 Contribution of the research work toward the problem domain

Current methods for authenticating herbal medicines, especially complex formulations, are inadequate for ensuring product quality and safety. This PhD research focuses on developing a reliable DNA-based method to authenticate Triphala, a traditional Ayurvedic herbal formulation. The project will involve optimizing DNA extraction techniques from Triphala ingredients and developing Species-specific PCR and Metabarcoding to accurately identify the plant species if present, for the individual and in the combined formulation. This approach aims to ensure the quality and safety of Triphala products for consumers, bridging traditional Ayurvedic knowledge with modern scientific quality control methods. Ultimately, the goal is to enhance the credibility and effectiveness of herbal medicines in the growing health and wellness market.

1.13 Hypotheses

This study hypothesizes that a combined approach of optimized DNA extraction, species-specific PCR/dPCR, chemical validation, and metabarcoding will enable

accurate and reliable identification of TB, TC and PE in a single or polyherbal formulation like Triphala. By validating this approach, the study aims to establish a standardized method for authenticating Triphala and its constituents, enhancing quality assurance and reliability in herbal products.

1.14 Objectives of the Research

- Optimize DNA extraction procedure for TB, TC and PE.
- Authenticate single herbal products of TB, TC and PE using species-specific PCR/dPCR assays.
- Validate species authentication results using chemical analytical methods.
- Designing of *ITS2* metabarcode and optimization of the metabarcoding approach for polyherbal formulation.
- Conduct metabarcoding analysis on *Triphala* herbal products to identify and authenticate individual species within the mixture.

1.15 Research approach

This Ph.D. thesis outline focuses on optimizing DNA extraction, authenticating species, validating with chemical methods, and implementing metabarcoding for Triphala components. Here's a breakdown of how each part could be approached (Figure 1.4):

1) **Optimization of DNA Extraction for TB, TC and PE:** Eleven DNA extraction protocols were evaluated to optimize DNA isolation from dried fruit samples of TB, TC and PE, focusing on buffer modifications, polyphenol removal, and lysis enhancement. Modifications included increased PVP concentrations, pre-lysis soaking, and adjusted lysis buffer composition to improve DNA yield and purity.
2) **Authentication of Single Herbal Products using Species-Specific PCR Assays (PCR/dPCR):** Species-specific assays for TB, TC and PE were conducted using PCR, with primers from prior studies and optimized conditions for DNA amplification (Bandyopadhyay & Raychaudhuri, 2010; Sharma et al., 2017). Primer efficiency was evaluated using varying DNA inputs across the leaf, fruit, and market samples for TB, TC and PE. digital PCR (dPCR) protocols were optimized to ensure specificity and sensitivity, particularly if samples are of low concentration or degraded.

3) **Validation Using Chemical Analytical Methods:** Perform HPTLC to validate the presence of gallic acid in market samples for TB, TC and PE.
4) **Designing and Optimizing a Metabarcoding Approach and Bioinformatics Pipeline:** Metabarcode targeting the *ITS2* region were designed to enable species identification from degraded DNA, optimized with barcoded fusion primers for library preparation. Mock controls with pooled genomic DNA from diverse plant genera and species were created to evaluate the resolution and universality of the primers for taxonomic discrimination. The metabarcoding pipeline was optimized by filtering read lengths, clustering OTUs, discarding low-read clusters, and assigning taxonomy via BLASTn, normalizing read abundance for accurate species analysis.
5) **Metabarcoding of Triphala Herbal Products:** Apply the optimized pipeline to complex Triphala samples, identifying individual species within the mixture.

Table 1.1 Summary of monographs and formulations in the Ayurvedic pharmacopoeia of India (API).

The Ayurvedic Pharmacopoeia of India (API)		**No. of monograph**	**Total no. of targeted plants**	**Total no. of formulation of targeted plants**	**Total no. of Vati, Churna, Gutika and Kvatha**	**Total no. targeted plants present in Vati, Churna, Gutika and Kvatha**	**Remark**
Part-I (Single drugs)	Vol. I to IX	645	24	130	37 (28.46%)	17	Lauha, Ghirta, Vati Arista, Rasayana, Churna, Taila, Lavana, Leha, Arista, Asva, Gutika
Part-II (Formulations)	Vol. I to III	152	22	152	30 (19.73%)	11	

Table 1.2 Advantages and limitations of quality control methods used for detection of adulteration in medicinal plants.

Technique	Advantages	Limitations
UV Spectrophotometry	Quick, simple, low cost for routine analysis	Lower specificity, limited to compounds with UV absorption
HPTLC	Cost-effective, simple, good for multiple compound analysis	Lower resolution compared to HPLC
HPLC	High sensitivity and specificity for multiple compounds	Expensive, requires sophisticated equipment
LC-MS	High precision, good for detecting diverse metabolites	Expensive, requires advanced expertise
Conventional PCR	Simple, cost-effective, requires basic equipment	End-point detection, semi-quantitative results
Quantitative PCR	Real-time monitoring, quantitative data, high sensitivity	Requires advanced equipment, more expensive, complex setup
Digital PCR	Absolute quantification, partitions the sample into many reactions, high-sensitivity	Expensive, requires specialized equipment, complex analysis
Barcoding	Species identification using DNA fragments (e.g., *rbcL, matK, ITS*), Simple, cost-effective	Single-locus may lack accuracy
Metabarcoding	Combines barcoding with NGS, Detects multiple species, high sensitivity	Needs advanced bioinformatics, PCR biases

Table 1.3 Recent advancement in DNA-based authentication of medicinal plant material.

DNA-based authentication method	Plant Marker	Application on Plant material	Reference
Species-specific PCR assay	SCAR Marker	*Mistletoe* species, *Taxillus chinensis* and *Viscum coloratum* leaves and branch material.	Noh et al., 2021
Species-specific PCR assay	*trnH*-psbA	*Isatis indigotica* dried leaves powder	Hsieh et al., 2021
Quantitative and Digital PCR	*ITS*	*Actaea racemosa* (Black cohosh)	Shanmughanandhan et al., 2016
Species-specific PCR assay	SCAR Marker	*Panax ginseng, P. quinquefolius, and P. notoginseng* root materials	Lu et al., 2022
Species-specific PCR assay and Digital PCR	*rbcL*	*Piper nigrum* and *Carica papaya* dried berries	Travadi, Shah, et al., 2022
Digital PCR	Diacylglycerol kinase 1 gene	*Panax notoginseng*	Yu et al., 2022
Species-specific PCR assay and Digital PCR	*rbcL*	*Ocimum basilicum and Ocimum tenuiflorum* dried leaves powder	Travadi, Sharma, et al., 2022
Species-specific PCR assay and Metabarcoding	SCAR Marker	*Bacopa monnieri and Centella asiatica* dried leaves powder	Shah et al., 2023
Species-specific PCR assay	*rps12 and clpP*	*Gastrodia elata Blume* and its commercial products	Shi et al., 2024

DNA-based authentication method	Plant Marker	Application on Plant material	Reference
Qualitative PCR and quantitative real-time PCR (qPCR)	*ITS2*	*Cinnamomum verum*	Rana et al., 2024
Species-specific PCR assay	psbA-*trnH and ycf1b*	*Moringa oleifera*	Wetters et al., 2024
quantitative real-time PCR (qPCR)	The flavonoid glucosyltransferase and *Ycf1* photosystem I assembly protein	*Bacopa monnieri*	Biltes et al., 2024
Barcoding	*ITS and matK*	Six *Momordica* species	Kumar et al., 2020
Barcoding	*ITS2, matK, rbcL and trnH*-psbA	DNA barcode library of plants listed in the Thai Herbal Pharmacopoeia (THP) and Monographs of Selected Thai Materia Medica (TMM) for 101 plant species	Urumarudappa et al., 2022
Barcoding	ITS2	Indian *Phyllanthus* species	Raghavendra et al., 2024
Barcoding	*rbcL, matK, ITS2, and mini-ITS2*	54 herbal supplements on the US market associated with the Ayurvedic treatment of respiratory symptoms	Harris et al., 2024
Barcoding and Metabarcoding	*ITS2*	30 raw material samples, 10 food products and 12 herbal products	Zhang et al., 2020
Metabarcoding	*ITS2 and rbcL*	39 Thai herbal products listed on the Thai National List of Essential Medicines (NLEM)	Urumarudappa et al., 2020
Metabarcoding	psbA-*trnH and ITS2*	15 herbal teas	Frigerio et al., 2021

DNA-based authentication method	Plant Marker	Application on Plant material	Reference
Metabarcoding	*ITS2*	62 products, containing basil, oregano, and paprika collected from different retailers and importers in Norway	Raclariu Manolică & de Boer, 2022
Metabarcoding	*ITS2*	71 herbal medicinal products were randomly purchased from Greek markets	Anthoons et al., 2021
Metabarcoding	psbA-*trnH*	3 Hedyotis herbal products collected from China and Thailand	Yik et al., 2021
Metabarcoding	*rbcL*	Polyherbal formulation of the Indian market	Pandit et al., 2021
Metabarcoding	*ITS2 and trnL*	4 TCM preparations from the Chinese market	Yao et al., 2022
Barcoding	*ITS2*	52 Licorice products from the Chinese market	Li et al., 2023
Metabarcoding	*ITS2 and rbcL*	Single and Polyherbal formulation of the Indian market	Travadi et al., 2023
Metabarcoding	*ITS2*	18 *Milk thistle* botanical formulations (teas, capsules, tablets, emulsion)	Raclariu et al., 2023
Metabarcoding	*ITS2*	51 Traditional Chinese Medicine (TCM) herbal products	Mück et al., 2024
Metabarcoding	SNP marker	9 Curcumae pieces	Xue et al., 2024

Table 1.4 Details of key genes and loci for medicinal plant barcoding.

Source	Major Genes Utilize for Plant Identification	Locus	Genome
NCBI (National Center for Biotechnology Information) & **BOLD** (Barcode of Life Data System)	*rbcL*	Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Large Subunit	Chloroplast
	MatK	Maturase K	Chloroplast
	ITS1/ITS2	Internal transcribed spacer 2	Nuclear
	trnH-psbA/ trnH-psbB	Chloroplast *trnH-psbA* intergenic spacer	Chloroplast
	trnL-F	trnL-F intergenic spacer	Mitochondrial
	CoI-5P	Cytochrome Oxidase Subunit 1 5' Region	Mitochondrion
	rpoC1/ rpoB	RNA polymerase C	Mitochondrion
	AtpF	ATP synthase membrane subunit c locus 1	Mitochondrion

Table 1.5 Details of the medicinal plants used in this study.

Plants	**Parts used in herbal formulations**	**Price (Rs. / Kg)**	**Collection in MT**	**Potential Adulterants**
Terminalia bellirica	Fruit	100	2000-5000	-
Terminalia chebula	Fruit	80	5000-10000	*Terminalia bellirica*
Phyllanthus emblica	Fruit	15 - 45	10000	*Ipomoea batatas*

Table 1.6 Overview of current quality control methods available for Triphala.

Technique	Key Compounds Analyzed	Reference
HPTLC	Gallic acid, Ascorbic acid, Chebulagic acid	Kondawar et al., 2011; Pallavi & Jha, 2021
UV Spectrophotometry	Rutin, Gallic acid	Modi et al., 2024; Pawar & Salunkhe, 2013
HPLC	Gallic acid, Chebulagic acid, Epicatechin, Emblicanin A & B	Pawar et al., 2017; Pawar et al., 2009; A. Singh et al., 2020
LC-MS	Gallic acid, Ellagic acid, Emblicanin A & B, Friedelin	Varma et al., 2016

Table 1.7 Quality control methods for *Phyllanthus emblica*, *Terminalia bellirica*, and *Terminalia chebula.*

Medicinal plant	Chemical constitute	Reference Standard	Methods
Terminalia bellirica	Bellericagenin A & B, Termilignam, Gallic acid, Ellagic acid, Ethyl gallate, Chebulagic acid, Corilagin, 1,3,6 - tri galloyl glucose, Bellericanin, Phyllemblin and Thannilignam.	Gallic acid	Microscopic, macroscopic, HPTLC and HPLC (API, Part I, Vol. IV, 2016)
Terminalia chebula	Tannins; Chebulagic acid, Chebulinic acid, Ellagic acid, Gallic acid, Terchebin, Ellagitannin, Tubulin and Syringic acid	Gallic acid and Chebulinic acid	
Phyllanthus emblica	Tannins, Gallic acid, Ellagic acid, Phyllemblic acid, Emblicol, Alkaloids - Phyllantidine and Phyllantine; Minerals	Gallic acid	

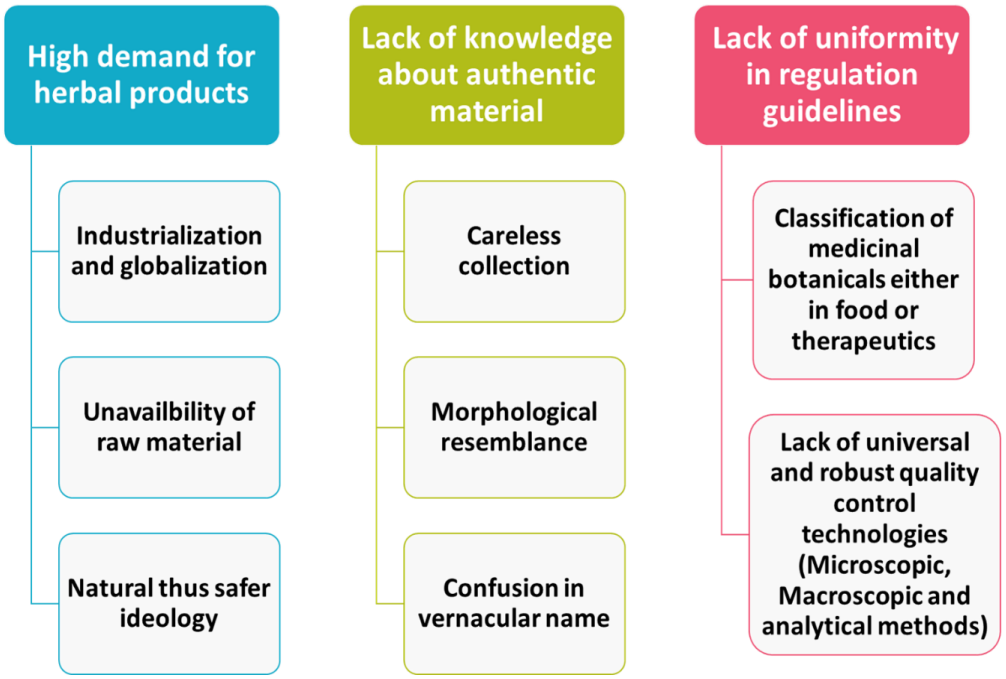

Figure 1.1 Key challenges in the herbal product industry.

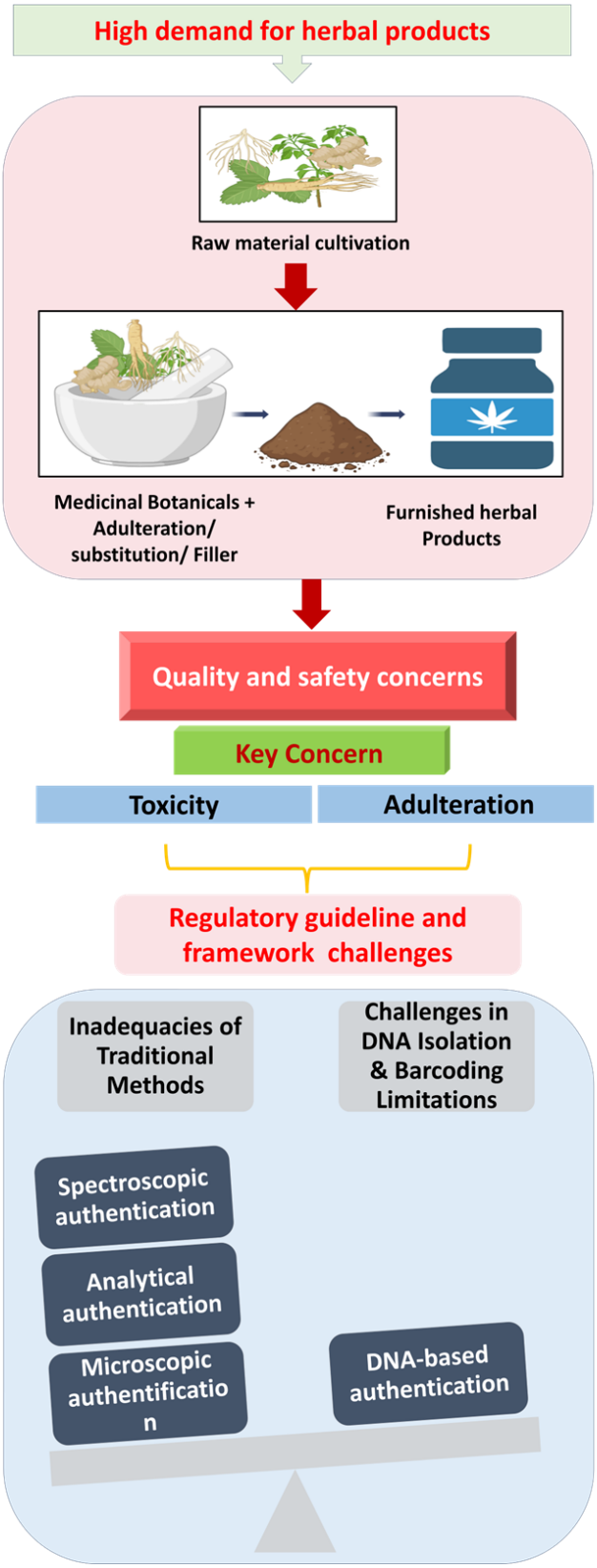

Figure 1.2 Key factors influencing the herbal products industry: demand, regulatory challenges, and quality control methods.

Figure 1.3 Overview of global regulatory frameworks for traditional medicines, highlighting the regulatory bodies, frameworks, and key safety standards.

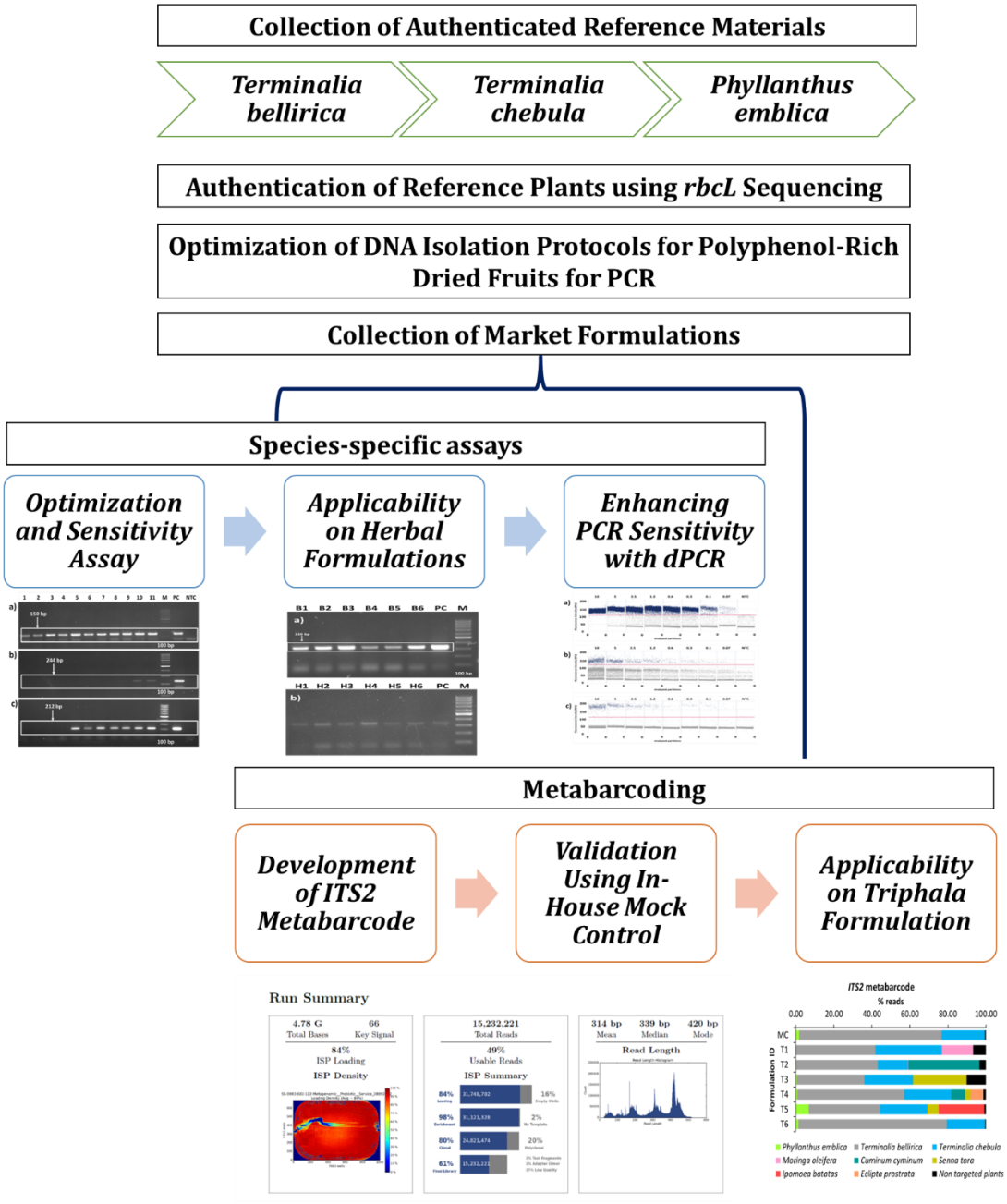

Figure 1.4 Research Framework