QUALITY CHALLENGES ENCOUNTERED IN THE DEVELOPMENT OF BOTANICALS INTO PRESCRIPTION MEDICATIONS

by

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(Under the Direction of Grace Gowda)

ABSTRACT

The 1994 Dietary Supplement Health and Education Act (DSHEA) recognized botanical products as dietary supplements, enabling their expansion into the pharmaceutical market. To further regulate botanical drug development, the U.S. Food and Drug Administration (FDA) issued the Botanical Drug Development Guidance in 2004, later revised in 2016, outlining pathways for botanical products to achieve prescription drug status through rigorous clinical trials. Despite these regulatory efforts, only a few botanicals have received FDA approval as prescription drugs. Key challenges include the lack of standardized quality controls, inconsistencies in safety and efficacy, and limited scientific validation.

This study examines the barriers impeding botanical drug development and explores potential solutions to enhance standardization, improve clinical validation, and refine regulatory frameworks. Findings may offer valuable insights for researchers, manufacturers, and policymakers to foster innovation, encourage investment in botanical drug research, and streamline approval processes, ultimately advancing the role of botanicals in modern medicine.

INDEX WORDS: Botanical product, Botanical drug, Active constituent, Phytochemical, Phytomedicine, Primary metabolite, Secondary metabolite

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DEDICATION

I dedicate this thesis to my family, especially to the cherished memories of those who are no longer with us.

To my late mother, **Selina**, whose unwavering support and encouragement were the foundation of my journey. She was my pillar of strength, always believing in my dreams even when I doubted myself.

To my late brothers, especially **Elias**, who never stopped believing in me. Even in the darkest moments, he pushed me forward, reminding me that perseverance was the key to success, "Youngman, loosing is not an option, you either win or learn."

To my dear brother **Watson**, whom I lost while in the midst of this research. His passing shook me to my core, and I nearly abandoned this work. But his words—*"Keep trying, my brother"*—echoed in my heart, giving me the strength to press on. This accomplishment is as much his as it is mine.

And to the future generations: Never give up. Keep trying. Always look forward with hope, for hope is the light that leads us through the toughest times. "May your choices reflect your hopes, not your fears."

"It always seems impossible until it's done." — Nelson Mandela.

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Definitions

Active constituents - are the chemical constituent(s) in a botanical drug substance that contribute significantly to a botanical drug's intended pharmacological activity or therapeutic effect.

Botanical drug – it is a drug product intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in humans (FDA, 2004a). It is a drug derived from botanicals and is regulated and sold on the US market as a pharmaceutical drug. These drugs are not highly purified or chemically modified substance.

Botanical product – it is a product made from plant or plant parts that are used for medicinal, therapeutic, flavor, or scent purposes.

CAMPFIRE – a community-based natural resource management program, aimed to promote sustainable management of wild resources by empowering rural communities to manage resources in their areas.

Dietary supplement – it is a product intended for ingestion that contains a "dietary ingredient" intended to add further nutritional value to (supplement) the diet. A "dietary ingredient" may be one, or any combination, of the following substances: a vitamin, a mineral, herb, amino acid, or other botanical (FDA, 1994).

Herbal medicine - is the art or practice of using herbs and herbal preparations to maintain health and to prevent, alleviate, or cure disease. A plant or plant part or an extract or mixture of these used in herbal medicine (FDA, 2004a).

Prescription drug (R*x*) - it is a drug product approved for marketing that can only be obtained with a prescription from an appropriate health care practitioner.

Over-The-Counter (OTC) drugs - An OTC drug is a drug product marketed for use by the consumer without a prescription from a health care practitioner (FDA, 2015d).

Primary Metabolites – they are compounds that are essential for the physiological functions of an organism during its growth, development and reproduction. They are the main products of metabolic processes that occur during the organism's active growth phase, examples are carbohydrates, proteins, lipids, amino acids and vitamins.

Phytomedicine – a medicine derived from plants in their original state and standardized for use in a dosage regimen, e.g., Echinacea, Garlic, Ginger, Ginkgo, and Ginseng.

Phytochemicals – are bioactive substances (chemicals) produced by plants including edible plants, e.g., beta-carotene, catechins, and carotenoids.

Phytohormone – they are small organic molecules or chemical messengers that are produced by plants to regulate or coordinate cellular activities like plant growth, development, and other physiological processes.

Secondary Metabolites - they are organic compounds produced by plants and are not necessary for the plants' normal growth and development but help them compete in their environments. They play a key role in plant defense, and have many applications in medicine and agriculture, e.g., terpenoids, alkaloids, tannins and flavonoids.

Definition of Acronyms and Key Terms

ABC – American Botanical Council

AE – Adverse Event

AER – Adverse Event Reporting

AHA – American Herbal Association

AHPA - American Herbal Products Association

AHP – American Herbal Pharmacopoeia

API – Active Pharmaceutical Ingredient

BDDGI – Botanical Drug Development Guidance

BHC – Benzene hexachloride

CAMPFIRE - Communal Areas Management Program for Indigenous Resources

CBD – Convention on Biological Diversity

CDER – Center for Drug Evaluation and Research

CFR – Code of Federal regulations

CGMP – Current Good Manufacturing Practice

CMC – Chemistry and Manufacturing Controls

CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora

DDT – Dichlorodiphenyltrichloroethane

DSHEA – Dietary Supplement Health and Education Act

DNA – Deoxyribonucleic acid

FD&C Act - Federal Food, Drug, and Cosmetic Act

GACP – Good Agricultural and Collection Practices

GACP - GMPBM - Good Agricultural and Collection Practices and Good Manufacturing Practices for Botanical Materials

GCP – Good Clinical Practice

GMP – Good Manufacturing Practice

CGMP-DS – Current Good Manufacturing Practice for Dietary Supplements

HPLC – High Performance Liquid Chromatography

HIPAA – Health Insurance Portability and Accountability Act

HIV – Human Immuno-deficiency Virus

IND - Investigational New Drug Application

IRB – Institutional Review Board

NDA –New Drug Application

NDI – New Dietary Ingredient

NIH – National Health Institute

NP – Nagoya Protocol

OTC – Over the Counter

OAM – Office of Alternative Medicine

PA – Pyrrolizidine alkaloids

PCBs – Polychlorinated biphenyls

PCNB – Pentachloronitrobenzene

PAH – Polycyclic aromatic hydrocarbons

Rx – Prescription Drug

SPE – Supercritical fluid extraction

TA – Tropane Alkaloids

TCM – Traditional Chinese Medicine

TLC – Thin Layer Chromatography

UNEP – United Nations Environmental Program

US-DSC – United States Dietary Supplement Compendium

USFDA – United States Food and Drug Administration

WHO – World Health Organization

CHAPTER 1

Overview

1.1 Background

Botanicals are plants or plant parts that are used for their various medicinal or therapeutic properties, flavors and scents. Those that are mainly used for medicinal or health purposes are often referred to as botanical or herbal products, as well as phytomedicines. Therefore, all three products are considered subsets of botanicals (https://ods.od.nih.gov/).[3] The single most important difference between a botanical, herbal or phytomedicinal product and a conventional pharmaceutical drug is that the three (botanical, herbal or phytomedicinal) are organic and only lightly processed, while a pharmaceutical product can be synthesized even if the active ingredient is derived from a natural source.

The use of botanicals as medicinal products for treating and or diagnosing disease has been in practice for thousands of years even though their scientific mode of action and safety profile has never been understood or investigated (Tony Yuqi Tang, et al. 2014).[4] It is only during the 20th century that efforts have been made to understand their composition, biochemical structure, safety profile and for some, how they work. Progress in this area has been slow compared to modern conventional pharmaceutical drugs which are based on a single chemical entity that can be modified in design to target a specific biological system where it can bind to a receptor and bring about a desired physiological response. Most researchers though, think that botanicals and botanical derived drugs have a great advantage over synthetic drugs even though there is yet to be any tangible scientific proof for that. In the book “Textbook of Pharmacognosy

and Phytochemistry", Shah, B.N. and Seth, A.K., 2010,[5(p17)] argue that the future of plant drugs and remedies is bright. His argument is based on the enormous progress that has been made in the development of new techniques to isolate and identify new curative agents that have brought about drugs such as artemisinin (antimalarial, taxol (anticancer), forskolin (antihypertensive), and piperine a bioavailability enhancer.

"It has now been universally accepted fact that the plant drugs and remedies are far safer than that of synthetic medicines for curing the complex diseases like cancer and AIDS" - Shah, B.N. and Seth, A.K., 2010,[5(p17)].

However, other researchers like Ali Karim et al,[6] in "Herbal versus synthetic drugs; beliefs and facts", argue that botanica/herbal remedies have a minimal and unproven role in disease treatment compared to synthetic drugs.

> *"Synthetic drugs address symptoms caused by specific diseases as understood by scientific pathology, herbal medicine usually directs towards aiding the body's own healing process" - Ali Karim et al.*[6]

In the US, botanical products are classified as dietary supplements under the DSHEA (Dietary Supplement Health and Education Act) of 1994, that is, products that have beneficial biological effects that can improve health outcomes but do not have disease modifying effects, meaning, they are regulated as foods (www.fda.gov).[7] While, on the other hand, "botanical" drugs, which are drugs derived from botanicals, are regulated and sold on the US market as pharmaceutical drugs (www.fda.gov, Botanical Drug Development Guidance for Industry).[7] By nature, botanical drugs are not highly purified or chemically modified substances, and can contain ingredients from plants, algae, macroscopic fungi, or a combination thereof (FDA, 2016). They are considered complex mixtures that may not have distinct active ingredients,

which further makes it more difficult to maintain their consistency and stability. By their nature, botanical plants' quality is mainly affected by the challenges of variable sources of raw materials, processing methods, dosage formulations and non-existence of credible standard criteria for quality control. These issues, in turn, affect the safety and efficacy evaluation of the botanical drug (Atanas G. Atanasov, et al. 2015).[8] Challenges like these are by far the biggest contributors to issues of drug quality during the development processes.

The DSHEA of 1994 allowed for "disease modifying" claims to be made for botanical/dietary supplements only if evidence to support that claim could be backed up by substantial clinical research data. The U.S. Food and Drug Administration (USFDA) further provided guidance on how botanicals can be further studied, with a view of seeking regulatory approval as a conventional prescription drug. In its revised botanical drug development guidance for industry of 2016, the FDA stated that -

> *"If a lawfully marketed botanical dietary supplement is studied for its effects on diseases in the proposed investigation (i.e., to cure, treat, mitigate, prevent, or diagnose disease including its associated symptoms), then it is an investigational new drug and will be subject to IND requirement" - (*Botanical Drug Development Guidance for Industry December 2016. *www.f da.gov*).[7]

It is, however, disappointing to note that the botanical and pharmaceutical new drug research industry has shied away from researching and developing botanicals into prescription drugs with a view to harnessing their promising health modifying effects. From 2006 to present, only five botanical products have been approved for marketing as prescription drugs by the FDA's CDER (Center for Drug Evaluation and Research), compared to over 680 pharmaceutical prescription drugs including biologics approved in the same period. These botanical drugs

are Veregen (sinecatechins), an ointment approved in 2006 for the treatment of perianal warts, Mytesi (crofelemer) an oral tablet approved in 2012 to treat drug-induced diarrhea resulting from HIV/AIDS medication, and Epidiolex (cannabidiol), an oral spray approved in 2018 for the treatment of seizures in children, Filsuvez (birch triterpenes) (2023), a topical gel for the treatment of epidermolysis (blistering of skin), NexoBrid (anacaulase-bcdb) (2022), also a topical gel for the treatment of thermal burns in adults. (www.fda.gov). Another comparison to be made is that over 400 botanical IND approvals have been processed compared to slightly over 20 000 pharmaceutical prescription INDs and over 50 000 dietary supplement products (https://www.cbo.gov/publication/57126).[9] The question therefore is, with a market value of over $34 billion (about $100 per person in the US alone), why is there a lack of interest in developing and establishing clinical efficacy of botanicals given their centuries and decades old and trusted use all over the world? What are the challenges holding this back?

1.2 Statement of the Problem

The hypothesis for this thesis is that we will see there are gaps in knowledge and unaddressed areas of concern that are critical in identifying quality challenges that hinder or limit the research and development of botanicals into drug therapies. This is partly due to inconsistencies that botanical drug research is being conducted. Knowledge about plant material composition and study design approaches varies considerably, and all this presents a low level of confidence in published data on potential biological and pharmacological targets of botanical. (Landis et al., 2012).[10] This lack of confidence in knowledge seems to be a factor in the research and development of botanicals (Shipkowski K.A. et al., 2018).[11] Because of this, far fewer botanical drugs (only 5) have been approved by the FDA since its issuance of the Botanical Drug Development Guidance of 2004 given that more than 800 INDs applications have been

submitted. This shows that there are some areas of concern that still need to be investigated before botanicals can be widely accepted and approved as prescription therapies.

This study will attempt to show that if these gaps in knowledge are identified and addressed, challenges associated with botanical drug research and development can be overcome. This may also help industry regulators to introduce better regulations and policies that can further support the development of botanical drugs.

The study results are also expected to show that botanical plant specifications, material composition, and substance characterization, contribute more to the quality challenges in the drug development process.

1.3 Purpose of the Study

The purpose of this study was to find better ways that botanical drug researchers, developers, and manufactures can use to overcome development challenges associated with the quality of botanicals that impact their development into prescription medications. The study also investigated what industry regulators can do to improve regulatory policies that can further support the development of botanical drugs.

In the development of botanicals into prescription medications, just like in conventional pharmaceuticals, the main purpose of the process is to minimize risk to the patient while maximizing efficacy, therefore the demonstration of safety and efficacy requires a showing that the benefits of the drug outweigh its risks (FDA, 2023).

During the development process of a botanically derived drug, drug quality must be guaranteed by following strict good manufacturing practices (GMPs). Since a botanical drug product is organic and not synthesized, the quality of the botanical substances from which the drug product is being made, to a larger extent, affects the quality of the drug product and

subsequently the drug that is derived from it. This is crucial in guaranteeing the drug product's safety and efficacy. To better understand the quality challenges that hinder the development of botanicals into prescription drugs, it is important to first investigate the quality of botanical substances, from which the products and drugs are made from. This study will investigate the quality requirements of the development processes of botanicals and the challenges that hinder their development into prescription drugs.

1.4 Research Question and Methodology

This study sought to explore the major botanical product quality challenges that hinder their development into prescription medicines. This was done by employing a secondary quantitative research method, that involved an extensive examination of published literature mostly from previous research reports, government resources, and the internet. One of the methods used to collect data involved a comparison of quality standards used to characterize botanical substances (ingredients) compared to pharmaceuticals. The data analysis included an evaluation of how the quality of botanical substances as described by the FDA's Botanical Drug Development Guidance for Industry (BDDGI)[7] of 2004 and as revised in 2016 affected the drug product, and also included a comparative evaluation of quality methods and processes used to evaluate the purity of botanicals compared to pharmaceuticals with regards to physical, chemical, biological, microscopic and organoleptic properties, which in turn play an important role in determining the quality of the drug vis-a-vis its safety and efficacy.

Furthermore, a comprehensive literature review of published research data evaluating considerations and challenges that remain unaddressed regarding botanical drug formulations and development processes that impact on the purity of the drug was also carried out. An in-depth investigation of how botanical plants are cultivated and harvested, and for those that grow

naturally as indigenous plants, how they are identified with a view of assuring their quality and purity, hence guaranteeing a safe and efficacious drug. The lack of chemical studies of the plant components or chemical constituents, purification methods and residue testing studies, scientific validation and standardization studies, and insufficient evidence-based studies on safety and efficacy which can be attributed to limiting the development of botanicals into drugs were also studied.

1.5 Importance of the study

Even though the use of most botanicals as medicinal agents has not yet been scientifically proven, over 250 000 plants have been documented for medicinal use. Of these, only about 50 000 have been investigated, with only a few hundred INDs having been submitted for scrutiny by the US FDA's CDER as potential drug candidates. Thousands of people around the world rely on them daily and in some countries, they are considered primary means of treatment. This is also because they are readily available and are less expensive compared to prescription medications.

This study will help botanical drug industry researchers and manufacturers to better understand the product quality challenges that hinder their development into prescription medications. Also, the study might help regulators benefit by refining some regulations that might be posing challenges to industry in clinical trials.

CHAPTER 2

Research Topic Context

2.1 Botanicals: A Historical Perspective

Medicinal plants have been in use since the Paleolithic age, and written evidence of herbal remedies dates to over 5 000 years when ancient civilizations like the Sumerians of Mesopotamia compiled lists of plants used for medicinal purposes like opium and myrrh (Batiha, G., et al, 2022).[12] When the populations grew, so did the civilizations, their need for curing prevalent diseases among people also grew. As they went about foraging and investigating food sources, they ate at random, plants or their parts like tubers, fruits, bark and leaves, and out of this inadvertent investigation of edible plants through experimentation by trial and error, they found plants that could cure ailments. These benefits of their foraging activities were passed on from one generation to the other, and new knowledge was added in the same way. These were the beginnings of what was to be later called pharmacognosy (drug –knowledge), the study of natural products and their potential for medicinal use. This later gave rise to another field of plant study, botany, which today is dedicated to the scientific study of how plants function, how they evolved, how they are related to each other, where they grow and how people use them.

The history of botanicals, their products and use cannot be complete without the mention of ancient plant use and contributions by early civilization like Chinese, Egyptian, and Indian. Ancient Chinese writings describe medicinal plant use as early as 3 000 BC (Pan SY, Litscher G, Gao SH, et al, 2014).[13] Early Chinese botanical plant use describes how emperor Shen Nung (about 2700 B.C.), investigated the medicinal value of hundreds of herbs. He is also known to

have tested many of them on himself and is credited for writing the first book or recording of 365 "drugs", the Native Herbal *(Pen T-Sao)*. Some of these drugs are still in use today, e.g. podophyllum, rhubarb, ginseng, cinnamon bark and ephedra (Shah, B. N. and Seth, A. K, 2010).[5] Modern Chinese and Japanese botanical medicine practices are strongly informed by their ancient traditional plant use and are mostly derived from early clinical manuals like the *Shang Hang Lun (Treatise on the Treatment of Acute Diseases Caused by Cold)* written by Chang Chung-Ching (142–220), the *Shang Han Lun* and the *Chin Kuei Yao Lueh (Prescriptions from the Golden Chamber).* Together, these manuals form the historical origins of herbal formulas that form the basis of today's famed Chinese and Japanese herbal practice known as *Kampo* (Shah, B. N. and Seth, A. K, 2010).[5]

Ancient Egyptians were among the first civilization to use plants as a source of medicines. Some of their earliest writings on papyrus describe medicinal plant use as early as 1500 B.C. The best-known and well preserved is the *Ebers Papyrus* of 1550 B.C., which is a collection of 700 drugs with details of 800 prescriptions. Others are the *Berlin Papyrus* (204 prescriptions), *Hearst Papyrus* (260 prescriptions), *Kahun, and Carlesberg VIII Papyrus* (both with gynecological prescriptions, *Ramesseum Papyrus* (medical prescriptions), and the *Edwin Smith Papyrus* of 1600 B.C., it contains surgical instructions and formulas for cosmetics. Some of the most used medicinal plants/herbs in most of these papyrus prescriptions were: senna, honey, thyme, juniper, cumin, (all for digestion); pomegranate root, henbane (for worms) as well as flax, oakgall, pinetar, manna, bayberry, aloe, garlic, wild lettuce, onion, peppermint, papyrus, poppy-plant, saffron, watermelon, wheat, myrrh. Also used and still being used today in the pharmaceutical industry is acacia gum (as an emulsifier, stabilizing agent and tablet binder), and turpentine, in veterinary medicine. These papyrus manuscripts are today considered as among the

most comprehensive and well-preserved documents of ancient Egyptian medicine in existence today, (David, R. and Forshaw, R 2023).[14]

India has the richest and probably the oldest medicinal plant knowledge. Extensive medicinal properties of plants and use are described in *Rigveda* and in *Atharvaveda* (3500–1500 B.C.). From these Vedas, the *Ayurvedic* practice, which is based on treatments that combine products derived from plants, animals and minerals evolved. *Ayurveda* is the term used to describe the traditional medicine of ancient India of which about 960 plant species are listed (Sahoo et al., 2010). The *Charaka Samhita* (6 - 7 B.C.), written by Maharshi Charaka, is considered to be the oldest writing on traditional medicine, and Charaka is assumed to be the founder and father of Ayurveda.

In the Middle Ages, further evidence of the widespread use of medicinal plants was also observed when plants like sage, anise, mint, savory, Greek seed, and later aloe, turmeric, pepper, and ginger became common (Sha, B. N., Seth, A. K, 2010).[5] Even Hippocrates (460 – 370 BC), considered the "Father of Medicine" categorized more than 300 medicinal plants according to their diagnostic and prognostic uses. It is after him that Aristotle (380 – 322 BC), and later his student Theophrastus (370 – 286 BC), often referred to as the "Father of Botany", through their research, gave rise to the field of botany as we know it today. Many more naturalists followed them, with Pedanius Dioscórides, Pliny the Elder, and the famous Aelius Galenus, better known as Galen (131 AD – 200), devising methods of preparing plant and animal drugs which are still known or referred to today as "galenicals" in his honor (Shah and Seth, 2010).[5]

2.2 Botanicals

In this study, the term "botanical" will be used in its broadest sense to refer to various products from plant materials obtained from leaves, roots, seeds, fruit, berries, flowers bark, and algae, including substances such as algae, macroscopic fungi, and combinations thereof that are processed into a variety of different end products.

It does not include:

a) Products that contain animals or animal parts (e.g., insects and annelids) and/or minerals.

b) Materials derived from botanical species that are genetically modified with the intention of producing a single molecular entity (e.g., by recombinant DNA technology or cloning).

c) Products produced by fermentation of yeast, bacteria, plant cells, or other microscopic organisms, including plants used as substrates, if the objective of the fermentation process is to produce a single molecular entity (e.g., antibiotics, amino acids, and vitamins).

d) Highly purified substances, either derived from a naturally occurring source (e.g., paclitaxel) or chemically modified (e.g., estrogens synthesized from yam extracts) *(Botanical Drug Development Guidance for Industry December 2016, www.f da.gov)*.[7]

2.3 Botanical Products

In this study, botanical products will be referred to under the FD&C Act's classifications as either –

a) foods – if they are primarily consumed as part of a normal diet and do not make any health claims.

b) dietary supplements – only when marketed to maintain health or provide nutrients.

c) drugs (including biological drugs and medical devices) - only if they are marketed with claims to diagnose, treat, cure or prevent a disease, and after being subjected to stricter regulatory requirements.

d) cosmetics – only when they are marketed as beauty products, e.g., skin care and essential oils products.

The classification of the product as a food, drug, medical device, or cosmetic depends to a larger extent on its intended purposes and use. For some product types, other factors can also be considered. Under section 201(g)(1)(B) of the FD&C Act, a botanical product intended for use in diagnosing, curing, mitigating, treating, and preventing disease would meet the definition of a drug and would be subject to regulations that pertain to a drug (Botanical Drug Development Guidance for Industry December 2016, *www.f da.gov*).[7]

Because of this increased interest in traditional medicines, a need for regulation of their production and use became necessary, leading to the DSHEA passing a statute in 1994 regulating them as dietary supplements, separating them from foods and drugs. According to DSHEA (1994),[15] botanical products are regulated and marketed as dietary supplements, which places them as a subset of foods under the USFDA. To be listed under foods, dietary supplements must contain a dietary ingredient intended to supplement the diet. Under DSHEA, Congress defined the term dietary supplement as a:

"Product intended for ingestion that, among other requirements, contains a dietary ingredient intended to supplement the diet." (US-103rd Congress, 1994).[15]

The dietary ingredients may include vitamins, minerals, herbs and other botanicals, amino acids, a concentrate, metabolite or extract. These botanical/dietary products can be found in a variety of forms that include - tablets, capsules, softgels, gelcaps, liquids and powders. Because of this categorization, they are not allowed to make any medicinal claims, for example, claims to diagnose, mitigate, cure, treat or prevent disease. The FDA does not approve dietary supplements (under which category most botanical products fall) for safety before they are marketed, but it has limited post market enforcement.

While the development and mass production of chemically synthesized drugs has changed health care provision in most parts of the world over the past decades, particularly in the western world, there remains a significant population around the world that are still largely dependent on traditional plant or herbal medicines for their primary care needs. In Africa and India, up to 90% and 70% of the population respectively still rely on traditional plant medicine to help meet their health care needs. In China, traditional medicine use accounts for about 40% of all health care delivered, while more than 90% of general hospitals in China have units for traditional medicine dispensing (WHO 2003, Watchtel-Galor, S. and Benzie, I.).[16,17] According to Ernst, S. and Winder (2005),[18] the use of traditional medicine has soared in the western world in the past two decades. Here in the US, it is estimated that about 38% of adults and 12% of children were using some form of traditional medicine in 2007.

2.4 Botanical Drugs

The term “botanical drugs”, also known as traditional herbal therapies, will refer to what the FDA describes as drugs intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in humans (Botanical Drug Development Guidance for Industry December

2016, *www.fda.gov*).[7] Botanical drugs are made from plant materials, algae and macroscopic fungi or a combination of the above.

> *"A botanical drug product is intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in humans. A botanical drug product consists of vegetable materials, which may include plant materials, algae, macroscopic fungi, or combinations thereof".* (www.fda.gov).[7]

They are regulated by the FDA and undergo an extensive review process and are also marketed in the same way as pharmaceutical drugs. To facilitate this process, the FDA, through the Center for Drug Evaluation and Research (CEDAR), issued a Botanical Drug Guidance for Industry (BDGI) (2004, 2016 revised),[7] which was meant to encourage and guide botanical drug developers on the expectations of this relatively new drug development pathway. To date, only five botanical drugs have met the BDGI requirements and have been approved for marketing as prescription drug therapies under the New Drug Application (NDA) and the Biologics License Application (BLA) pathways. These drugs are Veregen (sinecatechins) (2006), a topical ointment used to treat genital warts, Mytesi (crofelemer) (2012), an oral treatment for noninfectious diarrhea in adults with HIV/AIDS, Epidiolex (cannabidiol) (2018), an oral solution for the treatment of seizures in children two years or older, Filsuvez (birch triterpenes) (2023), a topical gel for the treatment of epidermolysis (blistering of skin), and the only BLA, NexoBrid (anacaulase-bcdb) (2022), also a topical gel for the treatment of thermal burns in adults.

Although the botanical drug pathway is lagging, the number of FDA approved drugs derived from natural products including botanicals has been going up. Between 1981 and 2014, 1 562 drugs were approved by the FDA, and 320 of them were derived from natural products, with 80% of these drugs coming from plants. More than 60% of Cancer drugs on the market or in

testing are based on natural products (Watchtel-Galor, S. and Benzie, I., 2011).[17] According to Sahoo et al (2010),[19] of the 252 drugs in the World Health Organization (WHO) essential medicines list, 11% are exclusively of plant origin.

A major characteristic of botanical drug products is that they have unique features, for example, they are complex mixtures lacking a distinct active ingredient and substantial prior human use. Another important and notable characteristic of botanical drugs is that they are organic, contain multiple compounds and are almost always lightly processed, compared to their pharmaceuticals counterparts, which are synthetic even if the active ingredient was derived from a natural product and they contain a distinct active pharmaceutical ingredient (API) that acts on a specific biological target in the body to produce a physiological response.(API). A botanical drug product may be available in the market as a solution, powder, tablet, capsule, elixir, topical, or injection.

There are, however, some botanical "drugs" that have been reviewed for safety and effectiveness including controlled clinical studies by the FDA and are listed in the Over the Counter (OTC) monograph. Examples of these are cascara, psyllium, and senna, all used for constipation. All OTC monograph drugs preparations must meet the FDA's review process of safety and effectiveness and must gain approval of one or more of its active ingredients. They, however, do not need to go through the rigorous IND process which results in an approval of an NDA.

2.5 Concerns Regarding the Quality of Botanicals, an FDA Perspective

In the United States, the US Pharmacopeia (USP) and National Formulary (NF) are the official U.S. compendia that establish quality standards for medicines, botanical drugs, botanical products/dietary supplements. The USP sets standards for active pharmaceutical ingredients

(APIs), drug formulations, and dietary supplements, while the NF focuses on inactive ingredients (excipients) and botanicals. The USP and the NF merged in 1906 as the USP-NF, which now serves as a regulatory benchmark to ensure drug identity, purity, strength, and quality. These requirements and standards are enforced by the FDA under the Federal Food, Drug, and Cosmetic Act of 1938.

Early pharmacopeias included monographs for botanical drugs, but, with the advancement in isolation techniques of active compounds from plants, pharmaceutical development shifted toward single-molecule drugs. This necessitated the establishment of the USP-NF in 1906 which brought in stringent quality and identity standards, favoring purified, well-defined compounds over complex botanical mixtures. This shift was reinforced by the FDA under the FD&C Act of 1938, which pushed for single-molecule drugs over botanicals because of their consistency, efficacy, and ease of regulation. This led to the decline in the use of whole-plant medicines in favor of modern pharmaceuticals.

The establishment of drug standards required formulations to conform to USP-NF specifications, mandating proof of safety before marketing. This requirement disadvantaged botanicals, as many failed to meet the purity and consistency requirements due to their complexity and lack of controlled clinical trials. As a result, the development of synthetic and purified drugs was encouraged over plant-based formulations, leading to the decline of many traditional botanical medicines. However, the 1994 Dietary Supplement Health and Education Act (DSHEA) provided an alternative path, allowing botanical products to be marketed as dietary supplements without requiring FDA drug approval. Further regulatory progress came with the FDA's Botanical Drug Development Guidance (2004, revised 2016),[8] which established a framework for botanical drugs to gain prescription drug approval. Despite these efforts, few

botanicals have successfully gone through the FDA approval process, largely due to complex standardization requirements and clinical trial challenges.

Further to this, when the World Health Organization recognized the importance of traditional medicine in providing essential care as a supplement to the modern pharmaceutical medicine in its WHO Traditional Medicine Strategy of 2002, more countries joined in in the development of botanical/herbal drugs. In the US, in particular, this resulted in the need to regulate the production and use of the botanicals regarding their quality, safety and efficacy to ensure their health claims and uses (Sahoo et al, 2010, WHO, 2005).[19,20]

To better understand the quality challenges encountered in the development of botanicals into prescription medicines, it is important to understand their pathway to approval as prescription therapies. Although there is a vast difference biologically and pharmacologically between botanical drugs, chemical drugs, and biological products, currently, the FDA does not have a separate criteria or requirements for botanicals to prove their safety and efficacy for regulatory approval. They must fulfill the same clinical trial requirements specifically designed for single entity drugs using the same CGMPs, there is no specific criteria that is designed to deal with the complex plant derived mixtures associated with botanicals. They can also be approved under the FDA 505 (b) (2) pathway which is a streamlined drug approval process that allows use of existing data. This lack of a specific regulatory and clinical trial criteria for approving botanical drugs - “the one size fits all approach”, is a limiting factor in their development and approval into drug therapies.

CHAPTER 3

3.1 Literature Review

Since early civilization, humans have recognized plants not only as a source of food but also as powerful medicines. Over time, the study of plant-based compounds has led to the discovery of new drugs and dietary supplements, expanding their role in healthcare. Advances in phytochemical research and pharmacology have further deepened our understanding of their therapeutic potential.

The passage of the Dietary Supplement Health and Education Act (DSHEA)[15] in 1994 and the FDA's Botanical Drug Development Guidance for Industry (BDDGI) in 2004 and 2016)[7] has provided new opportunities for developing plant-derived pharmaceuticals. These regulatory efforts have renewed interest in botanical drug research and underscored the need for innovative approaches to advance safe and effective plant-based medicines for disease treatment (Teoh, 2015).[21]

For those plants that have been used purely for medicinal purposes, they have never been characterized before to determine their chemical compositions and more, their mode of action. Their claims of safety have been based on age-old use, and clinically unsubstantiated claims of efficacy. This chapter will explore the quality challenges and risks encountered in the development of botanical drugs into prescription medications.

The quality of botanical (herbal) drugs can be described as the sum of all factors which contribute directly or indirectly to its safety, effectiveness and acceptability (Mollah, S. and Abu Bin Nyeem, M., 2021).[22] This means that the quality concerns start right from the time the plant is grown or cultivated through to the sourcing stage. This may continue to the product and drug

development stage as so many factors come into play. To understand these concerns, an in-depth analysis of the processes involved is required. In the case of cultivation and sourcing concerns, if they are not addressed properly, they later present product quality challenges during processing and development of the botanical to a drug product. Further to this, there is a lack of standardization of most parameters that guarantee a quality end-product starting with the sourcing of raw materials, processing methods, dosage formulations, which includes the ability to analyze, quantify and isolate the plant materials through to production. This lack of standardization makes it difficult to produce a consistent and batch-to-batch comparable product (Schilter et al., 2003).[23]

The quality of a botanical substance can impact the efficacy and safety of a drug product derived from a plant in several ways. This is so because the concentration of bioactive compounds or physiologically active substances within the botanical material can lead to inconsistencies in the therapeutic effects of the drug, either making the drug too potent or too weak to be effective, hence causing serious adverse reactions. Because of this, effective quality control measures must be built in throughout the whole development process to ensure consistency and safety across batches. Some of the important quality considerations include contaminants and impurities, chemical composition variability, identification and authentication, active ingredient variability (standardization challenges), impact on clinical trials, and stability issues and to some extent, regulatory agencies considerations.

Accurate identification of a botanical plant and plant materials is very critical during the sourcing stage. This process must be done following a recommended identification method or nomenclature that is universally accepted since botanical plants can be sourced from different places around the world, where names of similar plants can be different or vice versa. In the US, the use of scientific names is encouraged as it provides a greater degree of accuracy than common names. This is a key factor as it limits the number of species that are acceptable and

guarantees that the appropriate tests that are matched for those plants, can ensure that the recommended quality standards are met (Nam-Cheol Kim, 2021).[24] In some cases, further identification can be confirmed by macroscopic and microscopic examinations as well as chromatographic and spectroscopic examinations. Details of the identity specifications for the commonly used botanicals can be found in the American Herbal Pharmacopoeia (AHP) and other academic literature.

The main reason behind the need for evaluation of botanical raw materials before the development process is to ensure that the concentrations of substances with known physiological effects fall within predetermined limits. Evaluation should include proper identification of high-grade raw materials obtained from properly identified and harvested plants, identifying physiologically active constituents using standardized extraction methods, clinically relevant bioassays and purity tests, using third party testing or independent laboratories to verify the product's purity and absence of contaminants, and traceability. All these evaluation methods should be used with the aim of identifying the plant species, detecting adulterants, and assessing the overall quality of the botanical raw material based on its physical characteristics and chemical composition.

It is also important as a measure of safety, to evaluate the botanical raw material's safety profile to ensure that the final drug product produced will not adversely interact with other medications and cause unwanted side effects. These evaluations are important for maintaining consistent product quality, meeting regulatory standards, and providing a safe and effective drug product.

The quality of a botanical product largely depends on how it is classified, i.e., is it a prescription drug, Over the Counter (OTC) medication, or dietary supplement? The FD&C Act

Section 501(a)(2)(B) requires all drugs to be manufactured in conformance with the current Good Manufacturing Practices (cGMPs) (*www.fda.gov*). These cGMPs are found in 21 CFR 210 and 211 and they apply equally to prescription and OTC drugs.

However, OTC drugs that are marketed and used as food are exempt from 21 CFR part 211, instead of this part, parts 110 and 117 apply. Because of this, the benchmarks for assessment of quality, safety, and efficacy for prescription drugs and OTC medications are much higher and enforcement is stricter (21CFR 210 and 211, *www.fda.gov*) than those of foods/dietary supplements (21 CFR 110 and 117, *www.fda.gov*). Dietary supplements are regulated by the FDA under DSHEA quality expectations, which require manufacturers to ensure their safety, and that they are not adulterated and misbranded.

For the botanicals that are collected from the wild, there seems to be a lack of legal protection for these plants. Chaachouay, N. and Zidane, L.[25] observed that only a small percentage of these of plant species are protected by legal measures in places where they naturally exist. Unsustainable collecting practices of these plant species might result in their exhaustion and could threaten their survival. Chaachouay, N. and Zidane, L.[25] further argue that this lack of legal safeguards might lead to the degradation of the environment and eventually which might also lead to disturbances in the biodiversity that is so crucial in maintaining a healthy ecosystem that we depend on. Regulators should design and enforce laws that will protect wild botanical plants from over harvesting by ensuring that collectors adhere to international guidelines that seek to protect these plants.

There are several guidelines and standards enacted by international, regional and local regulatory agencies which are meant to improve conservation efforts of these crucial ecosystems through global cooperation. These include -

a) Guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plants (WHO, 2003), and it seeks to -

i) Ensure the quality of medicinal plants and herbal substances.

ii) Ensure safety and efficacy of botanical/herbal products.

iii) Promote sustainable cultivation of medicinal plants.

iv) Ensure compliance with regulatory standards and industry guidelines.

v) Ensure traceability, i.e., every amount of harvested material can be traced back to its primary producer and the land where it was cultivated.

b) Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)(UNEP), it seeks to -

i) Ensure that international trade does not threaten the survival of endangered species

ii) Conserve habitats and prevent habitat loss and degradation

iii) Address the impact of global warming and chemical pollution on endangered species

c) Nagoya Protocol (Nagoya, 2010), it seeks to –

i) Conserve biological diversity

ii) Use biological diversity sustainably

iii) Share the benefits of genetic resources fairly and equitably

iv) Create a transparent legal framework for implementing the objectives

v) It addresses genetic resources that indigenous and local communities have a right to grant access to.

These conservation efforts help ensure the continued survival of the plant species, and most importantly, guarantee the quality and safety of the plants for our use in drug discovery.

There is also, need to have standardization of plant extracts to ensure the quality and effectiveness of the plant-derived chemicals. Variations in concentrations of bioactive materials affect the quality of the drug products and it is important to implement quality control procedures that include thorough testing to ensure safety. The FDA requires all botanical drugs, just like regular pharmaceuticals, to be developed according to GMP standards and that clinical studies be carried out to ascertain the safety and efficacy of the drug, this results in increased time and expenses throughout the development process. Such a situation may not be favorable for small botanical companies as it places a huge expense on companies that are not well resourced.

Below is a diagram showing the various stages of the drug discovery process from collection to lead optimization stage. At every stage there is some standardized testing to ensure the quality of the plant substance – *source:* Shah, B. N. and Seth, A. K, (2010) *Textbook of Pharmacognosy and Phytochemistry.*[5]

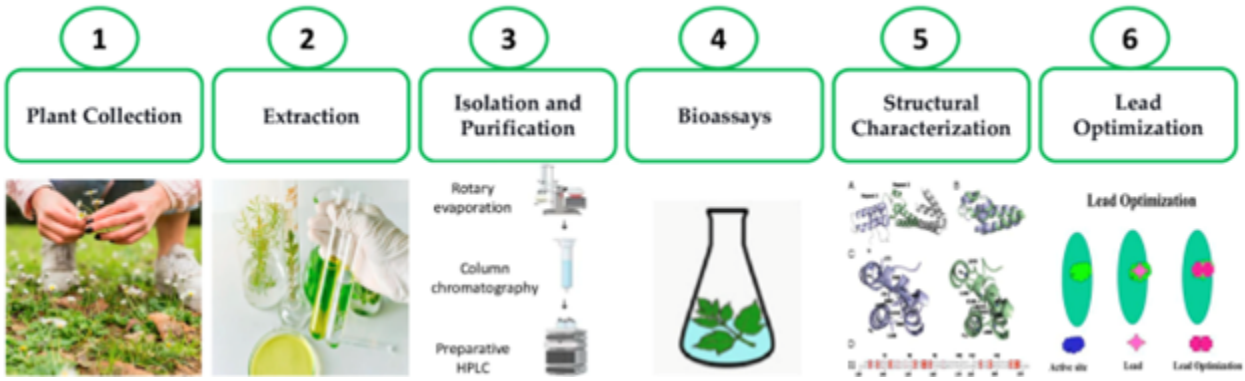


Figure 1. Various stages of the drug discovery process from natural products (1-Plant collection, 2-Extraction, 3-Isolation and purification, 4-Bioassays, 5-Structural characterization, 6-Lead optimization).

CHAPTER 4

4.1 Methodology

To better understand the quality challenges and limitations that are encountered during the development of botanicals into prescription medications, this study investigated how "the quality of botanical products and drugs", which is described above as the "the sum of all factors which contribute directly or indirectly to their safety, effectiveness and acceptability" (Mollah, S. and Abu Bin Nyeem, M., 2021)[22] is affected by conditions ranging from cultivation, harvesting, and through to the drug product development stage. The study looked at 6 areas of concern in which quality can be a determinant factor in the development process. These areas of concern ranged from the time the botanicals were cultivated through to the time they were processed and developed into drug therapies.

The investigation of these factors enables us to better understand the issues that have a direct bearing on the overall quality and quantity of the chemical compounds that the plant produces. Therefore, a good understanding and effective management of these issues, particularly for the cultivated botanicals, results in a crop yield of good quality that guarantees or ensures a good quality product.

To accomplish this, an in-depth secondary quantitative research method was utilized to uncover the shortcomings and gaps in knowledge that prevent the development processes of botanicals from being approved as prescription drugs. A detailed review of scholarly articles and academic literature published in scientific literature search engines like PubMed, Google Scholar, ResearchGate, Web of Science, Wiley Online, SciFinder, and Academia.edu, including

academic public and college library books were utilized to obtain data on what quality challenges and limitations are encountered in the development of botanicals into prescription medications.

This research method was found to be suitable to answer this research question because it allowed access to a large and diverse dataset from previous published research reports that might have been impossible through primary data collection. It also provided for analysis of a wider range of perspectives and experiences from different contexts. Using this methodology also allowed for each area of concern listed above to be investigated separately while allowing for cross tabulation of the collected data to be examined side by side to identify relationships between the issues that were observed. The study was more concerned with quantitatively analyzing the relationship between the six areas of concern, i.e., identifying the quality issues affecting the development of botanicals into prescription medication across all areas of concern, it however did not determine the statistical significance of the observed relation.

A purposive sampling method was used. This method was chosen because it allowed for selection of articles and books that were based on their relevance to the research question and it also permitted for a descriptive (cross tabulation) nature of data analysis to be carried out. The data gathered was grouped according to how each affected the quality of the drugs and products produced from them based on several factors that contribute to their cultivation, sourcing, and processing. They were further carefully evaluated and critically analyzed to identify the challenges that impede the development of botanicals into prescription medications.

There was no set target for the number of articles reviewed, but an inclusion and exclusion criteria based on publication dates between the year 2000 to the current was considered except for textbooks. The reason for limiting the publication dates for journal articles was that they usually have the most recent information on a subject area. More than 60 scholarly articles

and books published in credible journals were accessed through several scientific literature search engines. These articles and books were selected based on their relevance to the research topic and their currency (date of publication). To determine relevance, first, the abstract or summary of the article was considered to see if it was related to the research question. The second thing considered was knowing the discipline or subject area focus of the articles, which was done by looking at the title of the journal or book that published them. In this study, the discipline or subject area was botanicals, and the focus area was how their quality affects their development into prescription medications. These quality considerations ranged from botanical plant raising/growing, sourcing, raw material evaluation through to drug development. Article relevancy was also based on the currency of the information, which was done by limiting the publication dates to the year 2000 to present.

For final consideration, the articles were further narrowed down to 30 based on their relevance to one of the 6 research areas of concern listed above, i.e., 5 articles per area of concern, making it 30 articles in all considered for comparative reading. The grouping of articles was based on how an article focused on a specific aspect that directly related to one of the research areas of concern. These articles were also selected based on their research study type, the quantitative method, which was determined from the abstract and methodology section of the articles. This method was appropriate because it offered objectivity and the ability to identify cause-and-effect relationships which can also help identify trends and forecast future outcomes based on data analyses. However, other articles that had complementary data that further enhanced the ideas being investigated were also considered.

The first 5 articles considered were concerned with investigating the best possible environmental conditions that affect or influence the cultivation, harvesting and sourcing of

quality botanical plants and raw materials, i.e., these are conditions which in turn have a profound impact on the quality of the raw materials and the composition of their chemical compounds.

For the next 5 articles, consideration was given to articles that specifically investigated how the quality of botanical substances or lack thereof of them affects the drug product. The main part of this area of concern was investigating how contaminants and impurities in botanical substances affect drug quality. The articles also investigated how and when these contaminants and impurities were introduced to the plants. They also looked at how active ingredient variability can be controlled by using standards to minimize batch-to-batch variability. These articles collectively underscore the critical need for rigorous quality control and regulatory measures to detect and eliminate contaminants in botanical substances, thereby ensuring the safety and efficacy of derived drug products.

The criteria used to select articles that investigated drug product development and challenges were –

a) How to identify the right botanical plant.

b) Assurance of quality and purity.

c) Standardization to minimize batch-to-batch variability.

d) Determination of active chemical constituents.

Because these elements have a profound effect on the quality of a drug product, careful consideration was done in selecting the articles that best address these concerns.

To study the methods used to evaluate botanical raw materials, the five articles chosen were primarily investigating the physical, chemical and biological evaluation methods used to characterize botanical raw materials.

The articles used to study methods of extraction, isolation, and identification were chosen based on their in-depth and wide coverage of current extraction methods. Such as -

a) Extraction methods

i) maceration,

ii) percolation,

iii) supercritical fluid extraction (SFE)

b) Isolation of the active chemical constituents

i) fractional crystallization

ii) fractional distillation

iii) sublimation

iv) chromatography

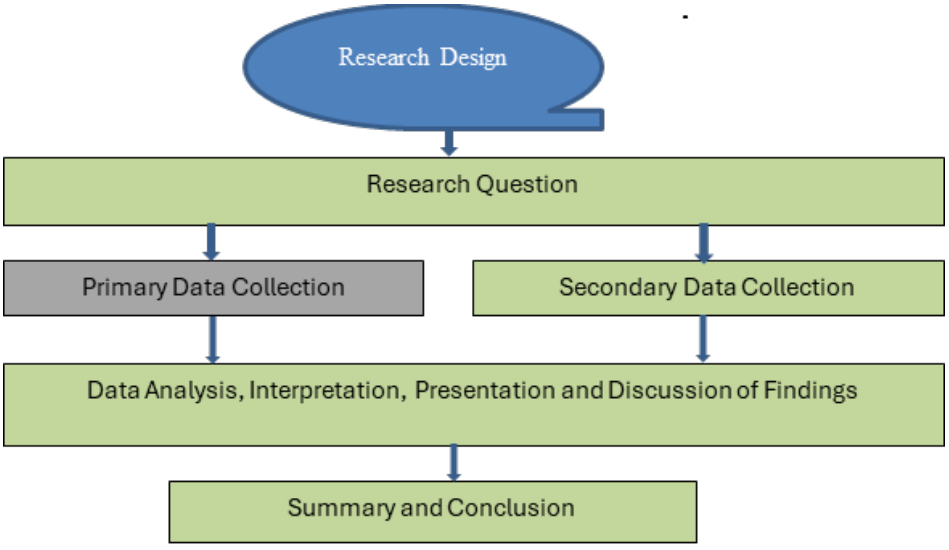
To study the challenges and limitations encountered in evaluating the quality of botanically derived drugs, articles chosen were primarily concerned with the investigation of quality and regulatory challenges, including resource demands and market challenges.

To ensure credibility, all the articles reviewed were checked for the authenticity and accuracy of the information they presented as well as the reputation of the university or organization that published them. This included a check on whether the publications were peer reviewed, and how often how often the article was cited by authors of other articles. The author's credibility was also based on their reputation in the field, qualifications, academic background, i.e., degrees they have obtained, their affiliations to academic and research institutions, and whether they have written other articles on the same topic as well as other associated publications.

Because botanicals are an increasingly growing source of drug discovery and development due to our expanding knowledge about the benefits of their constituent compounds, this review is therefore important in that it enhances our understanding of how best to optimize their cultivation, sourcing, harvesting, and processing techniques at ambient environmental and manufacturing conditions. The study also enhances our understanding of how to maximize quality and quantity of botanical compounds in an efficient and sustainable manner, while also being mindful of the challenges and limitations of quality that are inherent in their development into prescription medication.

The study has shown that botanical plant specifications, material composition, and substance characterization contribute more to the quality challenges in the drug development process, and that there is a lack of coordination between the regulators and the manufactures that is necessary to facilitate the development and approval processes of botanicals into prescription drugs.

Figure 2 - Flowchart of the Research Design



CHAPTER 5

Results and Discussion

5.1 Factors affecting the quality of botanical substances.

Botanical plants possess numerous chemical compounds that vary greatly with seasonal variations and horticultural conditions they are raised and harvested in. These conditions are also heavily influenced by the different climatic regions and geographic areas the plants are also raised and harvested in.

Therefore, in situations where the sourcing of botanicals is not from one place, i.e., where plants were not raised under identical conditions, there will be variations in the quality of the chemical compounds or phytochemicals found in the plants (Zargoosh Z. et al., 2019,).[26] The chemical compounds (phytochemicals), which are also referred to as physiologically active plant substances, are chemicals synthesized by plants through their primary and secondary metabolism, and they are primarily for their own use, i.e., they use them for growth, and for defense against pathogens and predators. Examples of these chemical compounds are flavonoids, phenols, alkaloids, saponins, and glycosides. These compounds or substances are found in plant raw materials that humans harvest and process for use as botanical medicines, foods, and cosmetics while some are further purified and used as derivatives of pharmaceutical drugs. Controlling the conditions that make up their environments to raise and harvest a desirable plant with the right phytochemical compositions necessary for controlling product quality during development can be challenging due to several factors (Pant, P. et al. 2021).[27] To better

understand the factors affecting the quality of botanical substances, the study looked at five research articles which investigated these factors.

The articles are -

- Pant P, Rijal B, Bhattarai S, et al. The influence of environmental conditions on secondary metabolites in medicinal plants: a literature review. *Chem Biodivers*. 2021;18(12):e202100345. doi:10.1002/cbdv.202100345. Accessed March 16, 2025. https://doi.org/10.1002/cbdv.202100345.
- Zargoosh Z, Tabrizi L, Mohammadpour M, et al. Effects of ecological factors on the antioxidant potential and total phenol content of *Scrophularia striata Boiss*. *Sci Rep*. 2019;9(1):16977. doi:10.1038/s41598-019-52605-8. Accessed March 16, 2025. https://www.nature.com/articles/s41598-019-52605-8.
- Chaachouay N, Zidane L. Plant-derived natural products: A source for drug discovery and development. *Drugs Drug Candidates*. 2024;3(1):184-207. doi:10.3390/ddc3010011. Accessed March 16, 2025. https://doi.org/10.3390/ddc3010011.
- National Cancer Institute (NCI). A Story of Discovery: Natural Compound Helps Treat Breast and Ovarian Cancers, https://www.cancer.gov/research/progress/discovery/taxol?
- WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants, 2003 https://iris.who.int/bitstream/handle/10665/42783/9241546271-eng.pdf

The articles examined two major areas of concern that heavily influence botanical substance quality, i.e., external and internal factors.

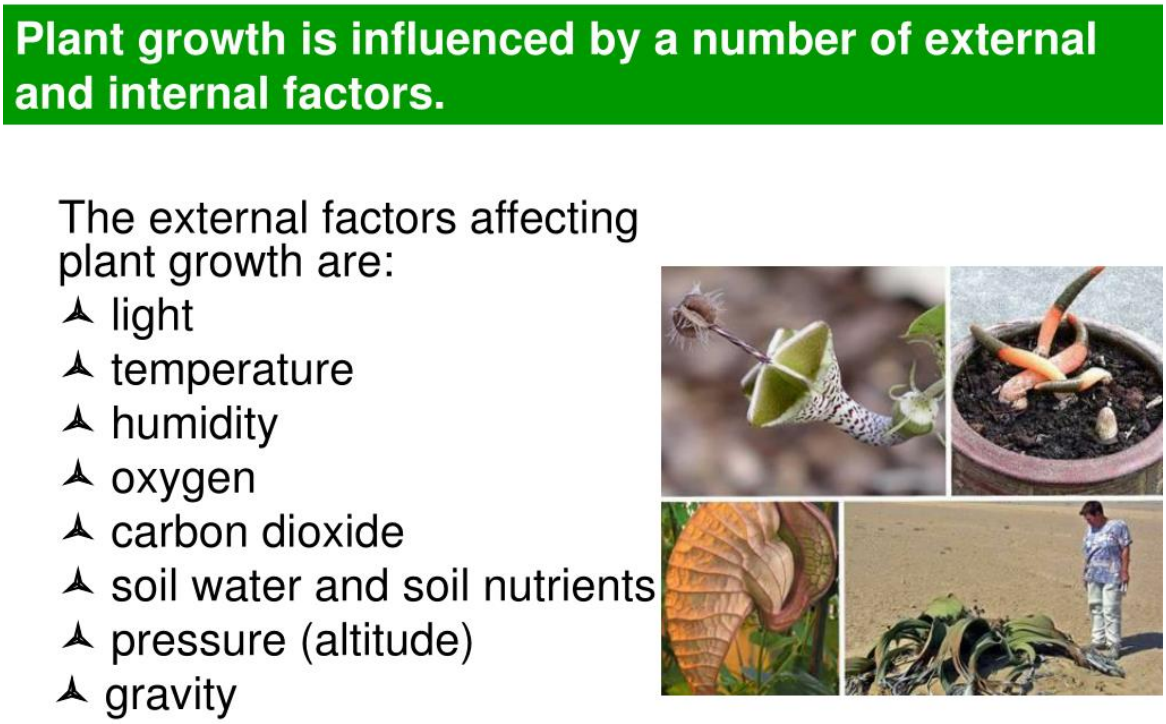
5.1.1 External factors

External factors that affect the quality of botanical substances include climate conditions, soil quality, pests and diseases, harvest time, harvest method, drying conditions, storage conditions, pesticides and herbicides residues, heavy metals, and microbial contamination. All these factors impact the growth of the plants, which in turn affects the quality of the substances (phytochemicals) the plant synthesizes (Zargoosh Z. et al., 2019, American Herbal Products Association, 2021).[26,28] Controlling these factors not only guarantees a consistent product that can be reliably used in research and therapy but also assures safety and product quality. The factors can be grouped into four categories namely growing conditions, harvesting practices, post harvesting handling, and contamination.

i). The environmental factors affecting plant growth (growing conditions)

Environmental factors are a major limiting factor for the survival and growth of medicinal plants. In plants, different medicinal plant species and plant parts (root, stem, leaves, flowers, fruits and seeds) express different secondary metabolites which are sources of natural bioactive chemicals. Therefore, understanding the role played by different environmental factors is critical in assessing their role in plant growth and the quality of metabolites they produce (Pant, P. et al, 2021).[27]

Figure 3 – External factors influencing plant growth



Source - Regulation of plant growth, https://www.slideserve.com/kolya/regulation-of-plant-growth#google_vignette

a). Climate: Temperature, rainfall, sunlight duration, humidity, and altitude.

These environmental factors are the major limiting factors for the survival and growth of medicinal plants, particularly for wild botanical plants. Since botanicals can be raised in different places, i.e., green houses and farms, conditions like temperature, rainfall (water), humidity can be varied or manipulated, thereby affecting the quality and quantity of the substances they produce. For those that grow in the wild, they are entirely dependent on the prevailing environmental conditions, hence, forcing the plants to produce a specified quantity and quality of phytochemicals to counter the environmental

stresses they are facing. A change in temperature, rainfall or sunlight duration greatly affects plant growth by interfering with the metabolic pathways involved in signaling, physiological regulation and defense responses which determine the quality and quantity of phytochemicals produced. It has been proven that plants of the same species grown in different environmental conditions have been found to have different concentrations of a particular phytochemical (Pant, P. et al, 2021).[27]

b). Soil: Nutrient content, pH levels, and soil structure.

Soil influences the growth and development of plants in many ways, and the production of phytochemicals is strongly dependent on the content of the nutrients found in the soil. Poor soil pH levels cause nutritional imbalances resulting in poor uptake of nutrition by the plant. The use of fertilizers in farmed botanicals can influence the quality of the botanicals. These imbalances affect the quality and quantity of the phytochemicals the plant produces. In some plants, e.g., Brassica napus var oleifera Del. (rapeseed), an increase in soil salinity causes a change in the contents of total phenolics, non-flavonoids, tannins and phenolic acids. Also, plants exposed to drought stress accumulate a higher concentration of chemical substances than those cultivated under well-watered conditions (Pant, P. et al, 2021).[27]

When studying the impact of soil and elevation, Zargoosh Z. et al., 2019,[26] illustrated the effects of ecological factors on the antioxidant potential and total phenol content of Scrophularia striata, a plant with various phytochemical components that have a wide range of therapeutic effects including treating infections, skin burns and respiratory illnesses. The plant was studied under different sites and elevations and the effect the interactions of both had on the plant extract yield, antioxidant capacity, and

total phenol content. The study found that there was a significant improvement in extract yield, antioxidant capacity, and total phenol content, confirming that soil, elevation and other environmental factors play a significant role in controlling quality of botanical plants.

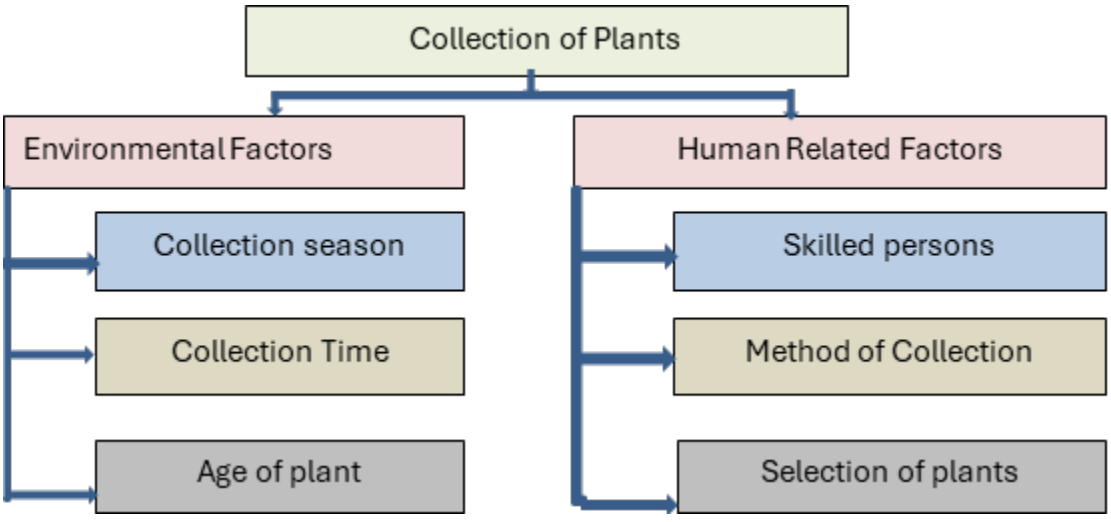
ii). Harvesting Practices

Where medicinal plants are harvested from the wild rather than cultivated, there are several threats to their survival. A specific threat of concern is over-collection to meet rising demand for medicines (Chaachouay, N., Zidane, L., 2024).[25] An example of this being the discovery of paclitaxel (Taxol), a potent anti-cancer compound derived from the bark of the Pacific yew tree (*Taxus brevifolia*). After the National Cancer Institute (NCI)[29] identified paclitaxel's effectiveness against certain cancers in the late 1980s, demand for the compound surged leading to over-harvesting of the wild populations of this species. Harvesting paclitaxel required stripping the bark from mature trees, a process that ultimately killed the trees. This practice was unsustainable and would eventually lead to the decimation of Pacific yew tree populations and other ecological issues (National Cancer Institute (NCI)).[29]

Plant collection or harvesting is the second most important step after cultivation of a botanical plant intended for drug development. To ensure quality, harvesting or collection practices must conform to the basic principles of good agricultural and collection practices as stated by WHO and other international regulatory bodies including American Herbal Products Association (AHPA) that have jurisdiction over areas the botanicals are collected. WHO published guidelines for Good Agricultural and Collection Practices (GACP) for medicinal plants[30] in 2006, a document intended to reduce the

natural variation and inconsistencies found in the collection of medicinal plants. This document provides technical guidance for obtaining medicinal plant materials of good quality for sustainable production of quality herbal products by establishing quality standards from cultivation to collection of medicinal plants and ensures identification and traceability of plants during collection. Also, AHPA, published its guidelines for Good Agricultural and Collection Practices and Good Manufacturing Practices for Botanical Materials (GACP-GMPBM)[30] in May 2021. This document provides guidance to growers, collectors, and processors of botanical crops on how to accurately identify adulterated and contaminated plant materials that may present a public health risk. *"Botanical identity and quality must be assured throughout the growing, harvesting, post-harvest handling, and further processing of botanical materials. Improper or careless practices at any stage may result in material that is misidentified, adulterated, or that fails to meet the necessary specifications".* - (American Herbal Products Association, 2021).[28]

Figure 4 - Factors affecting collection of botanical plants and plant parts



Adapted from: Factors Affecting Collection of Crude Drugs - Solution Parmacy,

https://solutionpharmacy.in/factors-affecting-collection-of-crude-drugs

The amounts of chemical constituents in plants vary throughout the year. Therefore, the timing for harvesting, i.e., the season, is important to maximize the amount of the quality, quantity, number as well as the nature of the active constituents. For example, Shah, B. N. and Seth, A. K, 2010,[5] explain that Rhubarb for example, is collected only in summer seasons because no anthraquinone derivatives would be present in the winter season. They further explain that the age of the plant should be taken into consideration since it not only determines the total amount of active constituents produced in the plants but also the proportions of the constituents of the active mixture (Shah, B. N. and Seth, A. K, 2010).[5]

For specifications and testing requirements, see 5.1.3 Regulatory consideration below.

iii). Post Harvest Handling

Preservation of medicinal botanical plants after harvesting requires a great deal of care if quality is going to be maintained. Several dried plant materials absorb moisture during their storage and therefore susceptible to microbial growth. This has a direct effect on the quality of the drug substances as it can also cause some chemical or enzymatic reactions that can change the nature of the active constituents. A good example is digitalis leaves, wild cherry bark, gentian and ergot which all get moldy due to excessive moisture. Direct sunlight also affects the quality of botanical substances during storage by causing the destruction of active chemical constituents. Temperature and air (oxygen) are also very important factors in the post-harvest preservation of botanical drug substances as they can accelerate several chemical reactions which can then lead to the

degradation of vital constituents (see specifications and testing requirements below under 5.1.3). Also, protection against insects is also important. Insects like worms, mites and nematodes are known to attack botanical plants not stored properly before being shipped to processors (Sagaya, R., 2020).[31]

5.1.2 Internal factors

Internal factors that affect the quality of botanical products are mainly due to biological processes within the plant. These biological processes influence the quality, quantity and chemical composition of the plant's substances like flavonoids, alkaloids, phenols and glycosides. Therefore, to control the quality and quantities of these phytochemicals will depend on several biological factors that include the plant's genetic make-up (plant variety), which is a botanical plant's key to producing certain compounds (secondary compounds) that are not only necessarily for the plant's survival, but necessary for its defense and determining the quality of compounds produced and their therapeutic effect. Other factors include plant hormones or phytohormones (auxins, gibberellins, cytokinins,) these can promote or inhibit plant growth, and also have a direct effect on the quality and quantity of the compounds produced. With favorable environmental conditions (external factors), a fast-growing plant produces more quality compounds compared to a slow growing plant (Zargoosh Z. et al., 2019).[26]

It can therefore be concluded that the production of some botanical plant compounds can be dependent on both internal and external factors.

Since wild botanicals cannot be physically protected from some environmental elements, like drought, pests, and predators, there is not much control that can be done to ensure their quality compared to cultivated botanicals. Because of this, measures to protect these valuable resources must be strengthened. Considering this, the WHO issued strict guidelines for the

collection and preservation of medicinal plants (GACP), but enforcement is light. It is left to individual countries to implement these guidelines. We know very well that most of these wild plants are found in forests of untamed wildlands, and we know quate well that this is for the most part in developing countries. It is also known that trade in wild plants for medicinal purposes is done clandestinely in these places. Village communities in these countries do not know or understand or even care about these laws and guidelines, and traders, who are after big profits do not care too.

Given all this, when such guidelines are issued by the WHO or other international and local regulatory agencies, they should take into consideration the needs of the communities where these resources are found. They should help educate them about the importance of preserving these natural resources rather than leave it to individual countries' governments who might not have total control of their communities. Above all, they must provide incentives and alternatives for them to find better ways to meet their needs. If it can be done for wildlife (fauna), then it can be done for wild plants (flora). We have seen this work very well with wildlife especially in the developing world where most wildlife is found. Examples of these are the enforcement of CITES and adoption of CAMPFIRE (Communal Areas Management Program for Indigenous Resources) and others. For WHO, enforcement guidelines for wild plants should not start and end in Geneva but should end with the encouragement of the countries were the resources are found to better develop mutual, persuasive cooperation with the local communities that live in these habitats and benefit from them. This can only be done by WHO working with individual countries to develop and adopt local solutions for local communities, solutions that people can identify with as indigenous.

5.2 How the quality of botanical substances affects the drug product

The quality of botanical substances is crucial in determining the safety and efficacy of derived drug products. Variations in the composition, purity, and potency of these substances can significantly impact the therapeutic outcomes of herbal medicines. The articles listed below collectively highlight the necessity of rigorous quality control and standardization in the use of botanical substances for drug development.

To investigate this, the following articles where used -

- American Herbal Products Association. Good Agricultural and Collection Practices and Good Manufacturing Practices for Botanical Materials, May 2021. https://www.ahpa.org/files/Document%20Library/AHPA%20Guidance%20Documents/GACGMP%20Guidance/2021_AHPA_GACP_GMP_for_Botanical_Materials.pdf
- Xue, J., et al. Overview on External Contamination Sources in Traditional Chinese Medicines, 2008 https://www.researchgate.net/publication/238473383_Overview_on_External_Contamination_Sources_in_Traditional_Chinese_Medicines
- WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, 2007. https://iris.who.int/bitstream/handle/10665/43510/9789241594448_eng.pdf?
- Sarma, N., Pharmacopeial Standards for the Quality Control of Botanical Dietary Supplements in the United States, 2023 https://www.tandfonline.com/doi/full/10.1080/19390211.2021.1990171?
- Guédon, D. et al. Impurities in Herbal Substances, Herbal Preparations and Herbal Medicinal Products, IV. Heavy (Toxic) Metals, December 2008.

https://www.researchgate.net/publication/288271349_Impurities_in_Herbal_Substances_Herbal_Preparations_and_Herbal_Medicinal_Products_IV_Heavy_Toxic_Metals

5.2.1 Contaminants and Pesticides/Herbicides Residues

Further to what was discussed above under - (i) *The environmental factors affecting plant growth (growing conditions).*

The effects of how contaminants and impurities affect the quality of botanical substances which in turn affect the quality of the drug product are being investigated more with a view to understanding their impact on the botanical products. Major impurities of concern include heavy metals, pesticides, microbial contaminants, and residual solvents.

A considerably large number of botanicals are susceptible to contaminants and residues found within their environments. Heavy Metals such as lead, cadmium, mercury, and arsenic are a result of metallurgic processing of ore, cement plants, uncontrolled discharge of sewage sludge, burning of fossil fuels and waste incineration plants, and lead petrol (Guédon, D. et al).[32] The main threat to human health from these contaminants is lead, cadmium and mercury. They can accumulate to toxic levels if proper cultivation standards and testing are not done. However, the levels to some extent vary depending on the plant species and growing conditions including environmental conditions. For instance, research indicates that contamination with lead and cadmium is subject to broad fluctuations depending on the genetic make-up of some plant species. Examples are cadmium, lead and mercury which have been shown to have significantly higher levels in the roots of a valerian plant grown on sewage sludge-amended soil, than on unamended soil (Guédon, D. et al).[32]

For those that are cultivated, both contaminants and residues mainly result from the agricultural treatments that are introduced during cultivation. A study by Xue et. al.[33] on pesticide

residues found that out of 280 Traditional Chinese Medicines (TCM) studied, 76% of them were contaminated with one or more pesticide residues at levels above the recommended ones. The most common and with the highest levels were pentachloronitrobenzene (PCNB), benzene hexachloride (BHC), and dichlorodiphenyltrichloroethane (DDT). For DDT, although it was officially banned in the US in December 1972, and worldwide in 2004, it is however, still being manufactured (China, India and North Korea), and used in some countries as a pesticide and insecticide. Its residual effects are far reaching, affecting drinking water, soil, plants and animals including birds. Contaminants and residues are among the most serious external factors affecting the quality and purity of botanical substances.

Because these contaminants can potentially be concentrated during extraction processes, it is important for raw materials suppliers to disclose the maximum levels in their raw materials, and for the buyers to also carry out quality checks to see if the materials they are purchasing conform to recommended standards. The GMP for Dietary Supplements issued by the FDA in 2004 (CGMP DS), are not as strict as those of pharmaceutical drugs, and enforcement is minimal.

Botanical products are susceptible to diseases caused by microbial contamination like bacteria, fungi, and pests. The contamination mostly happens during harvesting, processing, and storage. Bacteria and fungi are from naturally occurring microorganisms (microflora) and other aerobic spore-forming bacteria that live in the soil while Escherichia coli or Salmonella spp. contamination may be caused by poor methods of harvesting, cleaning, drying, handling, and storage (American Herbal Products Association, 2021, WHO).[28,30] Microbial contamination can result in spoilage and serious health risk if products are not properly processed. These contaminants and the diseases they cause have a substantial impact on the quality of botanical

products if they are not properly monitored according to rigid standards and testing as well as good manufacturing practices. Also, pesticide contamination is a major issue in cultivated medicinal plants, and it can lead to residual accumulation above recommended levels if it is not monitored during cultivation, see table 1 below for a list of contaminants and specific examples and their possible sources.

Solvents used in the extraction and preparation of herbal products may pose a great danger if they are not properly monitored. They cannot be entirely eliminated, but it is important that they are kept at recommended levels. Recommended guidelines for specific tests and acceptance criteria to ensure that residual solvent levels are within permissible limits are found in the USP and they stipulate tests and assays required, as well as provide analytical methods and acceptance criteria to control contaminants that may be useful for quality assurance. Table 1 below lists examples of solvents contaminants and their possible sources (Sarma, N. et al. 2023)[34]

To address these impurity concerns, comprehensive quality control measures are necessary. This includes adhering to standardized cultivation practices, implementing stringent manufacturing protocols, and conducting thorough testing for contaminants at various production stages. Regulatory bodies provide guidelines to help manufacturers establish appropriate specifications and acceptance criteria, ensuring the safety and efficacy of herbal medicinal products.

Table 1. Contaminants with specific examples and possible sources – source WHO

Contaminants					
General classification	Group	Subgroup	Specific examples	Possible sources	Stage of production at which detectable[a]
Chemical contaminants	Toxic and hazardous materials	Toxic metals and non-metals	Lead, cadmium, mercury, chromium (arsenic, nitrite)	Polluted soil and water, during cultivation/ growth, manufacturing process	1,2,3,4
		Persistent organic pollutants	Dioxin aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex	Polluted air, soil and water, during cultivation/ growth	1,2,3,4
		Radionuclide	Cs-134, Cs-137	Air, soil, water during cultivation/growth	1,2,3,4
		Biological toxins	Mycotoxins	Post-harvest processing, transportation and storage	2,3,4
			Bacterial endotoxins	Post-harvest processing, transportation and storage	1,2,3,4
Biological contaminants	Micro-organisms	Bacteria	*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* species, *Shigella* species, *Escherichia coli*	Soil, post-harvest processing, transportation and storage	1,2,3,4
		Fungi	Yeast, moulds	Post-harvest processing, transportation and storage	1,2,3,4
	Animals	Parasites	Protozoa – amoebae, Helminths – nematoda	Soil, excreta; organic farming/cultivation, manufacturing process	1,3,4
		Insects	Cockroach and its parts	Post-harvest processing, transportation and storage	1,2,4
		Others	Mouse excreta, earthworms, acarus	Post-harvest processing, transportation and storage	1,2,4
Solvents		Organic solvents	Acetone, methanol, ethanol, butanol	Soil and water, during cultivation/growth, manufacturing process	1,2,3,4
Residues					
General classification	Group	Subgroup	Specific examples	Possible sources	Stage of production at which detectable[a]
Agrochemical residues	Pesticides	Insecticides	Carbamate, chlorinated hydrocarbons, organophosphorus	Air, soil, water, during cultivation/growth, post-harvest processing	1,2,3,4
		Herbicides	2,4-D, 2,4,5-T	Air, soil, water, during cultivation/growth, post-harvest processing	1,2,3,4
		Fungicides	Dithiocarbamate	Air, soil, water, during cultivation/growth	1,2,3,4
	Fumigants	Chemical agents	Ethylene oxide, phosphine, methyl bromide, sulfur dioxide	Post-harvest processing	2,3,4
	Disease control agents	Antiviral agents	Thiamethoxam	During cultivation	1,2,3,4
Residual solvents		Organic solvents	Acetone, methanol, ethanol, butanol	Manufacturing process	3,4

[a] Stage of production at which detectable: 1, medicinal plants; 2, herbal materials; 3, herbal preparations; 4, finished herbal products.

5.2.2 Identification and authentication.

It is important that correct botanical substances or plant material are used to ensure that proper active compounds are present in the mixture. According to the AHPA and Kim NC,[28,35] post-harvest activities are amongst the most critical in ensuring that the botanical material meets appropriate quality specifications and testing requirements (for specifications and testing requirements, see 5.2.4 below).

It is therefore important that specifications and quality testing procedures are developed to ensure material authenticity, quality, and proper identification. These procedures are listed under 5.4.3 Table 1 below. The testing methods are essential in controlling the quality of botanical materials because they ensure accurate identification and verification of botanicals, and they also guarantee that the correct plant material is used. This is important because different species of plants or plant parts may have varying therapeutic properties and safety profiles (Lo and Shaw, 2019; Zhang et al., 2022).[36,37]

5.2.3 Standardization challenges (Active ingredient variability).

Regulatory requirements for the quality of dietary supplements, botanicals included, are found in 21 CFR part 111, according to these regulations' "quality" is defined as a way a botanical/herbal product consistently meets the established specifications for identity, purity, strength, and composition and adheres to the limits on contaminants. The specifications for quality consist of a list of tests, analytical procedures, and appropriate acceptance criteria. When tested using a specific analytical procedure, the substances must meet or conform to required specifications or standards designed as acceptance criteria (Sarma, N. et al., 2023).[34]

A very important step of controlling and ensuring quality in botanical product development process is to ensure that effective standardization and identification procedures are

built in the whole process by establishing product specifications. Botanical substances contain complex mixtures of compounds, which present difficulty in standardizing the concentration of the desired active compound. It is therefore necessary to have specified standards and guidelines that can be used to identify, isolate, and purify bioactive compounds. According to Aleksieva and Yordanov, 2018,[38] standardization and identification of botanical substances involves establishing consistent and reliable ways of measuring the levels of active compounds or markers in botanical substances. However, the CGMP for botanical/dietary supplements are not clear on which methods to be used specifically but leaves it to the judgement of quality control personnel to approve the use of those scientifically valid tests that will ensure a product's identity, purity, strength, and composition whether or not such tests are contained in a particular compendium (Sarma, N. et al., 2023).[34]

The aim of standardization is to minimize batch-to-batch variability and ensure that each product meets predetermined quality standards which further determine the quality and safety of the product (Ketai et al., 2000).[39] Therefore, establishing reference standards or reference materials that represent the desired levels of active compounds or biomarkers is important since these standards act as benchmarks for comparison during quality control testing and help ensure consistency across batches (Jin et al., 2018; Xiong et al., 2022).[40,41] Standardization provides a means to monitor and control the quality and efficacy of botanical medication products and is a key component of the whole drug/product development process.

Given that the CGMP for botanical/dietary products are not clear on which methods to use, i.e., leaving that decision to the manufacturers creates a problem with compliance. Without a specific criterion for use as a standard, different companies will use different standards they see fit for the same product being manufactured. Although it is understood that the difficulty in

adopting one testing standard for botanicals arises from the fact that botanicals are multi-complex substances and are also different in many respects within one plant species. Without a standard that is acceptable across the board, it will be difficult to effectively monitor the quality of products being brought to the market.

5.2.4 Specifications and standards

To assure quality and traceability of both the botanical and finished product, cultivators, harvesters, and processors of raw botanical materials should provide sufficient information on how the botanical was cultivated, collected, harvested, stored, and processed if any processing will have taken place prior to reaching the user. This also includes site location and post-harvest processing such as washing, cutting, dehydrating, packaging, storing, and transporting. To facilitate this, appropriate standards and specifications, that offer consistency in ensuring that materials, products, processes and services are fit for their intendent purpose must be developed (The International Organization for Standardization).[42] The USP Public standards has monographs that provide specifications (guidelines) for identity, assay for content (purity), composition of constituents, absence of contaminants, impurities, specific tests, and other requirements including labeling and packaging. These standards are consistent with GMPs for Dietary Supplements. Specifications for raw materials used in manufacturing of botanical drug products should also consider the processing procedures that will be used as some may affect the chemical composition of the active ingredients, stability and physical appearance of the material. Also, some specifications should consider the intended use of the finished drug product (Sam Jennings 2016, American Herbal Products Association, 2021).[43,28] These specifications can also be found in the US Pharmacopeia Compendial Standards (Botanical Dietary Supplements Quality Standards), and the American Herbal Pharmacopoeia. These include:

- Good Agricultural and Collection Practices (GACP)
    - Guidelines for cultivation, harvesting and processing of medicinal plants to maintain their quality and bioactive content.
    - Control over environmental factors such as soil composition, climate, and harvesting time.
- Other specifications that aid traceability of plant materials between cultivator/seller and buyer/processor.
    - Scientific name of botanical plant.
    - Common name.
    - Whether it was cultivated or collected/harvested in the wild.
    - Plant part or plant product collected.
    - Geographical origin - e.g., where relevant, country and province/state
    - Period/season of harvesting.
    - Appropriate information to enable traceability - e.g., information on source, lot number.
    - Signed certification/declaration, where required.
- Good Manufacturing Practices (GMP)
    - Ensures consistent production and quality control in the processing and manufacturing of botanical substances.
    - Covers extraction methods, contamination prevention, and packaging integrity.
- Pharmacopeial Standards
    - United States Pharmacopeia (USP) and others, set specific standards for botanical identity, purity, potency, and composition.

- Includes analytical testing methods such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS).

- Standardization & Chemical Profiling
  - Ensuring consistency in active constituents across different batches through marker compound analysis.
  - Use of reference standards for quantitative assessment of bioactive components.
- Contaminant & Safety Assessments
  - Compliance with limits set by regulatory bodies such as the FDA, WHO and others (regional and local).
  - Testing for heavy metals, pesticides, microbial contamination, and residual solvents.

    Examples include:
    - Heavy metals – cadmium, lead, mercury, and arsenic.
    - Mycotoxins – excreted by-products produced during the growth of certain fungi.
    - Environmental contaminants – these are organic contaminants found in the botanical matter, they include -
      - i. Dioxins, furans, polychlorinated biphenyl (PCBs).
      - ii. Polycyclic aromatic hydrocarbons (PAHs).
      - iii. Radioactivity – this kind of contaminant results from botanicals cultivated or harvested near nuclear disaster zones e.g. Chernobyl and Fukushima.

- Plant metabolites – pyrrolizidine alkaloids (PAs), tropane alkaloids (TAs).
- Residues - pesticide, herbicide and fungicide residues, ethylene oxide, and fumigants, (e.g. phosphine or methyl bromide).

Specifications of acceptable quantitative limits for the levels of contaminants and residues permitted in the materials can be found in the US Pharmacopeia Compendial Standards. See Table 1 above for types of contaminants and their sources.

- Stability & Shelf-Life Testing
  - Determining the degradation profile of botanical substances over time under various storage conditions.
  - Ensuring potency and safety throughout the product's intended shelf life, for examples, ensuring that -
    - The harvested plant material is handled and stored in a way that ensures no degradation.
    - There is no compaction, i.e., no stacking of harvest containers to levels that will result in physical damage as well as temperature build-up and overheating.
    - There is protection from external sources of contamination.
    - There is protection from the elements, i.e., protection from sunlight, rainfall, freezing, etc.

Considering these standards ensures that botanical substances meet regulatory requirements and maintain high quality for pharmaceutical use. But, given that the conformance to the USP-NF standards including cGMPs, is voluntary for dietary/botanical supplements, the

reality of conformance on the botanical drug development aspect using the CGMP for botanical drugs is not so clear, and at best seems confusing or burdensome. Because of this lack of clarity, it is probably one reason why there are so few big companies willing to take on botanical drug research, and the reason why small companies that are more likely to do so are struggling.

5.3 Development considerations and challenges

Botanical drug development faces significant challenges and threats from the substitution or contamination of botanical raw materials, and this compromises drug safety, efficacy, and regulatory compliance. Adulteration comes in many different forms, detecting and preventing it requires advanced analytical techniques. In this section, the articles listed below discuss the prevalence of adulteration in both raw materials and products.

Another issue the articles address is the various techniques used to determine the active chemical constituent of a botanical substance. Unlike synthetic drugs, botanical products often contain multiple bioactive compounds, making it difficult to pinpoint the exact therapeutic agents. The articles used are:

- Gafner, S. et al. Botanical Ingredient Forensics: Detection of Attempts to Deceive Commonly Used Analytical Methods for Authenticating Herbal Dietary and Food Ingredients and Supplements, 2023, https://pmc.ncbi.nlm.nih.gov/articles/PMC9972475/?
- Ichim, M.C., et al. Chemical Authentication of Botanical Ingredients: A Review of Commercial Herbal Products, 2021 https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2021.666850/full?
- Caesar, L. and Cech, N., Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2, 2019.

https://pmc.ncbi.nlm.nih.gov/articles/PMC6820002/

- Kronenberg F., Kennelly E, Phytochemical identity and stability of herbal products: challenges for clinical research, 2013.

  https://pubmed.ncbi.nlm.nih.gov/24120310/

- Shah, B. N. and Seth, A. K. Textbook of Pharmacognosy and Phytochemistry, ELSEVIER, 2010.

  https://pharmabookbank.wordpress.com/wp-content/uploads/2019/03/14.2.pharmacognosy-by-biren-shahavinash-seth-1.pdf

5.3.1 Identification of botanical plant

Proper identification of a botanical plant by collectors is perhaps the most important first step in identifying a pure botanical plant. The next important step is for the processors and developers of the plant's raw material to further identify and assure its quality and purity before it is characterized for constituent quality and quantification.

> *"Research on botanicals involves unique challenges as plant source materials frequently vary in chemical content and may contain unwanted pesticides, heavy metals contaminant plant species, or other adulterants. Ideally, a botanical formulation should be standardized, both chemically and biologically, by a combination of analytical techniques and bioassays".* - (Piersen et al. 2004).[44]

One of the most critical challenges in identifying botanical plants' raw material is being able to identify adulterated and contaminated plant materials. Adulteration is defined as the intentional substitution with another plant species or intentional addition of a foreign substance to increase the weight or potency of the product or to decrease its cost (Shah, B. N. and Seth, A. K, 2010).[5] Adulteration can result from intentional or unintentional practice. Intentional

adulteration is mostly for commercial reasons and is intended for improving profits. This can be due to the scarcity of drug products and the high price prevailing in the market. Intentional adulteration can result from using manufactured substances, substitution using inferior commercial varieties, using one part of the same plant instead of the other. Unintentional adulteration can be attributed to several reasons including confusion in botanical plant vernacular names, lack of knowledge about the botanical plant, similarity in morphology and or aroma, and carelessness in collection. Gafner, S. et al,[45] states that most of the time, adulteration is carried out for financial gain. The article further states that ingredients are intentionally substituted, diluted, or "fortified" with undisclosed lower-cost ingredients.

A study by Ichim, M.C., et al., 2021,[46] found that, out of 2386 commercial herbal products reviewed for adulteration across 37 countries around the world, 73% were found to be authentic, while 27% were adulterated. Given the growing use of botanical/herbal products and interest in botanical drugs, this high figure of adulterated products only shows how serious the issue is, and how important it is to develop appropriate detection techniques to counter this threat. Ichim, M.C., et al., 2021,[46] further states that, many cases of substituted or adulterated plant products sold in most marketplaces had botanical ingredient labels that did not match the chemically identified ingredients. These products also had other accompanying issues of low-quality which further affected the safety and potential efficacy of commercial herbal products. Most of the articles reviewed seem to agree that most of the adulterated cases of botanical products are from products acquired from unregistered market outlets and the internet, where it is difficult to trace the product manufacturer or distributor. While it is not a best possible option for the consumers to be able to differentiate from adulterated and authentic, buying products made with certified organic ingredients may offer some assurances regarding traceability, including

origin, cultivation methods and manufacturing practices in leu of formal regulations being introduced.

Because of this, it has been found that some adverse events reported from the use of botanical plants products are not due to the intended botanical, but rather due to the presence of an unintended botanical - "the adulterant" (Shah, B. N. and Seth, A. K, 2010).[5] Without proper identification methods and effective standardization procedures, quality challenges due to adulteration and contamination will be very difficult to manage.

Detecting adulteration has since become a complex exercise as some adulterants are disguised in a way to mimic the visual aspects and chemical composition of the labeled botanical ingredient to deceive the analytical methods that are used for authentication. Botanical ingredients most targeted for adulteration include the essential oils of lavender (*Lavandula angustifolia*, Lamiaceae), rose (*Rosa damascena*, Rosaceae), sandalwood (*Santalum album*, Santalaceae), and tea tree (*Melaleuca alternifolia.* Myrtaceae), (Gafner, S. et al).[45]

There are several ways that are used to detect adulterants, but the adulterators, who are aware of the testing techniques continue to device ways to beat the system. Depending on the plant substance being tested, i.e. fresh whole plant, powdered, liquid or dry plant parts, the techniques vary, but the most used is macroscopic identification, which relies on the examination of specific taxonomic features in a plant and comparison of these features with other species. Others include organoleptic evaluation, which relies on taste, look, feel, and smell, of an herbal ingredient, gas chromatography for essential oils, botanical microscopy for assessment of characteristic tissues in whole, cut, or powdered plant materials, Mass Spectrometry, Nuclear Magnetic Resonance Spectroscopy, High-Performance Liquid Chromatography and Ultra-High-Performance Liquid Chromatography, Genetic testing, and UV/Vis Spectrophotometry.

Being able to detect adulterants in botanicals helps improve product quality. According to Kronenberg and Kennelly, 2013,[47] one way to improve product quality which also assures product potency of active components is by using effective standardization procedures. Standardization minimizes batch-to-batch variability and ensures reproducibility. Therefore, without official standards, botanical drug manufactures may select to use one or more compounds of their choice as their standard against which to measure the effectiveness of their product, while another manufacturer might use a different compound for the same product. Kronenberg and Kennelly, 2013[47] further state that in a study of more than 300 botanical clinical trials registered on the clinical trials registration website, clinicaltrials.gov, in the U.S., several similar botanical research studies for the same indication had significantly different results that could only be due to the inherent variability of the products under test, that is, ruling out other issues like product contamination, adulteration, and processing errors. This variability made the interpretation of data to be different and can partly be due to the lack of standardization, which might have resulted in one manufacturer using a standard of their choice different from the other manufacturers, thereby affecting the interpretation of the results.

5.3.2 Determination of active chemical constituent

A single botanical plant, like many other plants, contains multiple compounds. Determining how many and what types of compounds a plant has is a very challenging process. The determination of the compounds depends on the type of plant, and the part of the plant being analyzed, i.e., root, stem, bark, or leaves. The analytic technique used in the process also depends on the plant material used. The most common is thin–layer chromatography, which is a general technique that simply determines how many and what kind of compounds are in a plant material. For a more specific determination of bioactive (active chemical) constituents, non-

chromatographic techniques such as Fourier Transform Infrared Spectroscopy (FTIR), which is a form of phytochemical screening assay, can be used. FTIR identifies and characterizes a plant's functional groups, thereby allowing for the identification of individual compounds present. Other forms of analyzing and identifying the bioactive components of a plant are mass spectroscopy (MS), which determines the molecular weight and fragmentation pattern of each compound, and by comparison with a known sample from a database, the bioactive component can be identified. Nuclear Magnetic Resonance (NMR) spectroscopy is also used for a detailed structural analysis of the active compound. These analytic techniques are described further in the next section under Methods used to evaluate botanical raw materials, 5.4.

Because of the potential that plant materials may be misidentified, contaminated, adulterated or be of substandard, it becomes important to develop reliable analytical methods that can precisely discriminate the content of active ingredients in a particular sample of a plant material. A botanical plant extract may contain hundreds of constituent components or phytochemicals, each capable of acting on a different physiological pathway as compared to a single molecule drug. In such situations, developing standardized analytical methods for testing plant materials and products, ability to validate analytical methods, ability to assess purity and potency, ability to assure shelf life and stability, and ability to find validated biomarkers may help in the evaluation and understanding of their physiological benefits (Shah, B. N. and Seth, A. K, 2010).[5]

To evaluate these chemical compounds separately, analytical techniques that are highly sensitive are required. This will allow for the identification and evaluation of the active components independent of the others, a step that is necessary to understand the pharmacological actions of individual compounds. Without separating them and studying them independent of

each other, it will be very difficult to know which compound in the mixture is responsible for a particular pharmacological action the plant might have. This is better seen or observed in the analysis of St John's wort *(Hypericum Perforatum)*, a very popular botanical/herbal promoted for several physiological conditions like depression, menopausal symptoms and anxiety. It contains several phytochemicals including flavonoids, phenolic acids, tannins, and phloroglucinols. Mass spectrometry (MS) has been used to identify most of the constituents, but it remains unlikely that all its chemical components have been fully characterized. Ongoing research is still trying to uncover the new constituents based on the complex compounds that are also believed to be undiscovered. Other analytical techniques like liquid chromatography coupled with MS have been able to identify and quantify some of the many bioactive compounds of St John' wort but not all. Despite these advancements, Chandrasekera, D. H. et al.[48] conclude that the complete chemical profile of *H. perforatum* remains incompletely defined. Also noted is that other factors like geographical location, environmental conditions, and extraction methods can also influence the plant's chemical composition, further giving reasons that suggest that additional compounds may yet be discovered.

Given that some compounds of the St John' wort and some and their constituents have not been identified, it is therefore impossible to point to one compound as responsible for the therapeutic effects of St John's wort on a disease or health condition. Caesar, L. and Chech, N. 2019,[49] in the article - "Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2, 2019", state that, in the case of the St John's wort, it is possible that some compounds are inert, but play a part in increasing the solubility, absorption, distribution and metabolism of the active ones. The reasoning behind Caesar, L. and Chech, N. 2019's[49] research article can be explained in 2 ways, 1). Because the individual compounds of St John's wort cannot be

separated and studied independently, it is best to assume that there is a possibility synergy might be at play here, whatever the effects of the most active compound, the others are helping or adding up theirs for a much higher combined effect, and 2). No one compound elicits a meaningful response on their own, but they enhance each other to elicit a response by either increasing solubility, absorption, distribution and metabolism for each one of them to work, an example of potentiation. It is a clear example of the need for specialized techniques to be developed that can effectively separate these compounds so they can be studied individually.

Another example is catechins found in several teas, they are known to benefit cardiovascular health, weight loss, and neuroprotection. The difficulty of isolating and evaluating each of the hundreds of chemical compounds independently leads most researchers to believe that they all contribute to the overall effect of the botanical by increasing the bioavailability of the compounds responsible for the desired pharmacological action in a synergistic way. This means that the beneficial effects of catechins cannot be attributed to a single compound unless otherwise proven (Kronenberg F., Kennelly E, 2013).[47]

These examples show that there is need for advanced analytical techniques to be developed for accurate characterization of the plant materials by identifying their chemical composition and physical properties to enable proper assessment of their pharmacological activities. For those that advance to clinical trials, understanding the profiles of individual active compounds will make therapeutic evaluation of the drug easier as the individual drug components will have been evaluated separately. Such measures can help avoid adverse events and unwanted drug interactions that are associated with some untested chemical compounds in botanical products and might lead to the development of effective new treatments.

5.4 Methods used to evaluate botanical raw materials

Ensuring the quality of botanical raw materials is essential for the development of safe and effective botanical products. Despite the inherent natural variability of botanicals, maintaining consistency across batches requires the use of high-quality raw materials, adherence to Good Manufacturing Practices (GMPs), and compliance with regulatory guidelines throughout the production process. High-quality botanical raw materials not only serve as the foundation for effective botanical products but also ensure that plant substances meet rigorous standards for safety, potency, and efficacy. These materials must contain a high concentration of bioactive compounds while remaining free from contaminants or adulterants.

Several methods and standards are employed to assess and verify the quality of botanical raw materials. One key resource is the United States Pharmacopeia for Dietary Supplements Compendium (US-DSC), introduced in 2009, which provides manufacturers and regulators with benchmarks and quality assessment guidelines. Additionally, the WHO Guidelines on Good Agricultural and Collection Practices for Medicinal Plants (GACP)[30] outline critical aspects of raw material management, including botanical identification, geographic sourcing, and harvesting practices, which are essential for ensuring consistency and traceability in botanical products.

According to Liang et al. (2023),[50] quality assessment begins with a comprehensive evaluation of raw materials, ensuring they meet required specifications before entering production. Benedetti et al. (2019)[51] further emphasizes that effective evaluation methods should involve identity verification, authentication, purity testing, and overall quality assessment, utilizing a combination of advanced analytical techniques to safeguard the integrity and efficacy of botanical-based medicinal products.

The articles listed below detail the major screening techniques that are used in industry today.

- Upton, R., et al. Botanical ingredient identification and quality assessment: strengths and limitations of analytical techniques, 2020 https://link.springer.com/article/10.1007/s11101-019-09625-z?
- Pratiwi, R. et al. Recent Analytical Method for Detection of Chemical Adulterants in Herbal Medicine, 2021 https://www.mdpi.com/1420-3049/26/21/6606?
- Salam, U. et al. (2023) Plant Metabolomics: An Overview of the Role of Primary and Secondary Metabolites against Different Environmental Stress Factors. https://pmc.ncbi.nlm.nih.gov/articles/PMC10051737/
- Wang, H. et al. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices, 2023 https://pmc.ncbi.nlm.nih.gov/articles/PMC10561302/?
- Simmler, C. et al. (2016). Botanical Integrity: Part 2 Traditional and Modern Analytical Approaches. https://www.researchgate.net/publication/303123494_Botanical_Integrity_Part_2_Traditional_and_Modern_Analytical_Approaches

It is important to ensure that raw botanical materials are screened by specialized techniques to ensure their authenticity, quality, and safety. Various methods are employed to assess these attributes, ranging from traditional techniques to advanced analytical technologies. However, some screening techniques and methods listed below are only for reference, as their descriptions are too technical and beyond the scope of this study.

5.4.1 Physical evaluation methods

Macroscopic analysis technique allows for immediate detection of foreign matter, filth, and potential adulterants. It provides valuable insights for determining if the quality of the material is acceptable for use in medicinal products. Examination is usually visual or by a naked eye or with a magnifying glass. It looks at basic morphological features and attests the material's authenticity of observable features by comparing them to a botanical reference material or against an authoritative description to determine if all observable features conform and that the sample lacks non-conforming features (Upton, R. et al., 2020).[52] See also Table 2 below.

> *"Visual inspection provides the simplest and quickest means by which to establish identity, purity and, possibly, quality. If a sample is found to be significantly different, in terms of color, consistency, odor or taste, from the specifications, it is considered as not fulfilling in the requirements.*" (WHO 1998).[53]

Another valuable technique of evaluating plant material is Microscopy. It is generally reliable, quicker, and less expensive than chemical and genetic tests. The technique is considered valuable in detecting admixtures of a different plant or different parts of the same plant where structural differences between root and leaf tissues (in powder form) are readily discernable (Upton, R. et al., 2020).[52] See also Table 2 below

5.4.2 Chromatographic Techniques

The most used chemical techniques in the identification and assessment of medicinal plant ingredients are thin layer chromatography and high-performance thin layer chromatography (TLC/HPTLC). According to (Upton, R. et al., 2020),[52] "both provide characteristic qualitative and quantitative patterns of constituents". TLC works very well for qualitative testing, while HPLC allows for both qualitative and quantitative analysis of individual constituents. The only

disadvantage of TLC is poor reproducibility and low sensitivity (Pratiwi, R. et al).[54] Other chromatographic techniques are described in Table 2 below.

5.4.3 Spectroscopic Methods

Spectroscopic techniques allow for the quantitation of single or multiple compounds that share similarities in their UV absorbance. Spectroscopic techniques offer good selectivity and sensitivity, and they also provide detailed molecular structure of compounds. Mass Spectrometry determines the mass-to-charge ratio of ions to identify and quantify molecules. It is often used together with other chromatographic techniques to enhance the analysis of complex botanical matrices, for example, GC-MS, LC-MS. Nuclear Magnetic Resonance (NMR) Spectroscopy provides detailed information about the molecular structure of compounds, which helps in the identification of specific phytochemicals within botanical samples, (Pratiwi, R. et al, Upton, R. et al., 2020).[54,52] Their disadvantage is that they are highly costly.

5.4.4 DNA – Based Techniques

DNA Barcoding is a testing used to authenticate and identify herbal/botanical species by sequencing a specific region of the herb's DNA, which is unique to each species. It then compares the obtained DNA sequence with a reference database thereby determining the genetic identity of the plant species. DNA testing provides a highly reliable method for herb identification and is particularly useful when the herbs are in processed or powdered forms (Wang, H. et al., 2023).[55] It is especially useful for detecting adulteration or substitution in botanical materials. In their analysis of the DNA method, Simmler, C. et al. (2016)[56] further states that - "it is the most unambiguous identification method of botanicals, and it represents the first critical step for the determination of botanical integrity".

"DNA authentication is increasingly considered to be a helpful tool accompanying traditional macro- and microscopic examinations to identify botanicals accurately" - Simmler, C. et al. (2016).[56]

There are several other raw material evaluation methods that quantify the concentration of main active compounds in the extracts, evaluate functional groups to assess their chemical composition, and some that detect the presence of heavy metals which ensure that they are within safe limits. It is important to note that the choice of a method to use for raw material evaluation will depend on the plant species, intended use, and regulatory requirements. However, it can be argued that, by using a combination of these raw material evaluation methods, it is possible manufacturers can ensure the quality, authenticity, and safety of the botanical ingredients used in their products. However, most of these evaluation methods have not yet been fully developed for large scale industrial use, and for those that have been developed, they are underutilized.

Table 2. - Methods used to evaluate botanical raw materials

Method	Use
Physical	
Macroscopic Analysis	This involves the visual inspection of raw materials to assess morphological features such as color, size, shape, and texture. It serves as the first line of identification, allowing for the detection of obvious adulterations or contaminants.
Microscopy Analysis	Microscopic Analysis: This analysis method utilizes light microscopy, and it examines cellular structures and plant tissue organization which is too minute for visual analysis. Microscopic evaluation is essential when dealing with powdered or processed materials where macroscopic features are not discernible.
Organoleptic	It involves the examination of raw materials using human senses of sight, smell, taste, and touch. This process helps identify any inconsistencies in appearance, odor, and taste.
Moisture content	It measures the amount of moisture present in plant material. Controlling moisture prevents microbial contamination and fungal growth which are encouraged by the presents of moisture.
Ash content	It is the amount of minerals in a plant and represents the plant's native mineral content. It is determined by heating the plant's

	material to vaporize organic compounds and evaluating the ash left behind.
Particle size	Multiple techniques used, proper control of particle size ensures product consistency, efficacy, and quality.
Chemical	
DNA barcoding	is a molecular technique used to authenticate and identify herbal species, it involves sequencing the botanical's DNA, and compares it to a DNA database for authenticity, ensuring the use of the correct species and detecting any potential adulteration or substitution.
Chromatographic Techniques	
Thin Layer Chromatography (TLC)	This technique separates compounds based on their movement on a stationary phase under the influence of a solvent. It provides a chemical fingerprint that can be compared to a reference standard, aiding in the identification and assessment of purity.
High Performance Thin Layer Chromatography (HPLTC)	This is an advanced form of TLC, HPTLC offers higher resolution and sensitivity. It is widely used for the authentication of botanical materials by comparing the chromatographic profiles of samples against authenticated references.
High-Performance Liquid Chromatography (HPLC)	This is used to separate, identify, and quantify individual components within a mixture. It is particularly useful for analyzing non-volatile and thermally unstable compounds in botanicals
Gas Chromatography (GC)	It is mainly used for the analysis of volatile compounds, such as essential oils. When coupled with detectors like mass spectrometry (GC-MS), it provides detailed information on the molecular composition of samples.
Chromatographic Fingerprinting	It is used for identifying specific marker compounds or active ingredients, ensures the desired potency and therapeutic efficacy of herbal medication products.
Spectroscopic Methods	
Spectroscopy techniques (UV-Vis, FTIR)	These screening techniques are used for identifying and characterizing a plant's bioactive markers or functional groups, they allow for specific identification of compounds.
Mass Spectrometry (MS)	This technique determines the mass-to-charge ratio of ions to identify and quantify molecules. It is often used together with other chromatographic techniques to enhance the analysis of complex botanical matrices, for example, GC-MS, LC-MS.
Nuclear Magnetic Resonance (NMR) spectroscopy	It provides detailed information about the molecular structure of compounds, aiding in the identification of specific phytochemicals within botanical samples.
DNA – Based Techniques	
Polymerase Chain Reaction (PCR):	This technique amplifies specific DNA sequences, enabling the detection of plant species within a sample.

Biological	
Morphology	It is used with taxonomic keys to distinguish between different plant species and varieties, identifying shared structural characteristics both internal and external, i.e., shape, leaves, stems, roots, and other plant parts.
Invitro Bioassays	Biological characterization and comparative screening.

5.5 Extraction and Isolation of Botanical Compounds

Extraction of a botanical raw material is a process of separating the soluble raw material of a plant from its insoluble residue. This process can be accomplished by use of a solvent and is based on the physical nature of the material to be separated. The physical state of the extract has to be taken into consideration as well. Each soluble plant material contains several chemical elements in varying proportions, and to separate them, a suitable method that maximizes purity, quality, efficiency, and does not destroy or interfere with the materials must be chosen. There are several methods to choose from, depending on the nature of the plant material, i.e., are the compounds being extracted from a freshly harvested material extract or a dry powdered material, or are the compounds volatile, oily, or soluble in water?

The process involves the transfer of soluble material from a solid to a fluid to form a liquid mixture. This transfer process is also referred to as diffusion. The process happens until equilibrium is reached, whereby the concentration of the contents inside the cells of the plant material equals the concentration of the solvent material outside the cell. To speed up the transfer process, agitation and varying the temperature can increase the concentration gradient thereby increasing the extraction process. Other factors that can be employed to improve extraction range from size reduction of particles to the choice of solvent and method of extraction.

The articles listed below discuss the methods and techniques used in the extraction processes of plant substances from the raw plant materials, and how the chemical compounds are isolated, see figure 1 in chapter 3 above.

- Ponphaiboon, J. et al. Advances in Natural Product Extraction Techniques, Electrospun Fiber Fabrication, and the Integration of Experimental Design: A Comprehensive Review, 2023
  https://pmc.ncbi.nlm.nih.gov/articles/PMC10343563/#sec2-molecules-28-05163
- Sasidharan, S. et al. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts, 2010
  https://pmc.ncbi.nlm.nih.gov/articles/PMC3218439/#abstract1
- Altemimi, A. et al. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts, 2017
  https://pmc.ncbi.nlm.nih.gov/articles/PMC5750618/#sec2-plants-06-00042
- Popova, M. et al. Contemporary methods for the extraction and isolation of natural products, 2023
  https://pmc.ncbi.nlm.nih.gov/articles/PMC10314546/
- Nguyen, T.L. et al. Innovative extraction technologies of bioactive compounds from plant by-products for textile colorants and antimicrobial agents. 2024
  https://link.springer.com/article/10.1007/s13399-023-04726-4

5.5.1 Extraction Methods

Extraction methods are essential for obtaining active compounds from botanical plants in botanical medicine production. There are two categories of extraction techniques, the conventional and non-conventional (modern technique). In the conventional group are

techniques like Soxhlet, maceration, percolation, decoction, and hydro-distillation are the most used and they are generally used in small research facilities. They have disadvantages that seem to make them unpopular. These include lengthy extraction times, high costs, low extraction selectivity, poor extraction efficiency, high solvent consumption, and bioactive compound degradation due to prolonged exposure to high extraction temperatures (Ponphaiboon, J. et al, 2023).[57] However, their counter parts, i.e., the non-conventional group, which includes techniques like supercritical fluid extraction (SFE), microwave assisted extraction (MAE), and ultrasound assisted extraction (UAE), have a several of advantages including increased separation efficiency, reduced the use of raw materials, solvents, and energy, and have minimal environmental impact. (Ponphaiboon, J. et al, 2023).[57] Optimizing these methods is key as it ensures the efficient extraction of bioactive compounds from the botanicals, and this step is important as it directly impacts the potency and efficacy of the botanical products.

It is important to select the appropriate extraction technique which must take into consideration factors such as the nature of the botanical, targeted bioactive compounds, desired product characteristics, and manufacturing scale. (Ponphaiboon, J. et al, 2023)[57] states that "the quality and quantity of bioactive constituents found in plant materials are largely dependent on the selection of an appropriate extraction technique". These will also depend on other factors that will influence the extraction process like solvent selection, solvent-to-herb ratio, temperature, time, and pH. According to (Altemimi, A. et al., 2017),[58] the choice of solvent (e.g., water, ethanol, methanol) is crucial, as different solvents extract different compounds based on the polarity, safety, and regulatory considerations. Also, controlling temperature and extraction time prevents degradation of heat-sensitive compounds, while pre-treatment methods like grinding,

milling, and drying improve extraction efficiency by breaking down plant structures to create an increased surface area for the extracting solvent to work on.

Common solvents used include water, ethanol, and methanol. It is important to control the extraction parameters such as temperature and time to maximize the extraction of bioactive compounds while maintaining their stability and preventing the loss of heat-sensitive compounds or the degradation of thermally unstable components.

5.5.2 Conventional Extraction methods

i). Maceration

This technique uses a method of solid–liquid extraction. It recovers bioactive compounds from plant materials by using solvents with or without heat and agitation or shaking to improve mass transfer and the solubility of compounds. In this technique, a solvent is added to a closed vessel containing the powdered solid plant matrix. It is allowed to sit for hours or days depending on the compounds being extracted. This allows for the solvent to diffuse through the plant cell wall in order to solubilize the chemical constituents present in those materials. The process occurs through molecular diffusion (Ponphaiboon, J. et al, 2023).[57]

Figure 5 – Maceration plant extraction

Source: Maceration Plant Extraction Explained: Unlocking the Potential, *https://pure5extraction.com/maceration-plant-extraction/*

ii). Percolation

Percolation is a continuous flow of a solvent through a bed of plant material. Percolation is the most common technique for extracting active plant ingredients to make fluid extracts or tinctures. The process usually involves powdered plant material or crushed layered plant material that forms a bed through which the solvent flows through to form an extract. The liquid extract is clarified by filtration or by standing and then decanted. This process usually yields products of greater concentration and better quality than the maceration process (Ponphaiboon, J. et al, 2023).[57]

iii). Decoction

A decoction process is a water-based extraction method which is used to extract water-soluble compounds from botanical plant materials. In this procedure, the plant material, which is usually fibrous plant parts, barks and roots, are crushed or reduced to

small fragments or powder. Doing this helps increase dissolution as more plant material surface area is made available to the liquid. The amount of water used in this method is dependent on the plant material's hardness (Ponphaiboon, J. et al, 2023).[57]

5.5.3 Non-Conventional Extraction Methods

i). Supercritical fluid extraction (SFE)

Supercritical fluid extraction uses gases, for example carbon dioxide, under high pressure and very low temperatures to allow it to flow freely like a liquid in the process of selectively extracting desired components. The advantages of using supercritical fluid extraction, like in the case of carbon dioxide, is that it is sterile and bacteriostatic. It is also noncombustible and nonexplosive. Carbon dioxide is harmless to the environment and no waste products are generated during the process, lastly, it is readily available in large quantities. Supercritical fluid extraction has many applications in botanical drug processing. Its best-known use is in the extraction and isolation of the active constituents from extracts. This process is also used in the decaffeination of coffee and extraction of pyrethrins. According to Nguyen, T.L. et al., 2024,[59] SFE technology has yet to be applied fully in the industry and is not used on a large scale because of the high cost of the equipment, huge technical investments, and difficulty controlling parameters.

ii). Ultrasound-Assisted Extraction Method (UAE)

Ultrasound-assisted extraction method is among the recently developed extraction techniques for botanical products that are more energy efficient and resulting in shorter extraction times compared to conventional methods (Popova, M. et al., 2023).[60]

The ultrasound-assisted extraction method uses acoustic energy or sound waves that exceed the human hearing threshold of 20 kHz. For this extraction process, raw

botanical material is subjected to ultrasonication treatments ranging from between 20 to 100 kHz and 10 to 1000 W/cm^2 in power density. The generated ultrasonic waves travel through the plant material based on their mechanical and physical characteristics, i.e., its composition and structure. Because of the waves travelling through, molecules from the plant material are displaced into the liquid. Extraction of plant compounds using ultrasound has grown during recent years due to its role in reducing the amount of solvent and energy used (Altemimi, A. et al., 2017).[58]

There are, however, various other methods used in the separation of soluble plant material from insoluble residue, these include infusion and microwave-assisted extraction. The efficiency and level of purity attained by these methods is dependent on the compounds to be extracted as they are affected by the substances' polarity, solubility, choice of solvent, and plant material consistency.

5.5.4 Isolation of the active chemical constituents

Extraction of a botanical plant material of interest is usually followed by a process of isolating the active constituents from the extract. The processes involved in isolation have led to the discovery of many unknown compounds contained in a plant extract. The isolation process, just like the extraction process, largely depends on the nature of the active constituents of the material to be separated (Sasidharan, S. et al., 2010).[61] The isolation process separates the known compounds from the unknown compounds for further identification and characterization. Techniques to identify and characterize these compounds have been enhanced by advances in various fields, particularly chromatography. Most of these separation techniques are still not yet fully developed or scaled up for large scale industrial use, where large volumes of plant extracts need to be processed for commercial drug manufacture. To increase the purity of the target

molecules, impurities like sugars, organic acids and proteins should be removed from the plant extract. Purification is plant extract can be time consuming and requires a lot of patience (Nguyen, T.L. et al., 2024).[59]

5.5.5 Methods of isolation:

i). Fractional Crystallization

Crystallization depends on the differences of the compounds in a mixture forming crystal on freezing at their super-saturation points. Many plant compounds dissolved in a suitable solvent tend to form crystals within the mixture at different rates, thus enabling them to be separated. Individual identification and characterization by other means can then follow (Al Gfri, S, 2020).[62]

ii). Distillation

Hydro-distillation (HD) and steam distillation (SD) are among the commonly used extraction methods for volatile oils. The mixtures are separated as they steam at different temperatures. Both methods of extraction have a disadvantage in that they can affect the composition and quality of some extracted oils (Bucar, F., et al, 2013)[63]

iii). Sublimation

Although its use is limited because of the nature of natural plants products or materials that can sublime, it is still used in separating solid plant materials that can escape into a gaseous state directly without turning into liquid upon heating. On cooling, they form crystals, which can then be collected for further analysis. Examples of botanical products obtained this way are camphor from chips of wood of Cinnamomum camphor, and the isolation of caffeine from tea (Shah, B. N. and Seth, A. K 2010).[5]

iv). Chromatography

Chromatography has a wide range of uses in separating and analyzing botanical compounds. Several kinds of chromatographic techniques employ the use of a stationary phase and a mobile phase, which separates the components of the material as they pass through based on their affinity to the stationary phase. The component's affinity to the stationary phase determines the time it spends passing through the column containing the stationary phase, this time is often referred to as the retention time. Because different components of a botanical plant extract have different retention times, it is on this basis that they are able to be separated into individual components. Further analysis and characterization can then follow to determine their chemical structures and potential pharmacological activities.

Column chromatography has remained the conventional method for separating impurities, purifying polyphenols and metabolite extraction from a variety of plant samples (Nguyen, T.L. et al., 2024).[59]

High performance liquid chromatography (HPLC) is a much powerful method of separating botanical compounds from crude extracts in a biological assay in order to be able to characterize the active entity. Both chromatography methods are mostly used in small facilities, and scaling up to large industrial settings for high volumes seems to be a challenge (Sasidharan, S. et al., 2010).[61]

5.6 Challenges and limitations

The botanical drug industry faces significant resource demands, market challenges, regulatory hurdles, and quality concerns. High research and development costs, complex supply chain management, and difficulties in standardizing plant-based ingredients create financial and

logistical burdens. Market competition from pharmaceuticals, dietary supplements, and herbal products, coupled with intellectual property challenges, makes commercialization less attractive. Regulatory uncertainties, strict FDA guidelines, and complex clinical validation requirements further complicate approval processes. Ensuring batch-to-batch consistency, compliance with Good Manufacturing Practices (GMPs), and preventing contaminants and adulteration add to the quality control challenges. Overcoming these barriers is crucial for the industry's growth and acceptance. The following articles discuss these challenges and limitations.

- Sagaya R. *Cultivation, collection and propagation of medicinal plant*. 2020. Accessed March 16, 2025. https://www.kngac.ac.in/elearning-portal/ec/admin/contents/2_18KP3BELB3_2020111801372148.pdf.
- Scotchmer, S. Intellectual Property—When Is It the Best Incentive Mechanism for S&T Data and Information? The Role of Scientific and Technical Data and Information in the Public Domain: Proceedings of a Symposium. https://www.ncbi.nlm.nih.gov/books/NBK221851/
- Cardellina, J.H. Challenges and Opportunities Confronting the Botanical Dietary Supplement Industry, 2002 https://www.researchgate.net/publication/11240001_Challenges_and_Opportunities_Confronting_the_Botanical_Dietary_Supplement_Industry
- Sissi Wachtel-Galor and Iris F. F. Benzie, (2003). An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs. The Role of Scientific and Technical Data and Information in the Public Domain: Proceedings of a Symposium. https://www.ncbi.nlm.nih.gov/books/NBK221851/

- Shipkowski, K.A., et al. Naturally Complex: Perspectives and Challenges Associated with Botanical Dietary Supplement Safety Assessment, 2018 https://pmc.ncbi.nlm.nih.gov/articles/PMC6087675/

5.6.1 Quality and Regulatory challenges

Botanicals by nature have multiple active compounds which are believed to offer a therapeutic advantage over synthetic small molecule drugs. However, there is a challenge of separating and identifying these compounds in order to be able to characterize and evaluate them. Upon evaluation of the active constituents, they are further studied through guided studies in animals before being tried on humans through controlled clinical studies. Because all these activities are critical to produce a quality drug product at the end, there are strict regulations to be followed. The FDA does not have separate criteria or requirements for botanicals to prove their safety and efficacy for regulatory purposes as prescription drugs. It mandates the manufacturers to fulfill the same clinical trial requirements specifically designed for single entity drugs using the same GMPs, meaning that there are no specific criteria that are designed to deal with the complex plant derived mixtures associated with botanicals (Botanical Drug Development Guidance for Industry, 2016.)[7]

GMPs are designed to provide a quality, safe and effective finished drug. However, with botanicals, sources of variability in finished products does exist and can range from differences in composition between batches of raw materials to differences in processing and manufacturing methods (Shipkowski, K.A., et al., 2018).[11] The composition of individual batches of botanical products can vary significantly due to multiple factors, including the geographical origin of the plant material, such as altitude, climate conditions, and the timing and growth stage at harvest of the botanical. Shipkowski, K.A., et al., 2018[11] Adds that the manufacturing processes and

proprietary techniques differ among companies, leading to minimal batch-to-batch variation within a single manufacturer but substantial variability between products from different companies, even when they appear identical. Given this inherent variability, chemical evaluation has become an essential complement to botanical manufacture, study, and regulation of botanical products. Because of this, most manufacturers have adopted standardization, a process that measures and adjusts key constituent levels and their ratios, ensuring greater batch-to-batch consistency and improved product quality (Shipkowski, K.A., et al., 2018).[11]

However, a key observation in the industry found that, to limit variations in the quality of key components in a plant extract and to minimize the costs of development as well as to ensure product quality, there have been situations where some botanicals have been cultivated to contain a standardized amount of a key component or class of components. Examples of these are ginseng products (ginsenosides) or bilberry products (anthocyanins). However, even when such key compounds have been identified and a standard content is agreed upon or suggested, it is surprisingly still difficult for producers to meet these requirements (Sissi Wachtel-Galor and Iris F. F. Benzie, 2003).[17]

> *"New methods and standards are needed to facilitate chemical characterization and to improve the identification of possible biological actions and relevant interactions" (WHO, 1998).*[64]

The lack of specific regulatory and clinical trial criteria for approving botanical drugs – i.e., "the one size fits all approach", is one of the biggest limiting factors in their development and approval into drug therapies.

There is a general feeling that the DSHEA, with regards to the regulation of botanicals, needs to be modified. It is generally agreed that the lack of market exclusivity for botanical

products under investigation and those with approved health claims is a big disadvantage as there is no protection from competing companies that may also lay a claim on a drug source (the botanical plant) that technically does not belong to anybody. There also seems to be a need for modifications of regulations that deal with intellectual protection rights for companies that will have developed a botanical product to exclude others from copying the product and benefiting unfairly.

On the overall, there is a general agreement that if DSHEA were modified to require botanical manufactures to make submissions that prove safety and efficacy, then more companies would be encouraged to develop botanical drugs as this will offer market exclusivity, intellectual protection, and insurance coverage for their drugs and products.

However, critics of DSHEA, for example (USP Botanical Dietary Supplements Herbal Medicines Expert Committee, 2016),[8] argue that the current regulations have far too many technical issues with the assessment and manufacture of botanicals and their chemical constituents. They further argue that, because each constituent requires its own unique way of analysis that separates it from others, the whole analysis process becomes too cumbersome also given the fact that some of the methods of analysis have not yet been fully developed for full scale industrial use. Overall, this affects the quality assessment and development processes (Kesselheim et al., 2015).[65]

5.6.2 Resource demands and Market challenges

Currently, most of the botanical drugs and products that are on the market or are being developed are through small companies that do not have the same financial capacities as big pharmaceutical companies. This alone, places a big burden on such companies as carrying out a clinical study requires huge sums of money to spend on drug discovery studies (research and

development), and to conduct clinical trials. The stringent GMP safety and effectiveness testing required by regulatory agencies also results in increased time and expenses, a situation that is also not favorable for the development of multi-complex compounds by small companies.

Another major concern that arises from the development of botanical drugs is competition from dietary supplements in the marketing space. This is a considerable financial challenge faced by small companies trying to enter the pharmaceutical drug market by way of botanically derived drugs. Even if a way to finance the research and development of botanically derived drugs can be found, manufacturers will still have to find ways to recoup the money they will have spent developing the drugs.

The FDA requires botanical drugs, just like pharmaceuticals, to be manufactured under strict compliance with GMP standards before seeking approval for marketing, yet by comparison, supplements even though they must follow GMP standards, do not have to seek approval for marketing. Because of this, there is a huge financial investment placed on botanicals to get through to a drug classification compared to a botanical supplement, yet it seems, on the market stage they are competing equally based on a drug or supplement derived from the same plant source (Sissi Wachtel-Galor and Iris F. F. Benzie, 2003).[17]

The main issue behind this challenge seems to be that some small companies involved in botanical products development do not have the resources necessary to carry out research and development studies, and carrying out human clinical trials. Investing in these resources or sourcing out the testing to contract research companies would place huge costs on the small companies in an exercise where there is no guarantee the candidate drug will make it or worse, if costs of research and development will be recouped from sales.

It is imperative that botanical drug sales need to be protected from competition by dietary supplement products based on the same plant source. To recoup their research investments, the manufacturers need to be protected from this competition. Many researchers agree that offering a way through which companies can recoup the costs of developing a botanical drug will encourage more companies to join in the research. The only possible way will be offering some guarantees of protection from competition for companies that manufacture these botanicals. Examples of such guarantees include the granting of intellectual protection (IP) rights (Scotchmer, S.),[66] and some market exclusivity protections coupled with maybe lowering the threshold of the approval processes. Market exclusivity offers protection by providing a length of time during which the FDA does not review and approve similar drugs for a given period.

Patents allow for recovery of expensive drug development costs by protecting the new drug from competition with generic products for 20 years from the time the patent was granted (FDA, 2014a), meaning, no other company can make a similar product or version of it and sale it until the expiry of the patent. Sagaya, R. (2020),[31] argues that patents can be used on "active ingredients", which he refers to as primary patents, then in later phases of the drug development, patents can be filed on other aspects of active ingredients such as different dosage forms, formulations, and production methods to protect those who will have developed them at that stage. These will be secondary patents. This suggestion still does not look at the bigger picture of what regulations are going to protect these patents as patent holders of the secondary ingredients will still be making claims that include or infringe on those of the original active ingredient "the primary patent" holder. All these protections methods can allow the companies to somehow recoup their investment (FDA, 2014a). The problem with these two methods of protection is that "botanicals" are considered to be naturally occurring plants, because of this, any drug made from

its naturally occurring chemicals or compounds would be difficult to get patented. Cardellina, 2002,[67] argues that the chemical entity aspect of it cannot be patented since the botanical has been in known use for decades. Anyone can lay claim to it; it is not born out of innovation.

The other challenge is that botanical products prescribed by doctors are not covered by insurance, some manufacturers think the same problem might exist with botanical derived drugs. This uncertainty seems to play a part in small companies trying to enter the drug market via the botanical route.

As described above, for all the challenges and limitations encountered in the evaluation and development of botanicals into prescription medicines, there are overlaps in all 4 areas involved in the process. Resource demands, which involve the ability of the company to research and develop the drug, and carry out clinical trials, overlaps with regulatory requirements, particularly in the manufacturing of the drug and clinical trials. While resource demands and market challenges overlap as the manufacturers need a way to sell their product. Such overlaps of challenges and limitations put small companies that are trying to make a breakthrough into the lucrative but highly regulated drug industry at a disadvantage.

CHAPTER 6

Conclusion

In both the international marketplace and local U.S. communities, herbal products are marketed under a wide range of commercial classifications, including botanical drugs, botanical products, herbal medicines, phytomedicines, traditional medicines, dietary supplements, nutraceuticals, and food supplements. These varying terms largely stem from differences in national regulations governing their sale. The terminology used not only affects consumer perception of plant-based medicines but also influences regulatory decisions regarding their classification and oversight. Since both consumer demand and regulatory frameworks shape the pharmaceutical industry, the botanical drug industry is no exception. Whether marketed as medicines or food products, botanical and herbal products are categorized based on their intended final use, as defined by manufacturers operating within diverse regulatory frameworks worldwide.

The development of plant-derived drugs and products relies on optimizing phytochemicals to create safe and effective drugs. As new drug research increasingly turns to natural plant compounds for drug discovery, advancements in cultivation, harvesting, screening, identification, isolation, and characterization techniques have enhanced the ability to harness plant-based bioactive compounds. These innovations help mitigate the technological challenges traditionally associated with natural product development, paving the way for novel treatments.

The widespread use and growing recognition of botanical products and botanically derived drugs highlight the critical role of plant-based medicines in modern healthcare. This

research identified one of the key challenges facing the botanical drug industry, i.e., ensuring the quality, consistency, and efficacy of plant-derived compounds used in manufacturing.

However, significant challenges persist in optimizing cultivation, harvesting, and processing methods to develop a high-quality botanical product, including botanically derived drugs. Refining these practices is essential to enhance standardization, efficacy, and regulatory compliance, and create interests in the research of these drugs. In the U.S., botanical products are classified as dietary supplements rather than drugs. If a naturally derived compound were to be developed as a drug, obtaining a patent would be extremely challenging since naturally occurring substances cannot be patented in their original form. While the use of patents or process patents may be possible, securing exclusivity remains difficult.

Clinical trials are often initiated for marketing advantages, as publicly stating that a product is under Investigational New Drug (IND) status can enhance its credibility. However, the burden of gathering extensive data and evidence to advance clinical trials to application status is a significant hurdle. Without the ability to obtain patent protection or market exclusivity, pursuing regulatory approval, whether through a New Drug Application (NDA) or Biologics License Application (BLA) becomes less appealing. Even if a license is granted, the absence of exclusivity protections allows competitors to replicate the drug, market it freely, and make identical claims without bearing the high regulatory costs of drug approval. This lack of protection discourages investment in botanical drug development, making it a less viable pathway for many companies.

Although faced with such hurdles and limitations, the dietary/botanical drug industry is growing, it seems the problem is not finding money to support research and clinical trials, but it is making sure that the money spent in this investment will be recouped in sales if the drug is

approved. If there was a clear path for recouping these investments, it is likely that some companies would find the investments more appealing.

The study also found out that new methods and standards are needed to facilitate the characterization of compounds and to improve the identification of possible biological actions and relevant interactions. These new standards and methods of quality cultivation (to minimize contaminants and adulterant), extraction, isolation, and identification using advanced and accurate techniques will lead to quality botanicals being produced, thereby assuring a safe and efficacious drug product. Changes and improvements in the regulatory environment will also likely encourage more companies to invest in the botanical industry.

Today, the potential of botanical sources yielding new biomolecules remains big, this offers unique opportunities for the discovery of innovative therapies for a lot of medical conditions. However, the growing demand for medicinal plants in both botanical and pharmaceutical drug research poses a significant threat to their sustainability. Natural medicinal plants are under threat worldwide. To ensure the long-term viability of these valuable genetic resources, it is essential to implement conservation strategies and responsible harvesting practices. Future generations, equipped with more advanced technologies and sustainable approaches, must be empowered to preserve, manage, and ethically utilize these botanical resources for continued medical innovation.

While this might not be an issue here in the US, I have personally observed that in some parts of the world, particularly in the sub-Saharan regions of Africa and probably in the rainforests regions around the world too, that is, regions were traditional medicine is almost the primary means of health care provision, and where its use is encouraged because conventional modern medicine is expensive and in some cases not available, and with all the unsubstantiated

claims of safety and efficacy that come with it, wild plants of medicinal value are being decimated by over harvesting. They are cut down to reach the medicinal fruit at the top, or the bark is stripped, the roots are dug out, and the leaves are over-harvested. In other regions, private operators with connections with big business companies and overseas marketers buy the plant products in large volumes to sell to companies and exporters. A case in point during my recent travels in Africa, I observed people over-harvesting the baobab *(Adansonia digitata)* tree fruits and leaves to be sold to overseas markets. The fruit is rich in vitamin C, potassium, magnesium, iron, calcium, antioxidants, and thiamine. It is sold and exported raw to overseas manufacturers who make various teas, vitamin C drinks and high fiber foods. Marula *(Sclerocayra birrea)* fruits from marula trees, are over harvested for making marula oils, skin lotions, drug powder capsule, wines and other highly intoxicating alcoholic beverages, which are mostly available at overseas markets including here in the US, but none on the local markets. All this leaves the tree with no means of surviving, hence threatening plant biodiversity and risking plant extinction, an exercise akin to “cutting/chopping the hand that feeds you or killing the goose that lays the golden eggs”.

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