

A SCOPING STUDY OF THE CHARACTERIZATION AND STANDARDIZATION TECHNIQUES OF HERBAL FORMULAS

A Capstone Project presented to the faculty of Five Branches University
In Partial Fulfillment of the Requirements of the Degree of Doctor of
Acupuncture and Oriental Medicine

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June 13, 2024

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ACKNOWLEDGEMENTS

First and foremost, I want to express my gratitude to Dr. Robyn Soddors, who is my Dean and Capstone Advisor for my Doctorate of Acupuncture and Oriental Medicine (DAOM). Her dedication, patience, guidance, and feedback were invaluable. While this was an exciting and enlightening academic research project, I cannot imagine having done this without Dr. Soddors' valuable assistance every step of the way. The support from the faculty and staff at Five Branches University truly helped to make this journey possible. I want to acknowledge my wonderful group of classmates and cohort members that helped to make this a life-changing and memorable experience. I also want to thank my family for their undying support. I especially want to thank my mother for her unbelievable encouragement and confidence in me and my father for the many hours of proofreading this research paper. Lastly, I want to thank my loving husband, Johnny Griffin IV, for the many sacrifices he has made in support of my pursuits to actualize my dreams. The contributions I hope to make to the field of Traditional Chinese Medicine would have only continue to be a dream without him.

ABSTRACT

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Number of Pages: 97

Introduction: Approximately eighty percent of the world population uses some herb for medicinal treatment. Herbal formulas must meet rigorous standards for characterization that validate high quality, safety, and efficacy. The objective of this scoping study was to inventory and identify existing methods and techniques used to standardize and characterize the bioactive phytochemical ingredients in herbal formulas.

Method: This scoping study conducted an intensive search of scientific literature on the standardization and characterization of phytochemicals in herbal medicine. Selecting specific keywords and MeSH terms, we used two databases, PubMed and Google Scholar, with a particular focus on studies released within the past 15 years.

Results: We found that there is insufficient information about the synergistic effect of the bioactive components of each herb in a formula. There were 47 different characterizations, and 48 standardization techniques identified. Three of the characterization techniques made up roughly 55% and eight standardization techniques made up approximately 49% of utilizations.

Discussion: The three techniques include Mass Spectrometry, High Performance Liquid Chromatography, and Liquid Chromatography. The prevailing techniques were used multiple times as complements to and pairings with the lesser utilized techniques. This falls significantly below what is required to keep pace with the growing demand for herbal formulas. There were about 58 standardization techniques used about 8 were the most prevalent. These include metabolomics. Standard operating procedures, pharmaceutical standards, markers, fingerprinting analysis, chemical profiling, good manufacturing practices and quality control assessments.

CHAPTER ONE

Introduction and Background

Early Uses of Medicinal Plants and Herbs

For millennia, humanity has turned to the natural world for remedies and treatments, with plant herbs at the forefront of these medicinal pursuits (Chevallier, 2016). The historical archives of different civilizations confirm the importance of plant herbs and provide useful descriptions on the various efforts aimed at using plants to cure human ailment (Chevallier, 2016). For example, the clay tablets from ancient Mesopotamia and the papyrus records from ancient Egypt, are filled with lists of medicinal plants (Christensen, 2018). Documents like the Egyptian Ebers Papyrus not only provide insights into early herbal practices but also reveal an understanding of ancient cultures regarding the therapeutic potential of herbs (Metwaly et al., 2021). These age-old texts list various ailments and their herbal treatments, reflecting early ventures to systematically categorize and apply herbal knowledge (Petrovska, 2012).

In the vast landscapes of India and China, many herbal traditions have a profound resonance. Ancient Indian Ayurvedic texts, such as the Charaka Samhita, offer intricate details about a myriad of herbs and their medicinal properties. (Charaka-Samhita -- Britannica Online Encyclopedia, 2019). The historical uses of herbs found in archaeological sites of ancient China offer a glimpse into the early origins of traditional herbal Medicine (Hardy et al., 2021). Legendary emperor Shennong, who is credited with documenting the uses of 365 medicinal plants in the seminal work entitled, “Shen Nong Ben Cao Jing,” tasted hundreds of herbs to discern their medicinal properties, documenting his findings around 200 CE in his book (Shou-Zhong et al., 1998). This rich and vast list of herbal wisdom, interwoven

across different cultures and epochs, underscores the profound relationship between humanity and the botanical realm (Shou-Zhong et al., 1998).

Traditional Chinese Medicine (TCM). is a holistic healthcare system with roots extending thousands of years back to Emperor Qin Shi around 221 BCE (Ji, 2014 pg1). It is a testament to China's profound understanding of health, wellness, and the interplay between humans and their environment (Ji, 2014 pg1). Traditional Chinese Medicine is not just a collection of remedies or treatments; it embodies an intricate philosophy that is built on principles of universal balance based on yin and yang and the interactions between the mind, the body and the environment (Maciocia 2014, pg. 41). This ancient system encapsulates various modalities, from acupuncture and moxibustion to Qigong and dietary therapy (Guang-ren 2014, pg. 9). Herbal formulas stand as a cornerstone for complimenting these diverse practices and combining the wisdom of ancient sages with the healthcare needs of modern individuals (Guang-ren 2014, pg. 9).

However, despite this rich legacy, herbal formulas face significant challenges in standardization (Parveen et al., 2015). As we begin to integrate traditional herbal knowledge with modern medical practices, the need for standardized method to characterize herbal formulas becomes paramount and crucial (Pandey & Tripathi, 2013). While ancient philosophers and practitioners may have successfully relied on empirical observations and oral traditions, today's conventional Western medicine demands a more rigorous scientific validation (Mandal et al., 2021). Unfortunately, there is a gap between the historical practices and the modern-day rigor of scientific validation (Mandal et al., 2021). Bridging this gap requires not only an appreciation for the historical depth of herbal medicine but also a forward-looking approach that scientifically verifies the safety, efficacy, and consistency of herbal formulas used in medicinal treatments (Kumari & Kotecha, 2016).

Classical Chinese Medicine Representation

For thousands of years, people have used plants as a way of maintaining good health and as remedies for addressing and preventing diseases. However, the most developed and successful use of herbs for health maintenance and prevention can be attributed to China (Shou-zhong, 1998). The earliest recorded emphasis on herbs as a means of disease prevention and promoting longevity dates to the Qin dynasty (221-206 BCE). (Zhong, 1999).

Around the Qin dynasty, records indicate that the earliest classic writings, generally deemed to be the greatest seminal works that centered on the use of plants to achieve a healthy, long life, include the work by Huang Di, “The Yellow Emperor’s Classic of Medicine”. This work, also recognized as the *Neijing*, is considered to consist of two parts: *The Suwen and Lingshu*, which focuses on the best way of life, with an ultimate focus on living in harmony with the universe (Ni, 1995). This work laid the foundation for medical theory (Ni, 1995).

Another seminal classic work, *Shen Nong Ben Cao Jin*, builds on *The Yellow Emperor’s Classic of Medicine* and focuses on achieving the best way of life through the use of plants and animals as herbal Medicinals (Shou-Zhong, 1998). A year later, another seminal work that complemented the works of Huang Di and Shen Nong, “The Shang Han Lun, On Cold Damage”, added to the focus on medicinal herbs, as a way of maintaining good health and as a way of preventing disease (Zhong, 1999). The development and expansion of these three seminal works set the stage for a written philosophy known as Taoism (Zhong, 1999). Taoism has provided an ethical foundation for guiding the evolution of Traditional Chinese Medicine and the expanded the ideals relating to the best way of life (Zhong, 1999).

While the different texts use different approaches, they all agree that Chinese herbs, or *materia medica*, are essential to maintaining

health and preventing disease. Each approach aims to help human beings achieve the best way of life (Ni, 1995). Traditional Chinese Medicine (TCM), is founded on the Taoist philosophy, which states that the universe stems from a potent force called qi (Ni, 1995). This qi is split into two; yin qi and yang qi, which are symbiotically connected (Ni, 1995). Taoism is based on the concept of yin and yang, which is believed to be the foundation of the universe (Ni, 1995). Yin and yang create life and signify both the beginning and the end (Ni, 1995).

The perfect yang qi rises to form the heavens, while the imperfect yin qi descends and hardens to create the planet Earth (Ni, 1995). Yin and represent two sides of energy (Ni, 1995). Yin is calm, dark and less active energy; while yang is bright and active energy (Ni, 1995). Yang is important for things that needs to expand and grow, whereas yin helps to shrink and tie things together (Ni, 1995). In their concepts, yang is the energetic side of life, while yin is the origin of this potential and is nourishing (Ni, 1995).

The interaction between yang qi and yin qi is essential to maintaining balance in the universe and within humans (Ni, 1995). The equilibrium and the interplay of yin and yang can be seen with the changes of cosmic energy (Ni, 1995). This philosophy illuminates a deep interdependence between human existence and the world around us (Ni, 1995). Humans are at the center of universe and are connected to the heavens above and earth below (Ni, 1995).

TCM views life holistically, seeing all things in the universe interconnected to form a united whole (Ni, 1995). It underlines that we need balance and harmony between humans and nature (Ni, 1995). Humans are born of the universe and are therefore subject to the laws of the universe (Ni, 1995). How we relate to the environment, how we live, and spiritual health are important to the quality of human life (Ni, 1995).

A brief explanation of these natural laws, related to the four seasons, offer a view of the core principles that are the basis of TCM (Ni, 1995). It points to these principles still being important since its start

in the 220s BCE, despite many pre-Western developments (Ni, 1995). The primary reason for their continued relevance is that Chinese scientific and technological approaches have always placed value on balance and harmony between humans and the environment (Ni, 1995).

The Earth's orbit around the sun creates four seasons and five climatic elements (Ni, 1995). These climatic changes also produce pathogenic evils that can lead to health issues (Ni, 1995). Therefore, achieving longevity and good health requires us to adjust our life patterns according to the changing seasons to follow the laws of the universe (Ni, 1995). Following these natural laws bring about health and long life, while disobeying them can lead to diseases (Ni, 1995).

The energy from the heavens naturally moves around and meets with the energy from earth; when higher energy descends and lesser energy ascends, they mix and bring about the four seasons (Ni, 1995). The cyclic changes of yin and yang across the seasons explains why life starts, grows, multiplies, matures, and ends. Respecting these natural laws helps to prevent diseases, as shown by the ancient Chinese who treated illness preemptively (Ni, 1995).

Each season causes different changes and impacts: spring starts a new beginning, summer is characterized by abundance, autumn brings ripeness for harvest, and winter is a period of dormancy (Ni, 1995). Not respecting these seasonal changes can cause unique health issues (Ni, 1995).

Students and practitioner of Chinese medicine may be amazed to find how little Chinese medicine has changed since the time the *Nei-jing* was written (Ni, 1995). Natural therapies and preventive approaches are ever as effective and even more pertinent in today's drug-oriented medical environment (Ni, 1995). They offer a heartfelt and viable approach to the perception and treatment of illness (Ni, 1995). This is especially true in the battle against chronic, degenerative, infectious, and deficient medical conditions (Ni, 1995). Traditional Chinese medicine is very promising as an effective alternative to conventional medicine. (Ni, 1995).

Ironically, throughout the Chinese-speaking world, Western medicine has become recognized as the mainstream medicine due to its scientific values, which dominate all fields of learning, with education closely modeled on Western models (Zhang, 1999). Despite this, there is hope in China that by discovering the bioactive scientific components of herbs, traditional medicine will evolve into a scientific discipline that can be integrated with modern medicine (Zhang, 1999).

It is important to note that the three seminal classical texts of Traditional Chinese Medicine, “The Yellow Emperor’s Classic of Medicine”, “Shen Nong Ben Cao Jing”, and “Shan Han Lun” all made significant contributions to TCM. Each of them has a different approach to achieving the health of humans.

Huang Di, The Yellow Emperor, addressed the subject in the most all-encompassing manner by expounding on a comprehensive medical theory that enables the best way of life (Ni, 1995). To achieve this way of life, one must live in harmony with the laws of the universe (Ni, 1995).

Shen Nong, one of the three greatest heroes in Chinese culture, observed that the Yellow Emperor’s discussion on the best way of life was primarily based on the food that one eats (Shou-Zhong, 1998). In the book, “Shen Nong Ben Cao Jing,” Shen Nong, recognized the importance of food because it provides the foundation of the post-natal qi (Shou-Zhong, 1998). Therefore, he focused on the Chinese herbs, which included both plants and animals (Shou-Zhong, 1998).

Shang Han Lun is a significant text in Traditional Chinese Medicine (TCM). The focus of the book entitled, “On Cold Damage,” provided an in-depth examination of diseases and was compiled by Zhang Zhongjing during the Eastern Han dynasty, around 220 AD (Zhang, 1999). The Shang Han Lun is devoted to externally contracted diseases and their treatments. This text does not focus on treatment using acupuncture. Instead, it focuses on a highly sophisticated use of medicinal herbs to address disease (Zhang, 1999). The Shang Han Lun classified diseases into six stages based on the external relationships of

Yin and Yang. These six stages ranged from the mildest to the most severe. Zhang Zhongjing developed 113 herbal prescriptions (Zhang, 1999). This work laid the foundation for understanding disease progression, treatment principles, and herbal formulations in Traditional Chinese Medicine (Zhang, 1999).

It is important to acknowledge that the practice of Chinese medicine is based on a philosophy that is thousands of years old, and the present-day practice has not significantly changed from that of its original creation (Shou-zhong, 1998). It is also instructive to note that Chinese medicine, rooted in the past, has for centuries looked back to its formative period, and even today a sound knowledge of the classics is essential for gaining a firm understanding of Chinese medicine (Shou-zhong, 1998).

Students and practitioners of Chinese medicine continue to rely on the classics as the best way for one to gain knowledge of the practice of TCM; and because of that, the Western world, with its many scientific and technological advances, tends to be lukewarm towards Chinese medicine. However, a simple comparison of life expectancy between the East and the West provides evidence of the success of TCM. According to the United Nations in 2021, United Nations World Population Prospects, the life expectancy in China was 85.49 years, compared to 78.9 for the United States (United Nations, 2021). While the East has been reluctant to change the practice of TCM for fear of losing its ethical and philosophical foundation, a narrow scientific focus on herbs and herbal formulas may allow for scientific advancement without changing the core philosophy and ethics (Tian et al., 2021). Through more scientific analysis, it may be possible to find a way to, precisely, measure the impact a specific formula has on a patient. While there have been some scientific advances in herbal use, there is still a lot of variation in the way individual practitioners prescribe formulas. Through scientific analysis, it is possible to standardize the use of herbal formulas and better characterize the specific impact a formula will have on a specific individual patient. This could increase herbal safety, efficacy, and reliability, thus enhancing the use

of Chinese herbs without losing important ethical standards (Tian et al., 2021).

However, the main challenge still facing herbal use is the absence of universally accepted methodologies to characterize all the bioactive compounds present in herbal medicines (Rebiai, 2022). This gap not only prevents the accurate identification of these compounds but also presents an obstacle to ensuring consistency across batches. Without standardization, two batches of the same herbal medicine could vary significantly, leading to inconsistent treatment outcomes. (Mishra & Tiwari, 2022) These inconsistencies undermine the trust in herbal treatments, potentially sidelining them and preventing future advances in TCM.

Evolution of Western Biomedicine

The evolution of Western biomedicine was deeply influenced by the teachings of the most notable and recognizable names like Aristotle and Hippocrates. Aristotle was born in 384 BCE and died in 322 BCE. He made extensive contributions across many disciplines, including ethics, and is recognized for providing the foundation for comparative anatomy and embryology. His views would go on to influence scientific thinking for the next 2,000 years (Lennox, 2021). Hippocrates marked a paradigm shift from spiritual and holistic interpretations of health to a more empirical and observation-based approach (Silvano, 2021). This philosophical foundation was further enriched during the Renaissance, a pivotal era characterized by groundbreaking advances in human anatomy and physiology, and the gradual embrace of dissection and autopsy practices. These developments not only transformed medical understanding but also paved the way for the modern scientific approach to medicine (Francia & Stobart, 2014).

The Renaissance was a turning point in the evolution of Western biomedicine. It was defined by the interest in human anatomy and physiology, which led to revolutionary advances like dissection and

autopsy (Bambach, 2001). These advances facilitated a fundamental shift away from the practices of medieval medical doctrines. This era played a significant role in the progress of anatomical knowledge and greatly advanced the sophistication of medical science. (Bambach, 2001).

The 19th century heralded a significant milestone in Western biomedicine with the decline of miasma theory and the rise of germ theory (Risse, 1993). This period saw a major shift in medical understanding, suggesting that diseases were caused by microorganisms. Such a shift profoundly impacted public health strategies, hospital management, and laid the groundwork for the fields of epidemiology and bacteriology (Germ Theory - World History Encyclopedia, 2023). This era was marked by significant advancements in laboratory techniques, vaccine development, and improved sterilization and hygiene practices, moving medicine away from superstitions towards a more evidence-based approach.

The discovery of X-rays by Wilhelm Conrad Rontgen in 1895 revolutionized medical diagnosis. This non-invasive technique for observing the body's internal structures initiated a new era in diagnostic medicine and paved the way for modern radiology (Panchbhai, 2015). This period also saw major strides in combating diseases like malaria, highlighting the remarkable success of medical research in public health crises and setting standards for future disease control and eradication (Silvano, 2021).

In 1796 Edward Jenner found a way to inoculate an eight-year-old boy against smallpox by using a vaccine. After this discovery, vaccines grew very quickly to combat the deadliest diseases, including smallpox, rabies, tuberculosis and cholera. With the advance of vaccines, millions of lives have been saved (King, 2023).

Western biomedicine continued to expand rapidly in addressing medical challenges, including providing esthesia to perform surgery on patients. In 1846, William T.G. Morton made history when he

successfully used an anesthetic during surgery. This was followed by the introduction of chloroform, a fast-acting anesthetic (Britannica, 2023).

Philosophical debates in medicine, as discussed in academic articles, emphasize the ongoing tension between reductionism and holism, and realism and antirealism (Francia & Stobart, 2014). These debates question whether diseases should be studied in their smallest components or as larger holistic systems, and whether disease-causing entities are tangible realities or mere conceptual constructs. Such discussions impact perception and treatment of diseases, forming the foundation of contemporary biomedical practice and research. (Basser, 2017).

In the field of bioethics, traditional principles have evolved, reflecting a shift from protective to proactive and participatory approaches in patient care and rights (Basser, 2017). This evolution signifies a transformative approach in biomedicine, emphasizing patient empowerment, the continual relevance of medical practices, self-expression in healthcare choices, and a broader understanding of justice in terms of global healthcare citizenship (Basser, 2017).

Today's biomedical model prioritizes a comprehensive understanding of health and medicine, acknowledging the importance of diverse perspectives and experiences in healthcare (Basser, 2017). It proposes a balance between individual and collective identities in healthcare, underscoring the influence of community and cultural factors. This model also emphasizes maintaining standards of objectivity, ensuring that biomedical practices are based on reliable and unbiased evidence (Basser, 2017).

The historical analysis of medicinal plants in Western medicine, particularly through the lens of traditional texts like "Shengnong Bencao Jing," highlights the evolution from ancient sensory methods to contemporary analytical techniques like HPLC and GC (Fitzgerald et al., 2020). This transition from traditional to scientific approaches underscores the importance of standardization in ensuring the efficacy and quality of herbal medicines.

In summary, Western biomedicine, from its roots in ancient Greek philosophy to contemporary scientific practices, demonstrates a remarkable evolution. This journey highlights the integration of traditional wisdom with scientific advancements, shaping the current approach to healthcare and underlining the importance of balancing historical knowledge with modern scientific methodologies in addressing the complexities of global healthcare challenges.

While Western biomedicine has advanced more rapidly than Eastern medicine, their histories show signs that they remain intertwined. Western medicine advanced toward science and evidence-based approaches and Eastern medicine rests its effectiveness on past practices. However, both rely on the use of herbal formulas.

In ancient times, identification, preparation, and analysis of medicinal plants relied heavily on ancient texts like "Shengnong Bencao Jing" (Shengnong Materia Medica, 25–220 CE). This text organized and evaluated 365 single herbs and 113 herbal formulas, mostly based on flavor profiles. The use of organoleptic senses helped in the function and application of the medicine. This was a period of empirical knowledge that was closely tied to human senses. While contemporary methods somewhat surpass these old methods, they still established a foundation for systematic plant analysis. This exploration into the earliest practices of herbal medicine, using common parts of the plant and simple preparation methods, mainly serves to highlight the foundational role of traditional knowledge in the development of Western herbal medicine. The use of leaves and roots, along with concoction and decoction techniques, underscores the historical knowledge of plant properties and their therapeutic applications, forming the basis for modern herbal medicine analysis and use.

CHAPTER TWO

Explaining The Scoping Study

This paper aims to contribute toward facilitating the formation of a bridge between historical TCM practices, which are often imprecise regarding their impact, and an evidenced-based system where the quantity and type of medicinal herbs are scientifically validated to determine their precise impact on specific illnesses or diseases. The paper will focus on existing methods and techniques currently being used for establishing the characterization and standardization of herbal formulas and will provide a structured framework for compiling the information. It will examine and compare the current methods and techniques that can be useful in advancing and enhancing the overall industry involved with characterization and standardization. The paper will illuminate the obstacles and challenges facing the industry. It is hoped that the paper will result in a small contribution toward the enhancement of scientific characterizations and lead to greater demand for medicinal herbal formulas.

Characterization and standardization are critically important because the information derived can be used to facilitate a more precise determination of patient medical treatment (Lutoti et al., 2021). The safety, quality and efficacy of herbal formulas can be enhanced through better methods of gauging the quantity of herbal use based on each person's unique constitution and balance. Information derived from the characterization and standardization of herbal formulas can be tremendously useful in determining the impact that each herb has on an overall medicinal formula. By determining the impact of each herb, it becomes possible to measure the synergistic affect that each herb brings to a formula, and this will allow for the customization of specific individual treatment plans aimed at achieving a balance between the yin and yang. (Maciocia 2014, pg. 3). Practitioners formulate mixtures

of herbs to alleviate the symptoms of disease and to serve as remedies to treat particular ailments (Chevallier 2016, pg 42). As we draw from these traditions, it is important to focus on the development of modern and objective standards for evaluating the safety, quality and efficacy of medicinal formulas. (Mishra & Tiwari, 2021).

Unlike conventional or Western medicine, TCM does not just focus on addressing specific symptoms or ailments. The primary focus of TCM is to understand the unique constitution and imbalances of everyone. Once this diagnosis is complete, the practitioner crafts a treatment plan that primarily utilizes acupuncture and Chinese herbs to not only alleviate the symptoms but also restore the balance and harmony to the body's intrinsic energies. (Chevallier 2016, pg. 42). In a world that is increasingly seeking personalized healthcare solutions, the tailored approach of TCM, generally based on herbal formulas, serves as a beacon, illuminating the path towards holistic wellness. However, while drawing from this rich heritage, it is important that herbal formulas meet rigorous standards that have been proven and validated to provide a consistent pattern of high quality, safety, and efficacy under all circumstances. In response to the need for rigorous validated standards, the World Health Organization (WHO). has successfully taken the first step toward creating a reliable foundation for establishing validated standards for the use of single herbs. The primary foundation upon which the standardization process was created starts with the identification of the botanical components of individual herbs. This system for standardization has made it possible to validate the quality, safety and efficacy of single herbs. As a result, the process of standardization has raised the single herb reliability and has enhanced the growth and expansion of herbal medicine. (NCCIH, 2002).

The WHO's method specifies the scientific framework for extracting ingredients. It also details a formula that is reproducible and maintains consistency. The work developed by WHO clearly shows the elements that are indispensable in sustaining the healing capacities of herbal extracts. This process is completed by determining the amount of potent bioactive ingredients. The method utilizes superior

analytical techniques, such as high liquid chromatography (HPLC), gas chromatography (GC) and mass spectrometry (MS), to establish important markers necessary for quality control.

Additionally, WHO necessitates the utilization of comprehensive documents that include botanical identification, extraction mechanisms, and constituent quantification. It also sets a high bar regarding foreign contaminants. This structured way of maintaining consistency results in stronger therapeutic outcomes. Moreover, it aligns with global regulatory norms, which helps make traditional medicine more relevant in modern healthcare systems. While WHO has made tremendous advances in standardizing and validating the quality, safety and efficacy of single herbs, there are still problems with standardizing herbal formulas. The primary problem with standardizing herbal formulas has to do with the innate complex nature of the multiple bioactive ingredients of each herb and their synergistic effect on the overall outcome of the formula.

As detailed as the WHO's guide may be, a closer look shows that the guidelines primarily benefit the characterization and standardization of single medicinal plants. Their method pays little attention to the complex dimensions of multi herbal formulas. However, these multi-herbal containing formulas are central to traditional medical practices for Traditional Chinese Medicine (TCM). Practically every herbal treatment consists of more than one single herb. Therefore, the need to understand the synergistic effect of each herb in a treatment plan is very important. These treatment plans require an understanding of the unique bioactive ingredients of each herb to capture the synergistic effect and the entirety of their properties to successfully determine the overall impact of a formula. This means that characterization and standardization become ever more critical. The goal is to standardize herbal formulas based on the concentration of their bioactive molecules, which is essential to the quality of drugs. (Choudhary & Sekhon, 2011).

In the ever-evolving landscape of healthcare, it is essential to continuously refine our understanding and methodologies to ensure that

medical practices are both effective and safe. Traditional Chinese Medicine, with its rich tapestry of herbal formulas, offers profound therapeutic potential. However, to integrate its ancient wisdom with the stringent demands of modern medicine, there's a pressing need for meticulous standardization and characterization of these formulas (NCCIH, 2012).

Characterization will allow a deeper look into herbal formulas and strengthen our understanding of how each individual component impacts the overall planned outcome of a formula. Enhanced characterization of plants allows for not only the traceability of molecules of new products, but it also creates an identifying “fingerprint” of each of the main bioactive molecules. In order to determine the “fingerprint” of an individual plant’s composition, in comparison with the new formula, it will require high and expensive technique levels. (Bunghez et al., 2013). By standardizing the characterization methods for herbal formulas, we can ensure that each formula is consistent in its composition and potency. This could also eliminate variances that could cause undesirable effects on patient outcomes or pose safety concerns. With this information, researchers can better navigate the journey ahead and ensure that TCM herbal formulas are both preserved in their authenticity and optimized for contemporary use based on scientific validation. The process that ensures every preparation of an herbal formula, regardless of where or when it's prepared, possesses consistent properties and healing potential and confirms the herbal formula’s identity, quality and purity throughout all phases of its cycle is called standardization (Mishra & Tiwari, 2021). It guarantees specific quantities of active constituents, and ensures the predictability and safety of the treatment. There are several steps involved in the standardization of herbal raw drugs. This list includes microscopic & molecular examination, botanical authentication, various chromatographic techniques; and also, the biological activity of the whole plant’s identification of chemical composition can be found. (Choudhary & Sekhon, 2011).

With advanced analytical techniques, it is possible to dissect complex herbal formulas and gain insights not just into primary active

ingredients but also secondary compounds. These secondary compounds often play a crucial synergistic role in treatment. In this paper, particular attention will be paid to recent developments in the use of analytical techniques for the characterization and identification of novel active components from plant extracts. This includes advanced techniques in phytochemical analysis, chromatography and spectrometry (Cieřla & Moaddel, 2016).

It is important to note that while merging ancient herbal knowledge with advanced analytical processes, it must be done in such a way that it maintains the precision and reliability that modern medicine demands. This will ensure that the age-old wisdom of herbal formulas remains relevant and trusted. It will also enhance the confidence of contemporary healthcare practitioners by ensuring consistent potency and safety of these medicines. Standardization is the blueprint, ensuring that each dose has the expected therapeutic value, free from harmful adulterants or inconsistent concentrations of bioactive compounds. (Lutoti et al., 2023). Historic records containing details of hundreds of medicinal plants and their uses, set the early foundation for what we now recognize as the science of pharmacognosy. Orhan (2017). states that pharmacognosy is a systematic science of morphological, chemical, and biological properties along with history, cultivation, collection, extraction, isolation, bioassaying, quality control, and preparation of crude drugs of natural origin.

The main challenge still facing herbal use is the absence of universally accepted methodologies to characterize all the bioactive compounds present in herbal medicines (Rebiai, 2022). This lack of accepted methodologies not only prevents the accurate identification of these compounds but also presents an obstacle to ensuring consistency across batches. Without standardization, two batches of the same herbal medicine could vary significantly, leading to inconsistent treatment outcomes. (Mishra & Tiwari, 2022). These inconsistencies undermine trust in herbal treatments, potentially sidelining them and their potential.

Recent research advancements in analytical chemistry have catalyzed the evolution of a multitude of analytical methods, each tailored for specific aspects of herbal medicine characterization. Figure 1 encapsulates a selection of these techniques, ranging from chromatographic and spectroscopic to advanced mass spectrometry, highlighting their unique functions in the analytical process. (Ma et al., 2018; Wang et al., 2023). These techniques are spearheading the standardization effort vital to the integration of herbal medicines into modern healthcare, ensuring the precision and consistency required by contemporary medical practices.

These methods offer promising progress toward achieving the much-needed standardization in herbal medicine characterization. Moving the field forward, it's important that the global research community begins to expand its efforts to adopt and standardize these methods, ensuring that herbal medicines have the precision and consistency modern healthcare necessitates.

Technique	Function	Citation
DART-MS	Direct Analysis in Real Time Mass Spectrometry; rapid, ambient condition analysis	Ma et al., 2018; Wang et al., 2023
LC-MS/MS LC-DAD	Liquid Chromatography with tandem Mass Spectrometry and Diode-Array Detection; separation, identification, and quantification	Ding et al., 2007
UPLC-QTOF/MS	Ultra-Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry; high-resolution mass spectrometry for complex mixtures	Lin et al., 2022; Li et al., 2021; Yang et al., 2022; Zhang et al., 2009
HPLC/MS	High-Performance Liquid Chromatography with Mass Spectrometry; separation and identification of compounds	Li et al., 2022; Sanchez et al., 2020; Taha et al., 2015; Liao et al., 2021; Ding et al., 2007; Sasidharan et al., 2010
UPLC-DAD-MS/MS-BCD	Ultra-Performance Liquid Chromatography with Diode-Array Detection and tandem Mass Spectrometry with Base Peak Chromatogram Decoding; detailed compound analysis	Li et al., 2014
UHPLC-QQQ-MS/MS	Ultra-High-Performance Liquid Chromatography with Triple Quadrupole Mass Spectrometry; highly sensitive and selective quantification	Hu et al., 2021
HPLC	High-Performance Liquid Chromatography; separation of compounds based on retention time	Roshan et al., 2021; Fonmboh et al., 2020
UV-Vis Spectroscopy	Ultraviolet-Visible Spectroscopy; measuring absorbance or reflectance in the UV-Visible spectrum	Roshan et al., 2021; Zhang et al., 2011; Ding et al., 2007
FTIR	Fourier Transform Infrared Spectroscopy; identifying chemical bonds in a molecule by producing an infrared absorption spectrum	Fonmboh et al., 2020; Sasidharan et al., 2010
TLC	Thin Layer Chromatography; separation of non-volatile mixtures	Fonmboh et al., 2020
UHPLC-Q-TOF-MSE	Ultra-High-Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry; high-resolution mass spectrometry with enhanced scanning capabilities	Guo et al., 2018; Zhang et al., 2017
HPLC/UV/Vis/FLD-EDA-MS	High-Performance Liquid Chromatography with Ultraviolet/Visible and Fluorescence Detection, Electrochemical Detection, and Mass Spectrometry; multi-detection techniques for comprehensive analysis	Taha et al., 2015
NMR	Nuclear Magnetic Resonance; structure elucidation of organic compounds	Mahrous et al., 2015
HPLC-DAD	High-Performance Liquid Chromatography with Diode-Array Detection; separation and UV/Vis spectrum analysis	Bunghez et al., 2013; Zhang et al., 2011
LC-ESI(+)-QTOF-MS	Liquid Chromatography with Electrospray Ionization and Quadrupole Time-of-Flight Mass Spectrometry; high-resolution analysis of ionizable compounds	Bunghez et al., 2013
HPLC-DAD-ESI-MS	High-Performance Liquid Chromatography with Diode-Array Detection and Electrospray Ionization Mass Spectrometry; separation and mass analysis	Zhang et al., 2011; Ding et al., 2007
Immunoassay	Technique using antibody and antigen interactions for detection and quantification	Sasidharan et al., 2010
HPLC-ESI-MS/MS	High-Performance Liquid Chromatography with Electrospray Ionization tandem Mass Spectrometry; separation and sensitive mass analysis	Zheng et al., 2010

These analytical techniques are the anchor of herbal formula standardization. They ensure that the bioactive compounds are understood completely. Each method of analysis has a specific characterization technique and function. The Ultra-High-Performance Liquid Chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (UHPLC-Q/TOF-MS). method is used to characterize chemical profiles systematically (Wang et al., 2021). The High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) stand out for their ability to separate, identify, and quantify the myriad compounds present in a sample.

Mass Spectrometry (MS) further refines this process, offering insights into the molecular weight and structure of these compounds. Delving deeper, Nuclear Magnetic Resonance (NMR) spectroscopy provides a unique window into the atomic environment and the chemical structures of molecules, while Thin Layer Chromatography (TLC) offers rapid, qualitative analysis of mixtures (Yan et al., 2022). (Wang et al., 2020). The non-chromatographic techniques such as phytochemical screening assay and Fourier-transform infrared spectroscopy (FTIR), can also be used to obtain and facilitate the identification of the bioactive compounds (Sasidharan et al.2010).

Chemical Fingerprinting of herbal medicines is utilized for the authenticity and quality control of herbal medicine and herbal preparations. Chemical fingerprints obtained by chromatographic, spectroscopic, thermogravimetric analysis, capillary electrophoresis, and polarography techniques have become the most potent tools for quality control of traditional herbal medicine.(Sasidharan et al., 2010).

These tools are powerful; however, they have limitations which are important to consider. The quest for the most effective method to identify and quantify compounds still remains elusive. Each technique has its own constraints and suitability depending on the nature of the compound and the features of the sample. (Mishra & Tiwari, 2022). In addition, the scarcity of advanced data analysis techniques, such as chemometrics, presents additional challenges. Without a clear understanding of the composition, chemistry and biological activities of

these herbal medicines, their integration into contemporary western healthcare will remain a challenge. This void does not just represent an academic gap, it raises serious concerns about the reproducibility and reliability of characterization outcomes. (Tripathi et al., 2022). Without standardized procedures, the risk of mischaracterizing or overlooking vital bioactive compounds becomes extremely high, leading to potential clinical misjudgments.

Liu and Zhang emphasize the need for research into standardized protocols to accurately identify and quantify bioactive compounds in herbal concoctions. (Liu (2013). and Zhang (2013). The bioactive compounds found in TCM formulas can fluctuate based on growing conditions, harvesting time, and processing techniques. Because plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds (Sasidharan et al., 2010). There's an urgent need to standardize these methodologies, and ensure they are comprehensive and adaptable to the complexities of herbal medicines.

According to an estimate from the World Health Organization (WHO), approximately 80% of the world population still uses herbs and other traditional medicines for their primary health care needs (Choudhary & Sekhon, 2011). The surging demand for herbal medicine reflects a global shift in health consciousness, with increasing numbers recognizing the profound benefits offered by these age-old remedies. The Global Nutraceuticals Market has been projected to increase over 8.3% annually and is expected to reach 30 billion dollars in the US by 2015. (Jadhav et al., 2020). As the world gravitates towards these natural alternatives, the economic implications are vast. The expansion of these markets allows industries to thrive and can create a new wave of economic opportunities. Overall international trade in medicinal plants and their products was 60 billion US dollars in 2010 and is expected to reach 5 trillion US dollars by 2050 (Jadhav et al., 2020).

However, beneath this potential rising market lie the subtle concerns about the quality, safety and efficacy of these products and their impact on public health and patient outcomes. As highlighted by (Tang et al. 2013), the safety and efficacy of these herbal products are paramount. While the demand increases, the absence of standardized methodologies creates uncertainty for the future of herbal medicine. Without standardization, the inability to accurately measure the therapeutic value and impact of these products could potentially block the public health response (Asian Pacific Journal of Cancer Prevention, 2002).

Researchers in the field have echoed the need for further investigation into standardized approaches. (Liu (2013). and Zhang (2013). Their research underscores the necessity of precise identification and quantification of the bioactive compounds in various herbal formulations. Failure to address this could lead to a market saturated with inconsistent products. These inconsistencies could compromise the very essence of herbal medicine and diminish its economic potential. Patients might receive varying outcomes from similar products. If two batches of the same herbal concoction exhibit disparate concentrations of active compounds due to the lack of standardization, the outcome for patients can vary wildly, leading to under-treatment or even adverse effects. This issue underscores the inadequate knowledge of their mode of action, potential adverse reactions, contraindications, and interactions with existing orthodox pharmaceuticals and functional foods to promote both safe and rational use of these agents (Ekor, 2014).

Developing a comprehensive, standardized methodology would further propel the socio-economic potential of herbal medicine, ensuring that its economic promise is infused with the guarantee of safety, efficacy, and quality. While there is a promising demand for herbal medicine, the lack of standardized methodologies causes delayed public health responses due to the inability to verify measurements and impacts. Lin and Zhang have emphasized the urgency of establishing standardized protocols. Without such protocols, both individual health and the broader socio-economic landscapes are jeopardized.

Inconsistent treatment outcomes can erode trust in herbal treatments, potentially pushing individuals towards more expensive alternatives or even dissuading them from seeking treatment altogether. (Liu (2013) .and Zhang (2013).

In the realm of healthcare, decisions pivot on the axis of trust, evidence, and consistency. The absence of standardized methodologies for characterizing the bioactive compounds in herbal medicine casts a long shadow over these critical pillars. Physicians, healthcare providers, and policymakers rely on consistent, reproducible data to make informed decisions. Without standardization, the data generated from studies can be disparate, leading to challenges in their interpretation. A lack of adequate or accepted research methodology for evaluating traditional medicines compromises safety by the lack of suitable quality controls, inadequate labeling, and the absence of appropriate patient information (Ekor, 2014). Inconsistency hampers the integration of herbal medicine into mainstream healthcare and creates reluctance among practitioners to prescribe or endorse such treatments. As a result, patients might be denied the potential benefits of these natural remedies, exposed to inconsistent doses that could have varied therapeutic or side effects, or worse be steered towards pharmaceutical treatments. The National Kidney Foundation has suggested that herbal supplements should not be used by patients because herbal products are under regulated, pose the possibility of contamination, and may interact with prescription drugs (Min-Yen Lin (2015).

The scarcity of data on the composition and quality of most herbal medicines stems from the lack of adequate policies or government requirements. Beyond individual healthcare decisions, the lack of standardization has broader implications for public health policy. Governments and health organizations draft policies based on robust scientific evidence. However, without standardized methods for characterizing herbal compounds, the evidence base becomes fragmented. Policymakers are then forced to navigate this fractured landscape of data, leading to potential delays in policy formulation or adoption. Additionally, without clear standards, regulatory bodies often adopt a more conservative stance. This potentially limits the access or use of

certain herbal treatments, even if they hold significant therapeutic promise. (Parveen et al., 2015). This not only restricts patient choice but may also inhibit growth of a potentially transformative sector within the healthcare market.

On the economic front, market dynamics are heavily influenced by consumer trust and the regulatory environments (Halizah et al., 2022). A market without standardized herbal products risks diminished consumer confidence. Patients and consumers seek products that are safe, effective, and consistent in their composition (Mubarak & Mufeeth, 2020). Without standardization, brands and manufacturers might find it challenging to assure consumers of these qualities, leading to potential market declines. Furthermore, new manufacturers could face heightened entry barriers as they grapple with ensuring the consistency product quality without clear guidelines. In this situation, the broader economic potential of the herbal medicine industry could remain under-realized and could be a missed opportunity for both patient healthcare and economic growth.

CHAPTER THREE

Problem Statement

The Population

The magnitude and importance of the growing global demand for herbal medicine is driven by the increased recognition of the potential therapeutic benefits of traditional medicine and its holistic approach to treatment (WHO, 2019). However, ensuring the quality, safety, and efficacy of herbal products remains challenging and unpredictable. Consequently, medical practitioners and patients often express reservations about the use of certain traditional practices, including herbs, choosing instead to rely on Western or conventional pharmaceutical treatments. (Liu et al., 2020). This issue affects a broad spectrum of individuals, including patients who use herbal medicines, healthcare providers who incorporate these remedies into treatment plans, and researchers at the forefront of traditional medicine (Zhang et al., 2018).

The impact is especially significant on the vast population that depends on Traditional Chinese Medicine for healthcare. The general population affected by this problem includes patients, healthcare providers, and researchers who rely on herbal medicine for the treatment and prevention of various diseases. The lack of standardized methodologies for the characterization of bioactive compounds in herbal medicine is of concern as it can lead to inconsistencies in research findings, hindering the development of evidence-based herbal medicine (Liu et al., 2020). Moreover, as global health systems continue to evolve with time, the integration of herbal medicine into mainstream healthcare frameworks remains completely hindered by the inability to ensure reliable and reproducible results across various populations.

The Problem

The complexity and diversity of the chemical compositions in herbal medicine necessitates a comprehensive understanding of their bioactive compounds to uncover potential synergistic effects and interactions (Li et al., 2021). Currently, there is limited information about the methodologies that are used to gain an understanding of the bioactive ingredients contained in herbal plants. The most recognized methodology for preparing herbs for medicinal use involves standardization and characterization. However, existing methods have not been fully tested or proven; and what little information is known indicates that such approaches have many challenges.

The lack of standardized methodologies to verify the quality, safety, and efficacy of herbal medicines undermines the integrity of TCM and its acceptance in the global healthcare industry (Zhang et al., 2018). Without any proper form of standardization, patients might be exposed to inconsistent treatments which can lead to varying therapeutic outcomes or even adverse effects. This issue could easily deter healthcare providers from recommending such treatments to other people and pushing patients towards more expensive pharmaceutical alternatives.

Additionally, the ethical dimension of this problem is multifaceted. On one hand, there is the potential for Western pharmaceutical companies to exploit the results of standardization by combining active ingredients from traditional remedies with synthetic compounds to develop new drugs. This raises new concerns about intellectual property rights and benefit-sharing (WHO, 2019; Zhang et al., 2018; Li et al., 2021). Ensuring ethical practices in such cases involves respecting the source communities' knowledge, maintaining equitable partnerships, and implementing fair compensation for the use of traditional knowledge (Chen, 2023). With time, as herbal medicine continues to gain attention globally, these ethical considerations become paramount in making sure that commercialization efforts do not

exploit or effect indigenous communities or undermine any cultural heritage of traditional healing systems.

Significance of the Problem and the Implications

The standardization of herbal medicine characterization is critical for preserving historical and cultural significance of Traditional Chinese Medicine (TCM) (Zhang et al., 2018;). Standardized methods are essential to the validation and realization of the full therapeutic potential of herbal medicines. This standardization will facilitate the integration of TCM into modern medical practice, as well as enhance patient outcomes (Liu et al., 2020).

Given the limited available information on this topic, the first step is to identify all existing methodologies for standardization and characterization and determine the best practices. This paper is significant because it will help to define what is happening or not happening in the industry. It will facilitate the identification of current industry patterns that can be used to provide a baseline for comparative analysis (Mishra & Tiwari, 2022). Through comparative analysis, we can better assess the strengths and weaknesses of the various methodologies (Ekor, M. 2014). Once an effective methodology is discovered, there will be a corresponding improvement in the quality, safety, and efficacy of reliable herbal formulas, thereby advancing the practice of holistic healthcare treatments.

However, achieving standardization and characterization in TCM is hindered by several challenges, including the need to validate treatments based on historical theories, the contested efficacy of existing methods, and language barriers due to the dominance of Chinese literature (Li et al., 2021). Additionally, there is the ethical quandary of potentially exposing traditional knowledge to exploitation by entities with more resources and different intentions.

This research aims to navigate these challenges by expanding available literature and paving the way for advancements that honor the traditions of herbal medicine while adhering to scientific and ethical standards (Liu et al., 2020). This research is crucial as it seeks to

balance the need for a verifiable evidenced-based standardized approach with the imperative to protecting and respecting the original sources of herbal knowledge. The findings will not only enhance the understanding of bioactive ingredients in herbal medicine but also contribute to improving their quality, safety, and efficacy within the framework of ethical considerations.

Addressing the Gap in Literature

There is a significant paradox between the historical practice of using natural medicinal herbs for treating health care ailments and the emerging trend of relying on evidence-based treatments involving chemicals compounds. The dichotomy lies in evidence-based demand for natural treatment which has fewer side effects than chemical-based treatments. However, the use of medicinal herbs has proven to be somewhat inconsistent as to their effect. While chemical treatments tend to have more side effects, they tend to have a more predictable outcome. One way of addressing this gap is to find a way to enhance the predictability and reliability of medicinal herbs. This approach would assure the public that herbal treatments, which are increasing in demand, can be trusted and that the need to rely only on chemical compounds can be reduced.

The objective of this paper is to address the vital paradoxical gap in literature by systematically reviewing and analyzing methodologies for the standardization and characterization of bioactive compounds in herbal formulas (Li et al., 2021; Liu et al., 2020). The gap hinders the assessment of the quality, safety, and efficacy in TCM (Zhang et al., 2018). This paper can make a small contribution toward solving a major problem facing Traditional Chinese Medicine by enhancing and increasing the available information relating to the development of a standardized methodology for the identification and quantification of bioactive compounds in herbal formulas. The paper aims to contribute toward overcoming the information gaps and limitations in existing literature. This can be achieved through the conduct of a scoping study to inventory and identify existing methods and techniques that have

utility in facilitating the development of systems for standardization and characterization of medicinal herbs.

Verifiable standardization and characterization methods will make it possible for herbal formulas to be trusted for their safety, quality and effectiveness. It is hoped that the scoping study will reveal the best practices and current methodological shortcomings that will create a pathway to the development of universally accepted standards.

Research Questions:

1. What are the prevailing methodologies and practices for the standardization and characterization of herbal formulas in traditional medicine?
2. What are the gaps or limitations within the current literature on standardization and characterization, and which specific areas are in need of further exploration?

Purpose of Study

The extensive body of information on various characterization methods presents a significant problem. Therefore, it is essential to create a standardized methodology for characterizing the bioactive ingredients in herbal formulas.

The quality, safety, and efficacy of herbal formulas rely heavily on the accurate identification of bioactive compounds (Zhang et al., 2012). There is a direct effect on patients, healthcare professionals, and researchers who all rely on herbal medicine to treat a myriad of diseases. The lack of standardized methodologies to extract these bioactive compounds introduce discrepancies in the research, thus creating sizable roadblocks in creating evidence-based herbal medicine (Patra et al., 2010). This paper is important because it provides an opportunity to examine all current methodologies for characterization and begin creating a standardized approach for the characterization of herbal formulas.

The demand for maintaining the quality, safety, and efficacy of these products is exceptionally high, given that 80% of Earth's population relies on traditional medicine, which also encompasses herbal medicine; (WHO, 2013). Moreover, chemical compositions in herbal medicine mandate a comprehensive characterization of bioactive compounds, to understand the synergistic effects and interactions (Li et al., 2008).

To bridge this gap, there is a pressing need for a standardized method to identify and quantify bioactive compounds in herbal formulas. The goal of this research is to compile and compare characterization methodologies. These methodologies will drive evidence-based herbal medicine and the integration of herbal medicine into healthcare practices globally.

The significance of this paper lies in its potential to illuminate the synergistic effects and interactions of bioactive compounds in herbal medicine, ultimately enhancing the quality, safety, and efficacy of these botanical remedies. By standardizing methodologies for characterizing bioactive compounds in herbal medicine, this paper offers valuable insights into the bioactive ingredients in herbal medicine (Zhang et al., 2012; Patra et al., 2010; Li et al., 2008). The most effective approach to begin to achieve standardization is to catalog and compare existing information through a scoping study.

A scoping study is a type of research that examines the extent or the range of available information, the nature of the research, and the activities surrounding a particular topic. They summarize and disseminate research findings while identifying gaps that exist in the current literature. As Arksey and O'Malley's framework denotes, it aims to quickly map the key concepts underpinning a research area, main sources, and types of evidence present. It's useful in complex areas not previously scoped out. This approach is different from systematic reviews as it focuses on broader topics, and not particularly on specific questions. It also does not assess the quality of included studies.

In this scoping study, particular attention will be paid to recent developments in the analytical techniques used for the characterization and identification of novel active components from plant extracts. This list includes advanced techniques in phytochemical analysis, chromatography, and spectrometry techniques, among others. (Cieřla & Moaddel, 2016).

The scoping study is significant because it will help to define what is currently happening — or not happening — in the industry. It will facilitate the identification of current industry patterns that can be used to provide a baseline for comparative analysis (Mishra & Tiwari, 2022). Once an effective methodology is discovered, there will be a corresponding increase in the quality, safety, and efficacy of reliable herbal formulas that will advance the practice of holistic healthcare treatments. (Ekor, M. 2014).

While adhering to scientific and ethical standards in conducting this scoping study, the intent is to navigate these challenges by expanding available literature and paving the way for advancements that honor the traditions of herbal medicine. (Liu et al., 2020). This research is crucial as it seeks to balance the need for a standardized approach with the imperative to protect and respect the original sources of herbal knowledge. The findings will not only enhance the understanding of bioactive ingredients in herbal medicine but also contribute to improving their quality, safety, and efficacy within the framework of ethical considerations.

Research Aims

- **Map the Existing Literature:** To create a comprehensive scoping study of the current literature and evidence related to the standardization and characterization of herbal formulas in Traditional Chinese Medicine (TCM).

- **Identify Gaps:** To identify gaps in the current knowledge and practices related to the standardization and characterization of herbal formulas, highlighting areas that require further research and investigation.

- **Identify and Recommend Best Practices:** To identify and recommend best methodologies for the standardization and characterization of herbal formulas based on the findings from the scoping study.

CHAPTER FOUR

Research Methodology

To execute this scoping review, an intensive research strategy was developed, focusing on scientific literature related to the standardization of phytochemicals in herbal medicine. Selecting specific keywords and MeSH terms such as "Phytochemistry," "Herbal Medicine," "Characterization Techniques," and in the "Standardization Methods," a wide search was accomplished using two databases, including PubMed and Google Scholar. Table 1 indicates the keywords use in PubMed: ("Phytochemicals" OR "Phytochemicals"[All Fields]). AND "Characterization" AND ("Herbal Medicine" OR "Herbal"[All Fields]). ("How to search," 2023). Table 2 indicates the keywords used in Google Scholar: "Phytochemistry" AND "Herbal formula" AND "Characterization" AND "Advance characterization methods" AND "Standardization".

Table 1.

PubMed MeSH Terms

<u>Keywords</u>	<u>Results</u>
("Phytochemicals" OR "Phytochemicals"[All Fields])	3,465
"Characterization"	17,724
("Herbal Medicine" OR "Herbal"[All Fields])	5,009
("Phytochemicals" OR "Phytochemicals"[All Fields]) AND "Characterization" AND ("Herbal Medicine" OR "Herbal"[All Fields])	141
("Phytochemicals" OR "Phytochemicals"[All Fields]) AND "Characterization" AND ("Herbal Medicine" OR "Herbal"[All Fields]) NOT "Synthetic Drugs" NOT "Homeopathy"	141
("Phytochemicals" OR "Phytochemicals"[All Fields]) AND "Characterization" AND ("Herbal Medicine" OR "Herbal"[All Fields]) NOT "Synthetic Drugs" NOT "Homeopathy" NOT "Allopathic Medicine"[All Fields]	141
("Phytochemicals" OR "Phytochemicals"[All Fields]) AND "Characterization" AND ("Herbal Medicine" OR "Herbal"[All Fields]) NOT "Synthetic Drugs" NOT "Homeopathy" NOT "Allopathic Medicine"[All Fields] NOT "Cosmetics"	134
("Phytochemicals" OR "Phytochemicals"[All Fields]) AND "Characterization" AND ("Herbal Medicine" OR "Herbal"[All Fields]) NOT "Synthetic Drugs" NOT "Homeopathy" NOT "Allopathic Medicine"[All Fields] NOT "Cosmetics" NOT "Disease"	117
("Phytochemicals" OR "Phytochemicals"[All Fields]) AND "Characterization" AND ("Herbal Medicine" OR "Herbal"[All Fields]) NOT "Synthetic Drugs" NOT "Homeopathy" NOT "Allopathic Medicine"[All Fields] NOT "Cosmetics" NOT "Disease" NOT "History"	110

Table 2.

Google Scholar Keywords

<u>Keywords</u>	<u>Results</u>
"Phytochemistry"	837,000
"Herbal formula"	30,000
"Characterization"	6,110,000
"Phytochemistry" AND "Herbal formula"	3,180
"Phytochemistry" AND "Herbal formula" AND "Characterization"	1,430
"Phytochemistry" AND "Herbal formula" AND "Characterization" AND "Standardization"	232
"Phytochemistry" AND "Herbal formula" AND "Characterization" AND "Standardization" -"synthetic"	131
"Phytochemistry" AND "Herbal formula" AND "Characterization" AND "Standardization" -"synthetic" -"homeopathic"	126
"Phytochemistry" AND "Herbal formula" AND "Characterization" AND "Standardization" -"synthetic" -"homeopathic" -"allopathic"	124
"Phytochemistry" AND "Herbal formula" AND "Characterization" AND "Standardization" -"synthetic" -"homeopathic" -"allopathic" -"cosmetic"	115
"Phytochemistry" AND "Herbal formula" AND "Characterization" AND "Standardization" -"synthetic" -"homeopathic" -"allopathic" -"cosmetic" -"disease studies"	115
"Phytochemistry" AND "Herbal formula" AND "Characterization" AND "Standardization" -"synthetic" -"homeopathic" -"allopathic" -"cosmetic" -"disease studies" -"historical review"	113

The MeSH terms and Keywords were designed to include all relevant articles. The aspiration was to encapsulate a vast spectrum of both current and foundational research, putting a particular focus on studies released in the past 15 years to capture the latest advancements. This strategy was further enhanced by applying specific filters to exclude irrelevant studies, such as those focusing on synthetic compounds or unrelated medical disciplines, ensuring the results were directly aligned with the review's goals.

An initial review of the literature resulted in a reasonably expansive collection of studies, which were then thoroughly examined to identify those that directly contributed to the knowledge pool of advanced phytochemical characterization techniques in herbal formulas. Titles and abstracts were initially reviewed, followed by a detailed review of full texts to make sure they met specific selection criteria. Empirical studies detailing analytical methods and their application to herbal medicine standardization were given the highest priority. This focused approach was highly focused and methodical, which helped in refining the search to a select group of studies that substantially enriched the understanding of herbal medicine characterization and standardization.

Criteria for Inclusion/Exclusion

This review considered systematic analysis reviews, empirical research, and analytical studies that explored the phytochemical properties, characterization methods, and standardization of herbal medicines. Studies not focused on areas of phytochemical analysis or standardization were excluded. The exclusion criteria included research on synthetic substances, non-herbal supplements, cosmetic uses, disease-specific studies that did not relate to analytical techniques, and historical overviews lacking relevance to modern practices.

Inclusion Criteria Clarification

1. **Empirical Research:** Focused on original studies that highlighted details on standardization and characterization of phytochemicals, integral to advancing herbal medicines science.

2. **Specific Subject Matter:** Extracted articles fixated on "Phytochemistry," "Herbal Formulas," and "Characterization," topics, aiming to further enhance the objective of characterization of herbal formulas through modern analytical methods.

3. **Recent Studies:** A critical emphasis is placed on the latest discoveries to make sure that the review encapsulates the most advanced methods in herbal characterization and standardization aligning with the rapid evolution of analytical technologies and techniques.

Exclusion Criteria Clarification

1. **Research Off-topic:** Studies focused on synthetic, homeopathic, and allopathic medicines were excluded, maintaining a focus on natural herbal formulas and their specific standardization complexities.

2. **Cosmetic-focused Research:** Excluded because the review's point of view was strictly medicinal, underlining the significance of therapeutic effectiveness and safety.

3. **Herb-specific Inquiries:** Focused only on techniques of characterization, discarding studies disease-focused unless they provided insights into the methods of standardization; this helped in concentrating on the analytical aspect of herbal medicines.

4. **Herb-specific Without General Application:** Excluded to prioritize research with broader implications for the field, to promote clarity on understanding the processes of standardization across a variety diverse of herbal medicines.

5. **Historical Analysis:** Though valuable, historical studies were excluded to focus on contemporary practices, ensuring the review propels a forward discussion in the field.

6. **Accessibility of Full Texts:** Only studies with full-text availability were included, ensuring a transparent and thorough examination process.

7. Lack of adequate Statistical Analysis: Excluded to maintain high scientific integrity, ensuring the studies included provided strong, evidence-backed conclusions relevant to the herbal medicine domain.

Data Collection

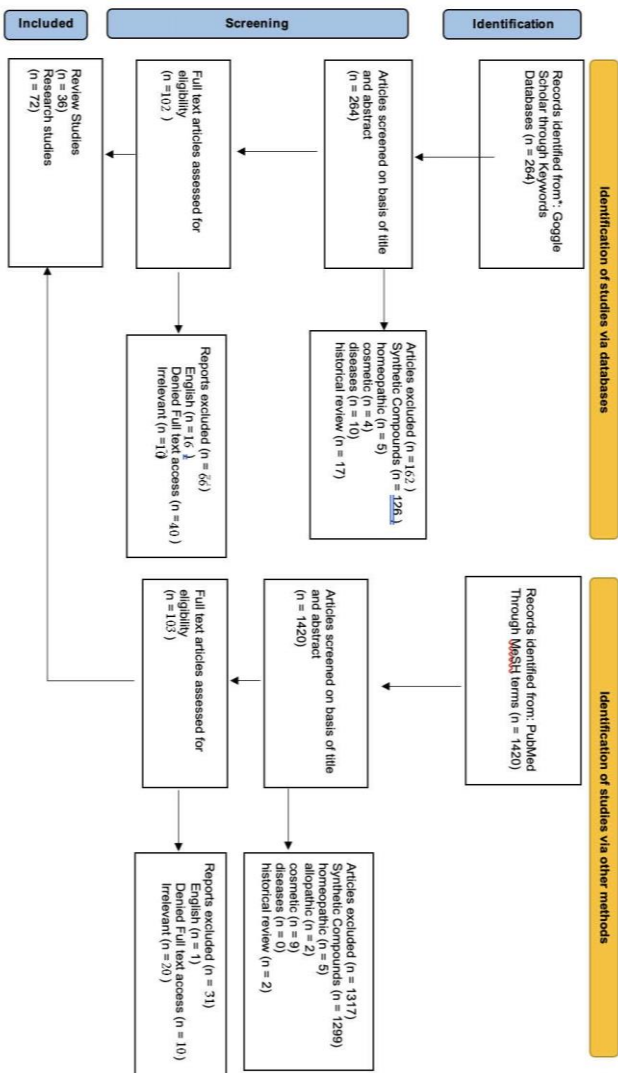
Data was pulled out methodically from the articles chosen. It's interesting, to note the publication year, the methods for analyzing phytochemicals (like chromatography and spectrometry); the assortment of herbal drugs under review, phytochemicals, and the expanse of herbal formulas investigated. The methods for characterization and standardization, the effectiveness of these approaches in figuring out active components, and qualitative and quantitative analysis of phytochemicals, were recorded in detail. For empirical studies, information such as sample size and experimental conditions were considered. Reviews regarding the number of studies was analyzed and significant conclusions were documented, illustrating the existing breadth of research in the field. This data assures a complete overview of the presented methods of phytochemical standardization and characterization of herbal medicines, that is in sync with the review's aim to accentuate progress and identify gaps in literature.

Analyzing Data

Figure 2 [PRISMA] shows the data from systematic reviews and studies were separately analyzed to recognize the developments and patterns in methods used for standardizing and characterizing phytochemicals. Major revelations regarding efficiencies of different characterization techniques, and capabilities in identifying and quantifying phytochemicals of herbal medicine formulas were identified. They were categorized by the method (e.g., chromatography, spectrometry), and the type of herbal formulation research. Themes being repeated, strong points in methodology, and possible inadequacies in the research were spotted, summarized, and interpreted based on their possible impact on improving herbal products' standardization and safety. The strategy for analysis aims to present a clear picture of the existing field that can guide future directions of research.

Figure 2

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers and other sources



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: <http://www.prisma-statement.org/>.

RESULTS

The scoping review was conducted with a focus on sifting through the expanse of scientific literature related to the standardization of phytochemicals within herbal medicine. Keywords and MeSH terms were meticulously selected to encompass a wide array of pertinent studies from the past 15 years. "Phytochemistry," "Herbal Medicine," "Characterization Techniques," and "Standardization" were used as search anchors. Figure 2 shows Google Scholar and PubMed databases were used to ensure a comprehensive search.

Google Scholar Search Refinement

In Google Scholar, the keyword string "Phytochemistry" AND "Herbal formula" AND "Characterization" AND "Advance characterization methods" AND "Standardization" returned 264 initial records. The title and abstract screening were scrupulous, maintaining all 264 records for full-text review. Exclusion criteria applied at this juncture led to the dismissal of 162 articles based on content focus areas: synthetic compounds (126), homeopathy (5), cosmetics (4), diseases (10), and historical reviews (17). Additional exclusions were applied for articles with language barriers (16), denied full-text access (40), and those deemed irrelevant (10), resulting in a curated list of 20 reviews and 16 research studies.

PubMed Database Inquiry

The PubMed search, utilizing MeSH terms like ("Phytochemicals" OR "Phytochemicals"[All Fields]) AND "Characterization" AND ("Herbal Medicine" OR "Herbal"[All Fields]), produced 1,420 records. After the title and abstract review, articles were excluded on the following grounds. The initial screening excluded 1,317 articles, predominantly for their content on synthetic compounds (1,299), homeopathy (5), allopathy (2), cosmetics (9), and historical reviews (2). The remaining articles faced further exclusions for English language

restrictions (1), lack of full-text access (10), and irrelevance (20), leaving a tally of 16 reviews and 56 research studies to be included from PubMed.

Consolidated Synthesis from Both Databases

After thorough screening and eligibility checks, the process concluded with 108 articles, comprising both review and research studies from Google Scholar and PubMed. This ensemble included:

- **Google Scholar:** 20 reviews and 16 research studies.
- **PubMed:** 16 reviews and 56 research studies.

The total included literature comprised 36 review articles and 72 research articles across both databases.

Inclusion and Exclusion Criteria in Relation to the Review Findings

The final curation of 108 articles directly corresponds to the stipulated inclusion and exclusion criteria set forth at the onset of this scoping review. Below is an elucidation of how the selected literature aligns with these criteria:

Inclusion and Exclusion: A Detailed Narrative

The review focused on empirical research and analytical studies that profoundly delve into phytochemical properties and characterization methods. The following inclusion and exclusion criteria were stringently applied:

- **Inclusion Criteria Clarification:**
 - Empirical research focused on "Phytochemistry," "Herbal Formulas," and "Characterization."
 - Recent studies emphasize advanced methods for standardization.

- **Exclusion Criteria Clarification:**

- Off-topic research, including synthetic, homeopathic, and allopathic medicines.
- Cosmetic-focused research.
- Disease-specific inquiries, unless contributing to characterization techniques.
- Historical analysis was overlooked in favor of contemporary practices.
- Research with inadequate statistical analysis was excluded to uphold scientific integrity.

Data Collection

Data was methodically extracted from the articles chosen. We covered the publication year, the methods for analyzing phytochemicals (like chromatography and spectrometry), the assortment of herbal drugs under review, phytochemicals, and the expanse of herbal formulas investigated. We recorded data on the methods for characterization and standardization, the effectiveness of these approaches in figuring out active components, and qualitative and quantitative analysis of phytochemicals. Empirical studies information, such as sample size and experimental conditions were taken into account. Reviews regarding the number of studies were analyzed and significant conclusions were documented, illustrating the existing breadth of research in the field. The data assures a complete overview of the presented methods of phytochemical standardization and characterization of herbal medicines. This information is consistent with our scoping study which aims to accentuate progress and identify gaps in literature.

Analyzing Data

Data from reviews and research studies were analyzed separately to identify the developments and patterns in methods used for standardizing and characterizing phytochemicals. Major revelations regarding efficiencies of different characterization techniques, and

capabilities in identifying and quantifying phytochemicals of herbal medicine formulas were identified. They were categorized by the method (e.g., chromatography, spectrometry) and the type of herbal formulation research. Themes being repeated, strong points in methodology, and possible inadequacies in the research were spotted, summarized, and interpreted based on their possible impact on improving herbal products' standardization and safety.

The context of the data utilized in the study provides a solid framework for understanding how the findings impact our analysis. By reviewing data in the context, we were able to compare the utilization of different characterization and standardization, the period covered, and how the data was distributed across various regions and countries.

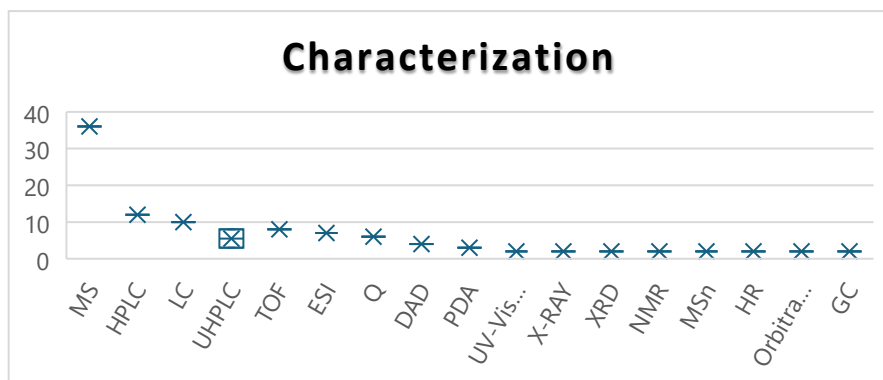
Table 3 outlines the methodologies commonly employed for characterization.

Table 4 presents the number of articles published per year over the study period.

Table 5 captures the frequency of techniques of the number of articles for standardization.

Table 6 provides a summary listing of the total number of techniques utilized in the data collected.

Table 3



Characterization Techniques: This chart outlines the most used techniques in the studies, with a specific focus on the continuous use of particular methodologies in the characterization process. It presents the reader with how instrumental these techniques are in analysis and characterization of chemical compounds. From the literature reviewed, it is evident that **Mass Spectrometry (MS)** is the most frequently employed technique, cited 36 times. It does so while further emphasizing the practicality of this approach, as well as the ability of mass spectrometry to identify and characterize molecular species. The bulk of its importance lies in high sensitivity, accuracy, and the capacity to provide a detailed molecular description, making it an exceptionally useful tool in both qualitative and quantitative works.

The next most cited technique to Mass Spectrometry is **High-Performance Liquid Chromatography HPLC** at 12 citations in total. HPLC continues to be valued for its effectiveness in analysis and identification, alongside quantifying constituents in multicomponent preparations. Its versatility of analyzing samples of different types, as well as the ability to work with different detectors, makes it one of the most widely used techniques in analytical laboratories. The utility of HPLC can be attributed to its efficiency, especially in research and

development, its ability to provide results which are comparable and consistent across all applications.

Mass spectrometry, Liquid Chromatography (LC) and capillary electrophoresis (CE) are mentioned frequently with twenty-three, ten and twenty-two references respectively which shows that analytical studies could not be carried out without these techniques. LC encompasses various techniques such as HPLC and **Ultra High-Performance Liquid Chromatography, Ultra-HPLC** to name but a few that involves the separation of compounds, depending on their interaction with the Stationary phase and the mobile phase. The LC is an important aspect that is prevalent in most of the separation sciences in accordance with the detailed mention of the term in the paper.

Ultra-High Performance Liquid Chromatography (UHPLC) referred to 8 times, is an improved version of the HPLC which offers shorter analysis time and better resolution. It should be noted that due to its high-pressure design, UHPLC offers both higher separation efficiency and shorter analysis times, which are invaluable in applications where throughput is paramount.

The tools mentioned 8 times are **Time-of-Flight (TOF) Mass Spectrometry**: a highly effective analytical method that meets users' high requirements for the speed of data generation and mass accuracy. Compared to other forms of mass spectrometry, TOF-MS is especially helpful in analyzing large biomolecules and compounds as well as complex samples or mixtures because it is designed to accurately measure mass-to-charge ratios.

Lastly, **Electrospray Ionization (ESI)**, cited 7 times, is a common ionization technique for mass spectrometry. It helps in the study of large biomolecules by creating ions from the macromolecules which are in solution form. Its significance is made apparent by its constant reference; it is discussed extensively and underlies several aspects of the current generation of tools, especially in proteomics and metabolomics.

Fig. 3

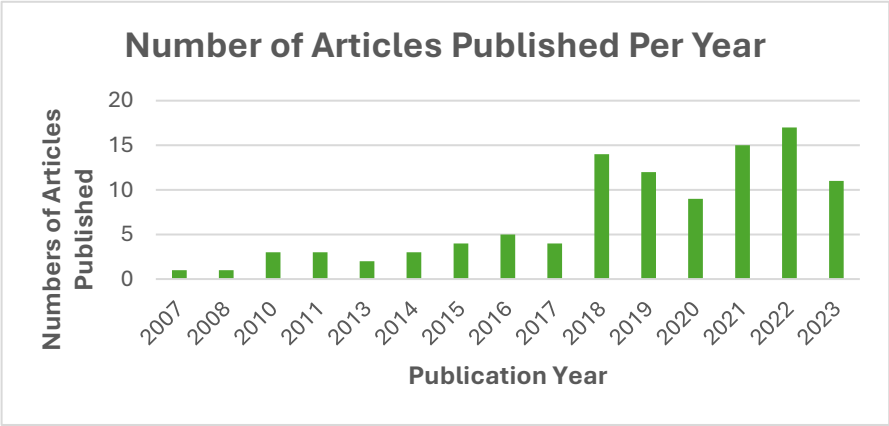
Research by Region



Geographic Distribution: The geographic distribution of the studies underscores a substantial body of research emerging from China, a leader in traditional medicine, with a notable body of work also originating from India. This significant output aligns with these regions' longstanding medicinal traditions and growing investment in herbal medicine research. Emerging contributions from countries such as Malaysia and Korea signal an expanding interest and an international acknowledgment of the value of traditional practices. Further afield, studies from Italy, Egypt, and Iran reflect the universal appeal and relevance of herbal medicine, and its integration into diverse cultural health paradigms. The research's global dispersion illustrates a dynamic exchange of knowledge and practices that enriches the collective effort to achieve standardization and quality control in herbal medicine, bridging traditional wisdom with modern scientific validation.

Table 4

Trend of years of research



Publication Trends: An analysis of the publication trend shows a discernible escalation in scholarly output over the years, with a peak in recent times from 2018-2023, which may denote increased recognition of herbal medicine's potential and a response to the pressing need for novel therapeutics. The consistent production of research, particularly the surge in the last decade, points to a concerted effort in the scientific community to address challenges in herbal medicine through advanced research. This enduring interest is possibly catalyzed by technological advancements in analytical techniques, enabling a deeper exploration into the phytochemical intricacies of herbal compounds and fostering a methodical approach to their standardization.

Table 5

Standardization Techniques



Standardization Techniques: In the context of the present paper, Table 3 shows the relative use of various standardization techniques in the contemporary literature on herbal medicine. **Quality Control Assessments (QCA)** emerge as the most frequently incorporated assessment type and rightly so since 9 articles covered this topic, before a product containing herbs reaches the market. Likewise, **Good Manufacturing Practices (GMP)** and **Chemical Profiling** methods (e. g., HPLC, GC, TLC) also take significant place, with 7 articles each. These methods are highly critical in establishing consistent standards for herbal products, ensuring their safety, efficacy and reproducibility.

Fingerprint Analysis and the use of Chemical and DNA Markers are highlighted in 5 articles, showcasing their importance in verifying the authenticity of specific herbal components. These techniques are very important for ensuring that the bioactive components are identified correctly. On the other hand, **Pharmacopoeial Standards** and **Standard Operating Procedures (SOPs)** are topics that are mentioned less often: There are 4 articles on each of these topics. Similarly, **Metabolomics**, with 3 articles indicates that there is at present

an increasing trend to apply highly complex analytical methods and regulations to the field of herbal medicine.

The focus of the studies included in this paper was aimed at identifying various approaches to delineating ways and methods for determining the bioactive components of herbs and herbal formulas. Table 6 provides a summary listing of the different techniques and the number of times the technique was used throughout this study. It is important to note that these techniques have been employed for this purpose over the 16-year period covered by this study. A review of the table shows that the studies incorporated 47 different techniques across all the 108 studies, in one form or another. Although the number of techniques totaled 47, the individual techniques were used multiple times throughout the various studies. The analysis shows that, collectively, the techniques were used 138 times. While the frequency in the use of the techniques total 138, Table 6 shows that the most used techniques are **Mass Spectrometry (MS)**, **High-Performance Liquid Chromatography (HPLC)** and **Liquid Chromatography (LC)**. They account for a total of 55% of all techniques used. This means that the most used techniques are not only used singularly, but they are also used in conjunction with other techniques.

SUMMARY OF TECHNIQUE

TABLE 6

#	Technique	Frequency
1	MS	36
2	HPLC	12
3	LC	10
4	TOF	8
5	ESI	7
6	Q	6
7	UPLC	5
8	DAD	4
9	PDA	3
10	UHPLC	3
11	FLD	1
12	CPC	1
13	UVI Vis Spectroscopy	2
14	X-RAY	1
15	XRD	2
16	SEM	1
17	TEM	1
18	DLS	1
19	RP	1
20	M	1
21	FRAP	1
22	UV -Visible Spectrophotometry	1
23	FT	1
Subtotal		109
Total		

#	Technique	Frequency
24	IR	1
25	GC	2
26	IT	1
27	NMR	2
28	1H	1
29	QAMS	1
30	LTQ	1
31	MSn	2
32	HR	1
33	EZP	1
34	Orbitrap Mass	2
35	NGS	1
36	UV-Spectroscopy	1
37	BCD	1
38	TLC	1
39	FTR	1
40	PDP	1
41	HLPC	1
42	DART	2
43	SFE	1
44	HPTLC	1
45	QQQ	1
46	MRM	1
47	2DNMR	1
Subtotal		29
138		

SUMMARY OF STANDARDIZATION TECHNIQUES

TABLE 7

#	Technique	Frequency
1	Good Manufacturing Practices (GMP)	7
2	Fingerprint Analysis (Including DNA and chromatographic fingerprinting)	4
3	Chemical Profiling (Utilizes techniques like HPLC, GC, TLC)	7
4	Markers (Chemical and DNA)	4
5	Pharmacopeial Standards	3
6	Quality Control Assessments	9
7	Metabolomics	2
8	Standard Operating Procedures (SOPs)	3
9	Development of global regulatory standards	1
10	Standard operating procedures for manufacturing processes	1
11	Spectroscopic and chromatographic techniques	1
12	Safety and quality control standards	1
13	Physical, Chemical, Biological Assessment	1
14	Quality markers (Q-markers)	1
15	Microbial contamination testing	1
16	Specific pathogen testing	1
17	Test for aflatoxins	1
18	Pesticide residue analysis	1
19	Heavy metals determination	1
20	Safety assessment	1
21	Efficacy assessment	1
22	Physicochemical analysis	1
23	Lyophilization (freeze-drying)	1
24	Viscosity measurement	1
25	Melting Point determination	1

(Continued)

#	Technique	Frequency
26	Solubility tests	1
27	Moisture Content and Volatile Matter determination	1
28	Optical Rotation	1
29	Refractive Index	1
30	Stability assessment and shelf life testing	1
31	Ash Values and Extractives	1
32	Determination of Extractable Matter	1
33	Bitterness Value measurement	1
34	Hemolytic Activity	1
35	Swelling Index	1
36	Foaming Index	1
37	Determination of foreign matter	1
38	Pharmacodynamics	1
39	Pharmacokinetics	1
40	Dosage	1
41	Stability	1
42	Shelf-life	1
43	Toxicity evaluation	1
44	Pharmacological parameters	1
45	Sensory evaluation	1
46	Rationalizing combinations in polyherbal drugs	1
47	Protocol-based standardization	1
48	Chelating agent testing	1
Total		79

These findings collectively underscore the intricate interplay between the methods used to characterize the complexities inherent in herbal medicines and the standardization efforts that ensure their safe and consistent application. The results of this scoping review provide a substantive foundation to be further discussed, contextualized, and built upon in the discussion chapter of this study, driving forward the

agenda for developing evidence-based standardization protocols that could be universally adopted within the field of herbal medicine

CHAPTER FIVE

Discussion and Prevailing Techniques

The Objective

The main purpose of this study includes the development of a scoping review of the range of methods that can be employed to standardize and characterize herbal formulas. This research seeks to better understand the applicability, shortcomings, and prospects of these techniques in the quality, safety and efficacy of herbal medicines. Thus, through a critical evaluation of both standardization and characterization techniques, it is the aim of the research to advance the state of the art and offer insights into future enhancement and development.

The Importance of Standardization and Characterization of Herbal Formulas

Standardization relates to the processes used for developing formulas for medicinal treatments in the practice of herbalism. This process aims to guarantee the stability and reliability of herbal preparations. When a proven standard operating procedure is implemented, it provides evidence-based verification that a formula has undergone rigorous screening and results in the production of an exact desired quality and reliability each and every time a formula is produced.

Conversely, characterization deals with the bioactive nature and behavior patterns of herbs and their impact on a given formula. An effective characterization process clearly distinguishes the various parts of an herb and how the components interact with other herbs contained in an herbal formula.

Once an herb has been accurately characterized, it can be processed through the standardization process. The processes of

enhancing coordination between standardization and characterization can result in herbal formulas becoming more consistent and reliable. As these processes advance herbal formulas will be better recognized as having the evidenced-based proof to demonstrate the quality, efficacy and safety necessary to become a viable treatment alternative that is fully accepted as a part of the universal health delivery system.

It is important to note that there are some key methodologies that offer potential applications which can examine medicinal plants for, among other things, their phytochemical and therapeutic properties. These methodologies are useful for consumer safeguards, enhancing the authenticity of the herbal products, and extending the utilization of the herbal remedies as a complement to the universal health system.

Based on the literature review for the period between 2007 to 2023, the most prevalent techniques that have been used repeatedly throughout the period include six characterization techniques and 8 standardization techniques. A summary of the six characterization techniques is provided in the following Section A and a summary of the eight standardization techniques is provided in Section B.

Section A: Summaries of characterization techniques

(1)**Mass spectrometry (MS)** is an analytical method that involves the determination of the mass of ions whose mass to charge ratio is determined to give information over the molecule composition and structure. In MS, a sample is ionized, especially through time, temperature or even exposure to a beam of electrons, for the purpose of making the molecules fragment or become positively charged. MS can be employed in forensic science for measuring the concentration of chemicals in body samples collected from crime scenes (Mumtaz et al., 2017), in food chemistry for identification of contaminants and suitability for human consumption (Wu et al., 2017), in pharma chemistry to assess new pharmaceutical compounds (Zhang et al., 2019), in environmental chemistry for determination of quantity of pollutants (Mumt et al., 2017).

(2)**High-Performance Liquid Chromatography (HPLC)** is another chromatographic method which is used to separate and identify chemically different substances in a mixture and possibly calculate their concentration. HPLC deals with the sample solvent and a stationary phase then separates the components; it plays an important role in the identification, separation, and determination of concentration of compounds. HPLC has been employed in various fields by people in pharmaceutical and biopharmaceutical research (Zhang et al., 2019), environmental research (Mumtaz et al., 2017b), the food and beverage industry (Wu et al., 2017), clinical diagnostics, forensic science (Mumtaz et al., 2017a), and academia.

(3)**Liquid chromatography** is a core analytical tool that helps in the separation, identification, and further purification of the compounds that are present in samples in both quantitative and qualitative capacities (Smith et al., 2020). This method operates by fractionating sample constituents by solvents wherein distinct components meet a liquid phase that is moved and a solid phase to which it is bound (Johnson & Lee, 2019). They observed that as the components pass through the column, they independently partition depending on their inclination to the mobile phase (Anderson et al., 2018). For example, while immobile phase has polarities diffusing about the entire body, parts with diverse polarities move uniquely when the mobile phase with polarity alike is layered, and thus generating diversified bands within the column (Taylor, 2021). These separations are essential for visualizing colored components and are widely adopted to analyze multi-component samples in all the major fields of application that include pharmaceuticals, biopharmaceuticals, environment, Food and drinks industries among others (Miller & White, 2017; Davis et al., 2016).

(4)**Ultra-High Performance Liquid Chromatography (UHPLC)** is a sub-type of HPLC that works under highly pressurized pneumatic or hydraulic technique through which solvents are forced through the column to allow for efficient separate at shorter times (Brown & Chen, 2020). With the help of UHPLC, it is possible to determine the presence and (or) concentration of individual compounds in binary and multicomponent mixtures; the technique is of major importance for the separation of amino acids, proteins, pesticides, antibiotics, etc. Furthermore, it plays a crucial role in evaluating the purities of the sample, as well as in the Quality Assurance (Miller & Taylor, 2018). This technology's applicability extends to a wide variety of fields, ranging from pharmaceuticals and biotechnology through ecology, food chemistry, and technology of chemical and petroleum products, forensic science and others (Davis & Green, 2017; Anderson et al., 2016). It is also widely used in academic and research organizations in different forms of analyses (William and Scott, 2022).

(5)**Quadrupole Time-of-Flight Mass Spectrometry (QTOF)** is a mass spectrometer which is comprised of both a quadrupole filter and a time-of-flight analyzer, where the quadrupole filter is used to select the ions based on their m/z ratio while the TOF analyzer measures the time taken by the ions to reach the detector, making the measurement of mass with higher precision (Johnson & Smith, 2021). This way, high mass resolution and accurate mass measurements can be obtained which is important in the identification of unknown compounds and also in the confirmation of particular analyte present in a sample (Miller et al. , 2020). QTOF has a high specificity and performance in large sample masses and can be suitable for proteomics, metabolomics, environmental analysis, drug discovery, and development (Brown et al. , 2019; Williams & Taylor, 2022). In addition, QTOF has extended capability of Data-Independent Acquisition (DIA) mode

which means that the acquisition of mass spectra is done randomly rather than selective and this is good for tackling complex samples and generating large data sets (Lee & Chen, 2018).

(6)**Electrospray ionization (ESI)** is a key ionization technique applied in mass spectrometry to resolve the molecular mass of biopolymers like peptides, proteins and nucleic acids is the Electrospray Ionization (ESI). The processes include In this process a drop of the sample solution containing a volatile solvent is introduced into the mass spectrometer, a high voltage needle in the instrument creates droplets (Smith et al., 2020). Because the solvent breaks up into ions in the gas phase as it is volatilized, the analyte molecules are left as charged ions in the gas phase (Brown & White, 2019). These ions are then directed into the mass analyzer, where they are sorted based on the intensity of the signal and their mass-to-charge ratio (m/z) (Davis & Green, 2018). ESI also has several advantages; it can efficiently ionize large complex biomolecules with minimum fragmentation which is vital in identification and quantification particularly in proteomics, metabolomics and drug discovery.

Effectiveness Based on Characterization Techniques

The above summary descriptions of characterization techniques serve as a demonstration that each technique is different and as a result, they have varying degrees of effectiveness and limitations. As noted earlier in Table 6, while there were only 47 primary techniques found, the frequency of utilization is almost three times the number of basic techniques. This suggests that the desired outcomes could not be effectively achieved through a single technique and needed the help and support of other techniques. This process of help and support is often referred to as pairing. The following are examples of common pairing relationships between the most prevailing techniques and demonstrate how these prevailing techniques are used together to overcome challenges and achieve greater effectiveness.

When Mass Spectrometry (MS) was used, the most common pairing occurred with Gas Chromatography (GC), High-Performance Liquid Chromatography (HPLC), and Nuclear Magnetic Resonance Spectroscopy (NMR).

I. Mass Spectrometry Pairing

1. Mass Spectrometry Paired with Gas Chromatography (MS-GC)

GC-MS has played a pivotal role in phytochemical works especially analyzing volatile compounds. Its utility is evidenced in works, including those of Smeriglio et al. (2019), and Anibogwu et al. (2021), where the publications focused on phytotoxicity and the identification of essential bioactive compounds. Still, GC-MS suffers from significant data inputs' variability of a complex protocol and additional sample preparation steps, which limits the method's reproducibility and accuracy (Fonmboh et al., 2020; Sharma et al., 2023). Related methods like NMR and FT-IR improve the identification given by GC analysis because it allows for a wider range of compound ionization and structural characterization as shown by Mumtaz et al. (2017). However, the use data integration results in challenges in methods for combining data from different analytical tools, which escalates the operation costs and makes it complex (López-Barrera et al., 2022). Moreover, the combination process of TLC and HPLC has given the opportunity to analyze a wider spectrum of substances that are uncommon when using either method alone but at the same time it is more challenging in terms of data interpretation (Elshafie et al., 2019). To this end, there are some major challenges that have been observed particularly in terms of standardization and methodological updating, especially when it comes to developing a unified protocol for increasing rather than facing the issue of replicability that is coupled with a high cost (Sharma et al., 2023). These areas should inform future research to make sure that GC-MS, alongside any linked strategies, are suited to the shifting requirements for the characterization of herbal medicines.

2. Mass Spectrometry Paired with High-Performance Liquid Chromatography

(MS-HPLC)

While the pairing of MS and HPLC are effective together, they are also coupled with HPLC-UV-Spectroscopy, MS/MS and FLD. This pairing and coupling are considered a top priority among the instrumental methods, which have been found to be very useful for the phytochemical analysis of herbal drugs. These techniques have enabled comprehensive compound profiling in many studies, including the pharmacokinetics study of *Gastrodia elata* by Wu et al. (2017). However, because of work such as that done by Taamalli et al. (2018) on Tunisian olive leaves, the variation in natural compounds present in these plants make the standards a challenge to achieve.

The use of enhanced versions of MS particularly PDA-ESI-MS/MS and LC-ESI(+)QTOF-MS has helped in increasing the sensitization and accuracy of mass measurements which are vital in complex natural product identification (Gao et al. , 2021; Zhang et al. , 2011). More complexity is observed when HPLC is combined with other techniques such as Q-TOF/MS, DAD-ESI-MSN, and QqQ-MS/MS, which identify and profile phytochemicals (Zheng et al., 2010; Rivera-Mondragón et al., 2019). However, these technologies are still relatively expensive and cumbersome, and data merging also depends on various data streams, for instance merging NMR and DAD-QTOF structures for better structure determination (Fonmboh et al., 2020).

However, the huge importance of the HPLC-MS summarized above and its ability to identify particular bioactive compounds in roots of *Osmanthus fragrant* and *Pueraria lobata* (Liao et al., 2021; Gao et al., 2021), shows a necessity for more standardized protocols. Future research should also concentrate on the improvement of these methods; but should also focus on discovering cheap protocols that are easy to repeat for the standardization of those herbal medicines.

3. Mass Spectrometry Paired with Nuclear Magnetic Resonance (MS-NMR)

The combination of Mass spectrometry coupled with Nuclear magnetic resonance spectroscopy imaging more and characterize complex intentional organic compounds making it a strong match for phytochemicals characterization. This association has been greatly embraced in works like that of Rivera-Mondragón et al. (2019) who employed High-Performance Liquid Chromatography-Diode Array Detection Quadrupole Time of Flight-Tandem High-Resolution Mass Spectrometry (HPLC-DAD-QTOF) hydrogen nuclear magnetic resonance (NMR) to elucidate the phytochemicals in ceptopharáidin species thus identifying diverse and likewise, Ha and his fellow authors also employed LC/MS linked with HR-ESIMS and NMR to elaborate specific constituents within *W. somnifera*, namely with anolide glycosides, to enhance its bioactivity investigation. Although all these methodologies are rich in detail and depth, they face several challenges, which include issues of geographical and biological narrowness and the lack of accommodation for general bioactivity assessments. These issues point to the fact that it is imperative to continually strive to refine the methods applied in such studies to increase the reliability of results, and also to untangle some of the major operational issues that study entails. Further studies should be made with an attempt to fine tune these methodologies to ascertain that MS and the incorporative designs keep on addressing the emerging needs of herbal medicine research satisfactorily.

II. High-Performance Liquid Chromatography Pairing

The three most common techniques paired with High-Performance Liquid Chromatography (HPLC), are High-Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC/MS/MS), Electro-Spray Ionization (ESI), and Mass Spectrometry (MS)

1.High-Performance Liquid Chromatography Tandem paired with Mass Spectrometry/Mass Spectrometry (HPLC-MS/MS)

Our review shows that the presented research employed High-Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC/MS/MS) which is one of the most common and primary methods of phytochemical analysis with possible supplementation of other methods depending on the particular research objectives. For example, Gao et al. (2021) employed a synchronized HPLC configuration with PDA detection, ESI, and MS/MS in a study to quantify and identify various isoflavonoids present in *Pueraria lobata*, thus illustrating the versatility of the mentioned approach for the analysis of complicated natural substances. Despite this success, certain drawbacks familiar to enthusiasts, namely concern over matrix sensitivity and typically elongated sample preparation processes, pose certain restrictions on wider use.

Additionally, some methods such as HPLC-DAD-MS/MS-BCD and HPLC-ESI-MS/MS have been used in several investigations for the rapid assessment and chromatographic studies where various groups of volatile compounds as depicted as noted, Li et al. (2014) and Zheng et al. (2010). These studies show that the developed method has higher sensitivity and specificity as compared to earlier traditional methods; however, at the same time they also point toward some potential problems such as non-specific enzyme inhibition and matrix effects which are necessary to have stricter calibration and to establish a more solid and reliable method.

The article citation also demonstrated the effectiveness of HPLC-MS/MS in herbal medicine research as shown by the following applications. However, some emerging issues like the requirements for time-consuming sample preparation steps and the susceptibility to diverse matrix interferences, demonstrate the potential for further enhancements in the methodological aspect. Subsequent research should deliberate on how to improve the sample preparation methods and implement better detection systems in order to overcome any future

weaknesses of HPLC-MS/MS in the advancing area of phytochemical study.

2. High Performance Liquid Chromatography paired with Electro-Spray Ionization (HPLC-ESI)

High Performance Liquid Chromatography (HPLC) paired with Electro-Spray Ionization (ESI) in plant investigation is ubiquitous and often supported by secondary techniques to improve the elucidation of substances. Altogether, as evidenced by diverse investigations in the present study, the combination of these two methodologies is highly synergistic for accurate chemical characterization of sundry herbal medicines. For instance, HPLC-PDA-ESI-MS/MS used by Gao et al. (2021), effectively detected several isoflavonoids in *Pueraria lobata* indicating that the chosen method is effective for analyzing complex molecules in natural products.

Extension of UHR-FT-MS technologies, ESI-TOF as used by Shi et al. (2021) extends the mass accuracy and resolution that is crucial in achieving quantitative and structural analysis of the organic molecules. Moreover, DAD-ESI-MSN, which integrates DAD's high sensitivity with the more specific information about the ions given by ESI-MSN mass spectra, improves the specificity and, to some extent, the quantification of the analyses, though this is occasionally restricted by ion suppression.

3. High Performance Liquid Chromatography paired with

Electro-Spray Ionization, and in conjunction with ESI-MS/MS and DAD/ESI-MSN

When used in conjunction with HPLC-ESI, techniques such as ESI-MS/MS and DAD/ESI-MSN demonstrate versatility in application, as observed in this study. However, they are faced with the following drawbacks which include complex sample preparation, matrix

interferences, and the need for highly skilled personnel. These issues indicate that there are vast opportunities for improvement in method development to tweak the HPLC-ESI to provide a broad range of analysis in the studies of natural products.

4. High-Performance Liquid Chromatography paired with Mass Spectrometry (HPLC -MS)

High-Performance Liquid Chromatography (HPLC) along with Mass Spectrometry (MS) serve as a very powerful weapon in phytochemical investigations of intricate herbal samples, often complemented by UV-Visible Spectroscopy, Tandem MS, or Electro Spray Ionization to increase the strength of the technique. This is a multifaceted approach, and examples of such approach include (Wu et al., 2017). which used both HPLC/ UV-Vis and MS/MS for the pharmacokinetic analysis of *Gastrodia elata* for phytochemicals; however, there were no proper in vivo drug interaction assessment. Similarly, (Taamalli et al., 2018). HPLC was improved through the addition of ESI-TOF and IT/MS for analyzing phenolic compounds which present in the Tunisian olive tree leaves; the authors were concerned about regional variations in the concentration of phenolic compounds that could hamper standardization of the procedure.

Additionally, (Tran et al. , 2017), HPLC-MS was used to extract metabolites that possess antiviral properties against influenza virus from *Polygonum Chinese*, proving that the method is efficient for the identification of active compounds; although, there are issues concerning toxicity at higher concentrations of the magnitude of the metabolites. (Zhang et al. (2011). Also when HPLC was employed with DAD-ESI-MSN for a characterization study in *Belamcandae Rhi-zoma*, there was excellent quality control because of the identification of thirty-five flavonoids. However, the authors recommended further study to help standardize natural products in plants.

These examples can justify the importance and utility of applying HPLC-MS and other secondary techniques in solving research problems in herbal medicine. Conventional analytical methods are highly

specific and sensitive and renowned for their ability to accurately determine the chemical profile and quality of raw materials and products; however, they are often difficult to operate, particularly when dealing with complex matrices and issues such as ion suppression can significantly impact their performance, underscoring the need for constant development and improvement of analytical tools to further refine and expand their use in phytochemical studies.

III. Liquid chromatography Pairings

Liquid chromatography (LC) is commonly combined with Tandem Mass Spectrometry (MS/MS) and Mass spectrometry (MS) techniques.

1.Liquid Chromatography paired with Tandem Mass Spectrometry (LC-MS/MS)

Liquid Chromatography paired with Tandem Mass Spectrometry is one of the most fundamental methods for analyzing large numbers of herbs, demonstrating high efficiency when combined with other techniques like High-Resolution Electrospray Ionization, Mass Spectrometry and Nuclear Magnetic Resonance Spectrometry. For example, in a study by (Lai et al., 2015), LC-MS/MS can be used alone to find biomarkers in Huangqi-Guizhi-Wuwu and validate the method for biomarkers such as astragaloside I and IV. Although the diagnostic accuracy was high, variation in biomarker concentration within different brands raised several questions which revealed limitations and the necessity for strict quality control and standardization of commercial drugs for delivering their optimum potential (Lai et al., 2015).

In another study by Ding et al.,2007, they used a system that combined the LC-MS/MS and Diode Array Detection (DAD) to analyze the levels of alkaloids in *Corydalis yanhusuo*. This method proved that it presented high sensitivity and specificity, yet the drawback of this method was that it could only screen specific alkaloids and could quietly remove other bioactive factors (Ding et al., 2007).

Moreover, optimizing LC-MS/MS supported by high-resolution ES-IMS in the positive ion mode and NMR analysis, Ha et al 2022 and his team provided a detailed study of withanolide glycosides isolated from the root of *W. somnifera*. It led to the structural elucidation and identification of various active compounds thus showing the kind of biological activity they possess. Whereas the use of HR-ESIMS, which gives high-resolution mass accuracy that is vital in confirming the molecular formulas, the use of NMR can also significantly contribute to extracting structures at a depth that is important for the elucidation of structures that have complex natural products (Ha et al., 2022).

These analyses reflect versatility and efficiency of LC with aided by MS/MS and other secondary steps. Despite the advanced chemical resolution and the high degree of analytical sophistication employed by these methods, they also underscore the issues raised by the molecular complexity of herbal matrices and the extensive validation and pre-standardization steps that may be required. To overcome these issues, there is a need to develop advanced analytical methods as well as research that will be beneficial in maximizing the usefulness of LC-MS/MS in natural product research, in efforts to improve the standardization and quality control in herbal medicines.

2.Liquid Chromatography paired with Mass Spectrometry (LC-MS)

LC coupled with MS is suggested to be a versatile technique, that is used frequently for various and sophisticated analyses of phytochemicals. This is further complemented by secondary methods like High-Resolution Electrospray Ionization Mass Spectrometry (HR-ESIMS) and Nuclear Magnetic Resonance (NMR), which do not have the specific ability but help to provide deeper chemical characterization Pang et al. (2016). For instance, review of LC-MS conducted by Pang et al. (2016). observes that LC-MS is highly sensitive and selective when it comes to the analysis of TCM and that it is of

paramount importance especially when it comes to deciphering complex formulations. There are however some key issues that are encountered while using the technique such as inability to identify compounds that are not available as standards besides the fact that one requires high purity sample hence the need for focus on better detection methods and sample preparation.

For instance, (Ha et al. 2022), when LC-MS/HR-ESIMS was used with NMR to analyze *Withania somnifera*, with the aim of confirming new and existing withanolide glycosides, this combination not only offered structural elucidation but also the opportunity to establish the causal relationship between certain structures and biological activities. Nevertheless, the study indicates some drawbacks in the broad application of biological assays where low activity has been reported against the *Helicobacter pylori* bacteria and this confirms the need for further biological analysis coupled with clinical research in order to confirm the therapeutic utility of the compounds found.

These examples demonstrate the utility of LC-MS/MS when combined with other techniques with a high level of sensitivity such as HR-ESIMS and Nuclear Magnetic Resonance, providing enhanced analytical resolution and important insights into molecular characteristics of bioactive compounds. However, challenges remain, including the complications of herbal matrices as well as the need for the multifaceted biological authentications. Such challenges reveal that future improvement for the dimension and practice of phytochemical research for complex herbal substances depends on the enhancement of analytical methods and better integration of those methods.

IV. Ultra-High Performance Liquid Chromatography paired with Quadrupole Time-of-Flight Mass Spectrometry (UHPLC – QTOF)

The combination of UHPLC or UPLC and QTOF-MS, Quadrupole Time-of-Flight Mass Spectrometry (QTOF), and researchers gain access to a highly effective pairing for the qualitative and quantitative analysis of important matrices need in analyzing herbs. (Wang et al. 2019), this method has been employed independently and in conjunction with other techniques, demonstrating its efficacy, weaknesses and potential for improvement.

For instance, Zeng et al. (2018). showed that the efficiency of UPLC-QTOF-MS enables the assessment of herbal components in a rapid and high-resolution manner; it is important in a single run to determine a closely related large number of the chemical entities at the same time. Because of its high sensitivity, it has the advantage of undertaking both qualitative and quantitative analysis, making it ideal for the profiling of various herbs.

Despite these advantages, UPLC-QTOF-MS is not immune to complexities such as high data density and ion suppression effects, requiring advanced software support and expert analysis to perfectly distinguish and quantify analytes (Li et al., 2021). Wang et al. 2019 explores some of their functions in metabolomics pointing out that it is widely used in the identification of known compound and finding new metabolites which are crucial in drug discovery.

However, it is prone to require constant enhancements to improve its efficiency in the analysis of trace components and complex mixtures present in herbal medicines (Chen et al., 2020). Further developments are needed because current methods present variability in the measurements and thus require greater analysis of herbal products. Future work should focus on enhanced data analytic techniques and improvements as well as fusion of other related analytical methods to further expand the versatility of UPLC-QTOF-MS in natural products research.

1. Ultra-High Performance Liquid Chromatography paired with Quadrupole Orbitrap Mass Spectrometry (UPLC - Q-Orbitrap)

UHPLC and Q-Orbitrap MS system stands for Ultra-High Performance Liquid Chromatography, and Quadrupole Orbitrap Mass Spectrometry, respectively and has been known to be a powerful analytical tool necessary for a detailed differentiation of the quantity and quality of different components present in herbal formulations. (Jia et al., 2018). The use of this technique in the methodical identification and confirmation of TCM, especially in the study on EZP, validates the tool's efficiency and its inherent backlash. This study used UHPLC/Q-Orbitrap-MS with high-speed data-dependent full-scan acquisition, which with selective ion monitoring (SIM) ensured a comprehensive and definitive elucidation of EZP and its bioactive constituents (Jia et al., 2018).

Due to the increased resolution and mass accuracy of UHPLC/Q-Orbitrap-MS in the differentiation and accurate quantification of the compounds in the analyte, this method becomes very viable in establishing the purity, quality and authenticity of the herbal products. However, the technique has been proven to have some drawbacks, which include the following: Because of the complexity in the pulsed excitations and the difficulties in achieving higher densities in rapid polarity-switching, the technique demands high operating skills and good quality equipment, hence presents some of the operational challenges particularly in everyday usage and applications (Jia et al., 2018). Finally, due to the costs that are incurred in constructing such systems, the technique is out of reach for most people.

These limitations underscore the need to optimize methods by reducing complexity that may hinder smooth workflow at the same time not affecting quality of data obtained; there also remains a need to conduct further investigations with a view of fine-tuning description and measurement of the parts of the herbs. By furthering this technology, it will enhance the exploration of the therapeutic prospects and offers concrete evidence on the benefits and risks of TCM products.

In conclusion, when incorporating technological improvements and methodological modifications, UHPLC/Q-Orbitrap-MS is a

valuable platform for TCM analysis. However, it remains crucial to broaden its applicability and efficiency across multiple sectors as well as clinical and academic disciplines.

2, Ultra High-Performance Liquid Chromatography paired with Tandem Mass Spectrometry (UHPLC-MS/MS)

This study employed Ultra High-Performance Liquid Chromatography in conjunction with Tandem Mass Spectrometry (UHPLC-MS/MS), which is a versatile technique used frequently in analyzing various components of herbal matrices. The fact that this technique can be coupled with conventional secondary techniques including Triple-Quadrupole Mass Spectrometry (UHPLC-QQQ-MS/MS) and Diode-Array Detection (UHPLC-DAD-MS/MS) elucidates its versatility in the thorough characterization and absolute quantification of phytochemical components.

For example, Hu et al. 2021 has shown the efficacy of the application of UHPLC-QQQ-MS/MS in determining the concentrations of 43 bioactive ingredients in Chinese herbal spirits, which adopted MRM for high selectivity and sensitivity; this is particularly important to ensure that the quality of the end products in subsequent batches of production is comparable. However, although this approach proved quite successful, issues such as the effect of matrix constitution on ionization efficiency which required various chromatographic conditions, were observed and improvements might be achieved.

Likewise, Okińczyc et al. 2022 made use of a targeted analytical methodology, namely UHPLC-DAD-MS/MS, in the characterization of Georgian propolis, whereby the authors' approach proved efficient in the identification of a wide array of phytochemicals. Geographical variations and types of vegetation also emerged as limitations of the study and the investigators stressed the need for conducting extensive future research effectively to investigate these factors and their effects on healing powers.

Such examples highlight the strengths of UHPLC-MS/MS in a system that can be complemented by technologies like QQQ and DAD, enabling supreme analysis of herbal medicines. However, there are still some difficulties in dealing with a complicated sample matrix

and consequently there is an urgent need for the enhancement of a methodology for identifying and quantifying compounds of interest. Relating the chemical structures of newly detected compounds from natural products to their biological activities can help to discover new drugs and new targets of existing drugs, while improving the accuracy of data collected from UHPLC-MS/MS. It can also increase the breadth of the detectable compounds and ensure the safety and effectiveness of herbal products.

As mentioned earlier, the effectiveness of these combinations can also vary greatly. We have utilized the information from various studies to determine the effectiveness of each technique and the different combinations and pairings.

For Mass Spectrometry effectiveness of these combination of other techniques like NMR and FT-IR with mass spectrometry in the study of plant organisms was helpful in the determination of the structure of complex organic compounds that originated from plant materials. These combinations provided general information about the profiling and biological activities; however, they contain some methodological limitations, including the scope and the replicability of the study. For example, published in 2019, Rivera-Mondragón et al. explained that the conjunction of MS and NMR and FT-IR were applied by the authors in their study for a profiling analysis and bioactivity investigation, as pointed out also by Ha et al. in 2022.

High-Performance Liquid Chromatography's (HPLC) effectiveness showed that the improvement of HPLC with various modes of mass spectrometry is crucial in the assessment of phytochemistry and natural products. The method showed high efficiency in several aspects with regards to the identification of numerous bioactive compounds; however, the authors pointed out that there are discrepancies with the matrix sensitivity and that sample preparation poses some complications. For example, Gao et al. (2021) applied HPLC to determine isoflavonoids present in *Pueraria lobata*; the technique was found to be efficient, although some matrices are sensitive to the method.

For Liquid Chromatography-Mass Spectrometry the effectiveness especially when combined with HR-ESIMS and NMR was proven to be a strong tool in unearthing the herbal matrices in its complex form. It was accurate in terms of quantitative measurement and gave detailed structural information of bioactive compounds; however, oscillations in biomarker levels called for enhanced quality assurance. For example, In another study, (Lai et al. 2015). employing LC-MS/MS successfully establish and validate biomarkers in Huangqi-Guizhi-Wuwu but the study acknowledged that there is a problem with the variations in concentration of the biomarkers, thereby calling for improved quality control.

Ultra-High-Performance Liquid Chromatography (UHPLC-MS) effectiveness techniques including UHPLC-MS techniques especially in combination with QTOF-MS were effective in providing high resolution and faster identification of bioactive compounds in the herbs. This technique was immensely powerful for comprehensive profiling but also encountered challenges like high data complexity and ion suppression effects. For example, according to the study by (Zeng et al. 2018), UHPLC-QTOF-MS offers high-speed, and the high-resolution analysis to support the herbal profiling.

Since there are so many characterization techniques and combinations there is a need for standardization. The literature illuminated eight primary standardization techniques.

Section B: Summary of standardization techniques

(1) Chemical Profiling:

Chemical analysis, especially with chromatographic and spectroscopic techniques, is central in the establishment of harmonized identification of herbal medicinal products and quality, safety, and efficacy. Azhar et al. 2017 reviewed some of the advantages of chromatographic fingerprinting in reducing the issues associated with batch-to-batch variation and

improving the reliability of the products, given the complex chemical nature of herbal medicines.

Furthering this, 2021 reviews from the *Journal of Herbal Science* integrate various chromatographic techniques like TLC, GC, and HPLC with spectroscopic techniques ensure comprehensive quality control (Research & Reviews: By: Adeleke A. S, Ige C. E, Aliyu M. D And Nafiu A. A (Published in *Journal of Herbal Science*, 2021). This integration is depicted as efficient in playing a routine role in ensuring the best quality and safety of herbal products.

A notable example of using chromatography in separating chemicals is seen in the HPTLC analysis of budmunchi- amines in *Albizia amara*. This method serves as an identification and separation tool of macrocyclic alkaloids, aiding in the quality control and standardization of herbal formulations (Roshan et al., 2022). This method clearly indicates how high-performance thin-layer chromatography can be a useful tool in the preservation and enhancement of the quality and stability of the extracts from the herbs.

Despite these advancements, the complexity of fully characterizing diverse and complex herbal matrices remains a challenge, necessitating extensive validation and adaptation to the complex nature of herbal medicines (Azhar et al., 2017; Research & Reviews: which were also supported by previous studies done by Han et al. (*Journal of Herbal Science*, 2021). The demand for using high-end equipment and the delicate balance when it comes to chromatographic conditions add to the challenge.

In response to these issues, Shukla et al. (2021) argue that better chromatographic technologies and techniques should be harnessed to enhance the standardization processes while advocating for extending more comprehensive authentications of the herbal medicines (Shukla et al., 2021). In the same vein,

the research conducted by Govindarajan, Tejas, and Pushpangadan (2019) shows that using the HPLC technique is an efficient approach to obtain the detailed chemical analysis of the investigated multicomponent *stigmatostemonis* herbal drugs which contributes to quality control in the production and manufacturing of the drugs (Govindarajan, Tejas, & Pushpangadan, 2019).

Additionally, the work by Kushwaha et al. (2010) emphasizes how chemical and DNA markers can aid in standardization thus increasing the safety of the use of herbal medicines. These markers aid in all the phases, from selection of raw materials up to evaluation of the final product; however, development and differentiation of these markers can be complicated by environmental and genetic volatility (Kushwaha et al., 2010).

Therefore, with complementary chromatographic and spectroscopic methodologies in chemical profiling, it is an important turning point for the quality standardization of herbal medicine (HM). Despite its high efficiency, the analysis of the realized complex and variable HMs required further innovations in the field of analytical technologies and practices. It is suggested that the combination of traditional knowledge with state-of-the-art analytical tools is the way forward to improving on the precision and practicality of these standardization techniques.

(2) **Standard Operating Procedures:** SOPs play a crucial role in the formulation, administration, monitoring, and evaluation of herbal medicine practices. Santosh and Jyotiram (2016) have also shown how SOPs are being applied under the guidelines of Good Manufacturing Practices (GMPs), significantly contributing to the issues on standardization of poly-herbal formulation. These SOPs cover operations right from harvesting, extraction and purification and play strategic roles in reducing variability and enhancing the

reproducible impacts of the therapeutic effects. (Santosh & Jyotiram, 2016).

While the advantages of SOPs are clear, poly-herbal formulation complicates the matters due to the variability of plant constituents where SOPs facilitate contribute to setting quality control profiles for the herbal materials and formulations (Santosh & Jyotiram, 2016). Among the different strategies discussed by Patra et al. (2010) in the development of SOPs, protocol-based standardization is described by Essler & Roberts (1998). Furthermore, SOPs for preparing extracts and finished products are also highlighted. This suits their 'sample analysis menu' that covers a wide range of analytical services such as elemental impurities and heavy metals, microbial, pesticide residues, aflatoxins and other basic and advanced scientific analytical services. This conventional approach successfully incorporates elements of quality control requirements with folk practices while presenting a structured approach to harmonizing the use of herbs. But the variable nature of compounds which make up the herbal medicine system often create major challenges when reconstructing the complete make-up of herbal formulas. (Patra et al., 2010).

Zhang, and Liang (2022) used *Scutellaria baicalensis* in their study on the

impacts of post-harvest processing. They also illustrated the influence of various processing techniques, including sun drying and oven drying to various active compounds including baicalin and baicalein. In this regard, they have developed the following SOPs for post-harvest processing of the herb to improve the efficiency of these techniques and quality of the final product. The study indicates an area for further research in this regard that includes the exploration of and documentation on less conventional drying methods which could be beneficial, and the need to standardize production methodologies to enhance quality across the different levels of production (Zhang, Zhang, & Liang, 2022).

Thus, the requirement of SOPs is paramount in meeting the standard of herbal medicinal products. They also offer lessons on procedures necessary to ensure the proper quality, safety,

and efficacy of these products. Nevertheless, the application of SOPs when signing contracts and orders for cooperation faces some challenges such as high variation in the composition of the herbs and the difficulty in standardizing the preparation of multiple compound preparations. These points support the conclusion that constant improvement and enhancement of these procedures is imperative to proceed in presenting the technology of herbal drugs to be adopted by the health care systems.

(3) **The Role of Markers:** The use of markers in the development of standardization of herbal medicines is important as it helps in the quality control, safety and effectiveness of the products as well as the consistency of the brands found in the market. Noviana, Indrayanto, and Rohman (2022) have pointed out that fingerprints analysis has recently become more prominent. This procedure is done through chemical and DNA that acts as a marker for quality assurance of medicinal herbs. They are therefore important in ascertaining the authenticity of herbal medicines and in identifying species of plants emanating from different sources. However, they strengthen the argument that interpretation of data is not very easy and there is heavy reliance on some electrical instruments or software to analyze the data (Noviana et al., 2022).

(4) **Metabolomics:** Metabolomics is the analysis of metabolites in a scaffold, is a modern technological tool that is widely used as the procedure for standardization and quality assessment of Ayurvedic formulations. This approach helps in the identification of the major and minor percentages of chemical compounds present in medicinal plants, thus assisting tremendously in the standardization and quality assurance of herbal products (Mukherjee et al., 2016).

However, as highlighted by Mukherjee et al. (2016), there is little information available on these Indian medicinal plants even though metabolomic investigations of famous Ayurvedic mixtures such as Triphala and Trikatu remain limited. This is a research area that has

not been widely explored, yet it is very important in order to gain a better understanding of the pharmacological characteristics of such extracts and enhance the requirements for standardization and quality assurance. Among the research imperatives highlighted in the study, the empirical ground should examine the metabolomic profiling of Indian medicinal plants and Ayurvedic formulations in detail (Mukherjee et al., 2016).

In another context, Fu et al (2022) describe the traditional uses, chemical compounds, and pharmacological effects of non-alkaloid natural products, including polysaccharides and phenolic compounds in *Aconitum carmichaelii*. They opt for the UPLC-Q/TOF-MS, which facilitates classifying and summarizing numerous chemicals and their potential impacts. They also use it for polymeric analysis of polysaccharides and examining their structures and functions (Fu et al., 2022).

Fu et al. (2022) highlights the limitations in their study; the authors elaborate on the chemical profiling standardization processes but rarely discuss other standardization issues. They suggest carrying out new extensive examinations concerning detailed structural definition of polysaccharides and the additional research of pharmacological effects of these compounds as immunomodulatory agents in the intestinal tract (Fu et al., 2022).

In conclusion, the metabolomics can be used as a global and specific approach to complete the standardization of herbal medicines by using traditional knowledge together with scientific advances. It plays a role in assessing compounds in a sample and gives the quality and the efficacy of the herbal products which are produced. However, the field needs more advancement in order to address the remaining gaps in knowledge and to ensure the optimal use of this field in the herbal medicine sector. Further advancements to the field of metabolomic research will without doubt play a role in improving the overall standardization and quality of herbal medicinal products.

(5) **Pharmaceutical Standards:** Pharmaceutical standards are essential in the standardization of the herbal medicinal

products, majorly concerning identity, quality, and purity of the products. As pointed out by Pandey and Tripathi (2014), it is wise to apply the pharmacopeial standards the specific numerical value for structural, analytical and physical qualities to validate obtained characteristics of the herbs in the herbal drugs. This method helps in the control that herbal drugs once produced must meet certain value standards that are chemical, botany and spectroscopy.

Lutoti et al. (2020) particularly reviewed the requirements of pharmacopoeia for standards of raw materials and the final products. The role of quality assurance measures is stressed, strong focus on the production formulation and recipe creation, and highly important safety assessment concerns are highlighted. These standards are qualities that are important in establishing and implementing standard measures that are to be followed when developing herbal medicinal products (Lutoti et al., 2020).

Nevertheless, due to the fact that these compounds are sourced from natural products, the compositions vary greatly, and this is a major limitation. Another intensive challenge is the selection of the most effective technique in extracting the drug with optimum yield and quality of the active ingredients. Such challenges call for more concerted studies to purify the extraction techniques, as well as working on a more complete and defined protocol for the Pre- Phytochemical screening (Pandey & Tripathi, 2014).

The medicinal standards for herbal products can be useful in maintaining the quality and reversing the consequences of false works but the issues like variation in the raw material along with the inability to standardize complex combinations have not been resolved yet. These involve complex analysis and present real challenges to achieving consistency of quality. (Pandey & Tripathi, 2014). The standards in the field of pharmaceuticals are vital factors that define the standardization of the products that are derived from the herbs and, thereby, it can be concluded that it has a prominent role in providing safety, effectiveness, and quality of the herbal medicinal products. In addition to overcoming the variations in natural constituent profile of herbs and

the complications, there is a need for embracing continued enhancement of standardization methods.

(6) **Quality Control:** One of the major concepts that form part of the standardization of herbal medicinal products is quality control, which pertains to the safety, reliability and quality of products. According to Zhou et al. (2019) to deal with the challenges mentioned above concerning the safety and effectiveness of Chinese herbal medicine, new stringent quality and safety measures should be put in place. There is a call for the development of international standards and regulation of Chinese herbal medicine equivalent to those of western drugs (Zhou et al., 2019).

Chopra and Sindhu (2020) document the standardization of crude drugs assessing the quality and purity by several physical, chemical, and biological tests. They give emphasis to the adaptation of these techniques in avoiding the process of adulteration as well as in increasing the healing potency of the herbal medicines. But as indicated they observe high complexity coupled with the cost of using some secondary characterization techniques which requires high end equipment and profound knowledge (Chopra & Sindhu, 2020).

Kushwaha et al. (2010) describes the identification of specific markers for quality control and stability evaluation. They discuss how chemical and DNA markers aid in standardizing and maintaining good quality herbal medicines starting from the selection of raw materials, going through the extraction and formulation process to evaluation of the final product. However, the production and identification of markers are not without difficulties, such as temperature and light which affect them profoundly (Kushwaha et al., 2010).

That is why Patil et al. (2018) discuss various types of the physical and chemical procedures aimed at the maintenance of the quality, safety, and therapeutic efficacy of the drugs based on the herbal medicine. Some are viscosity measurements, melting point determination and solubility tests amongst others for detecting adulterants and the therapy relevancy. The importance of the continuity of

standardization is highlighted by the necessity to adapt and improve research and production (Patil et al., 2018).

Quality control plays an essential role with regards to quality and standardization of herbal medicinal products thus making an impact on their safety and efficacy. It is therefore pertinent that standardization work progresses in parallel with globalization and that new approaches are developed to adequately combat the problems which arise from the natural variability of the herbal constituents and the complexity of the formulations. Combining traditional knowledge about plant utilization with the contemporary approaches will be vital for optimizing the standardization procedure and maintaining the healing effectiveness of the herbal medicines.

(7) **Fingerprint Analysis:** Fingerprint analysis is now becoming one of the promising tools for standardization of herbal medicinal products, which are facing challenges arising from their chemically diversified nature, along with effectiveness, safety, and quality issues. Azhar, Tahir, Sohn, Kim, and Bahadur (2017) stated that fingerprinting reduces the inter-batch difference and improves the product quality. This method becomes more useful in analyzing herbal medicines in detail and making sure that the measurements are consistent and the quality reliable (Azhar et al., 2017).

According to Noviana, Indrayanto, and Rohman (2022), fingerprinting analytics, especially using chemical and DNA approaches, are essential in evaluating the quality of HDMs. These techniques are greatly beneficial for distinguishing between genuine herbal remedies and fake ones and for tracking down what species of plants are being utilized. Such capabilities are important for the purpose of replicating predictability, efficacy and safety of the herbal medicines (Noviana, Indrayanto, and Rohman, 2022).

Shukla et al. (2021) focuses on using chromatographic fingerprinting as a new scientific approach in the standardization and quality control of traditional medicines. This technique helps to maintain identity, official recognition and the lot-to-lot similarity of herbal

formulations, which, in turn, affects quality control and consumer trust more positively. (Shukla et al., 2021).

However, fingerprint analysis seems to have a litany of drawbacks. Outstanding issues that can be highlighted include heterogeneity, variability of process, complexity of handling different and complicated matrices, and substantial reliance on specialized equipment and software to support the data analysis of the research results (Azhar et al., 2017; Noviana, Indrayanto, & Rohman, 2022). These scenarios could be labeled as rather intricate.

In other words, current fingerprinting techniques in the standardization of herbal medicines have limitations that require better technologies and analytical data to improve and become more useful. The limitations of current standardization processes are described by Shukla et al. (2021) who also urge for further developments of chromatographic techniques for that purpose.

Additionally, Kushwaha et al. (2010) have also elaborated on how markers are useful in the standardization of medicines formulated from herbs. Clearly, when it comes down to the actual process of using herbal medicine, both chemical and DNA markers are important tools when it comes to evaluating safety and medicinal properties at every step of the process – from procurement of raw materials to final product testing. Although, some opportunities that exist in the production and identification of the reliable markers experience challenges because of their ability to respond to the environmental and genetic factors (Kushwaha et al, 2010).

In conclusion, fingerprint analysis is a valuable tool which contributed to the current breakthrough in standardization of herbal medicinal products. Despite this, breakthrough, there is still a need for further refinement of the technique and its further extension in which it can be applied in order to improve the quality control and safety of the existing and used herbal medicines. It is also recommended that maintaining the practice of traditional indigenous knowledge with

modern analytical tools is commendable for fine tuning of the standardization methodologies.

(8) Good Manufacturing Practices (GMP): Good Manufacturing Practices (GMP) plays a significant role in determining the standardization of herbal medicines with an intent of assuring herbal products and medicines on their quality, safety, and efficacy. GMP addresses the issue of quality control through ensuring that every single detail of the manufacturing process is controlled, from the acquisition of the various raw materials to the final product (Horri et al., 2022). The major objective of GMP is therefore to ensure that the batches of any herbal product manufactured are consistent, safe, and of optimal quality to help in establishing public confidence and market acceptance and to enable herbal products to be accepted into the formal health care system (Zhou et al., 2019).

The benefits of GMP can be understood through studies that have been conducted where it was observed that these practices are a key to establishing good product safety and quality standards. (Zhou et al., 2019). This is especially important when blending traditional remedies with the orthodox health systems since there are rigorous production processes that must be met to enhance quality (Kumari & Kotecha, 2016). Innovative extraction methods under GMP, including microwave assisted extraction, and supercritical phase extraction, have also been demonstrated to improve on the bioavailability as well as pharmacological effectiveness of herbs used in medicine (Choudhary & Sekhon, 2011).

Nonetheless, GMP has its challenges mainly attributed to the anticipated variation of natural products and the holism of herbal products. This variability becomes problematic in guaranteeing quality, especially when there are high levels of production and manufacturing (Mishra & Tiwari, 2022). Due to the natural variation in the plant formulations used in herbal formulas there are some serious challenges in standardization. It is difficult to define active ingredients for certain herbs, due to the complexity in the formulation of many other herbs (Santosh & Jyotiram, 2016). This is where challenges emerge, making

it extremely important to create new extraction and analytical methods that are more selective. These enhancements can potentially lead to an increase in the yield and the decrease of the amount of solvent used during extraction. The increased purity of extracted compounds provides a closer link between the chemical composition of the extract and its therapeutic effects (Mishra & Tiwari, 2022). Also, there is suggestion for continuing the improvement of analytical method and a good coordination of western knowledge and indigenous science to create standardization framework for herbal products (Kumari & Kotecha, 2016).

Effectiveness Based on Standardization Techniques

While standardization provides consistency, the efficiency of the above-mentioned methods and combinations is important. Literature on the efficiency of these techniques demonstrates that there are a broad range of benefits for each method that needs to be considered.

(1) **Chemical Profiling's Effectiveness:** Chemical profiling techniques like LC-MS and GC-MS have been instrumented in establishing quality control by providing comprehensive chemical profiles of the herbal medicines. They were essential in determining things like which bioactive compounds to focus on and to ensure the quality of the herbal products. For example, Zhang says, it is extremely important to have a consistent chemical composition of the raw materials in herbal remedies. (Zhang et al., 2022).

(2) **Standard Operating Procedures (SOPs) Effectiveness:** SOPs play a vital role in the analysis process of herbal products that maintain consistency and reduce variations from one study to another. Writing SOPs reduced variation and the accuracy of the result, ensuring credible findings due to the increased standardization of the process.

For example, they help in reducing variability in analysis leading to reliability of the results produced. (Zhang, Zhang, & Liang, 2022).

(3) **Marker Identification Effectiveness:** Thus, specification of certain chemical constituents within some of the herbal remedies assisted in the process of ‘standardizing’ and ‘stabilizing’ such commodities. The use of marker compounds when preparing and testing herbal formulations helped to define its efficacy and genuineness, hence the need to always have the marker compounds. For example, all herbal formulations are extremely reliable and powerful, and marker compounds serve as the confirmation of their efficacy. (Noviana et al., 2022).

(4) **Metabolomics** effectiveness gives broad pictures of the metabolic constituents of herbal medicines as critical for evaluating their pharmaceutical effectiveness and quality assurance of their preparation. These techniques help to determine metabolic pathways and active substances in case of herbal products’ effectiveness. For Example, metabolomic technologies detect metabolites as well as compounds in foods that function as bioactive. (Mukherjee et al., 2016).

(5) **Quality Control’s** effectiveness. While conducting the R & D, other chromatographic and fingerprinting techniques were undertaken which made the process of herbal medicines consistent and safe. Stringent measures were required to ensure that the formulations met the right pharmacological, and therapeutic specifications of herbal remedies. For example: Manufacturing standards and controls are necessary to preserve the efficacy and healing potency of natural products. (Pandey & Tripathi, 2014).

(6) **Good Manufacturing Practices (GMP)** effectiveness shows rules guaranteed the quality, standard and control of herbal products thus being manufactured in a right and standardized manner. It was important to mention that GMP was crucial to implementing safety in the preparation and production of herbal products Production. For example, adoption of GMPs is vital to maintaining the quality of herbal products in today’s market (Zhou et al. 2019).

(7) **Pharmaceutical Standards** effectiveness is relying on-setting of standards on pharmacopeia for herbal medicines was vital in providing a clear indication of quality as well as recognition of adequate compliance. These standards facilitated the revelation of herbal products that met safety and efficacy standards and played a crucial role in enhancing consumer confidence and acceptance. (Azhar et al., 2017).

(8) **Fingerprint Analysis** effectiveness is based on the biochemical fingerprinting of herbal products. This is the foundation of discovering the identification and quality assessment part of fingerprint analysis techniques. These methods have made significant improvements to the consistency and the authenticity of the herbal formulations which are instrumental in herbal medicine research effort. For example, latent fingerprinting helps in maintaining the quality as well as the actuality of the herbal formulation. (Mishra & Tiwari, 2022).

CHAPTER SIX

Findings, Conclusions and Recommendations

Findings and conclusions

The study revealed that 47 different techniques were utilized by the studies reviewed. However, roughly 55% of the studies relied on three key techniques: Mass Spectrometry, High Performance Liquid Chromatography and Liquid Chromatography. These three techniques, due to their multiple uses across various techniques, accounted for a collective total of 138 users.

It is important to note that many of the studies undertook very different projects but were primarily using the same techniques. For example, HPLC was used as the main technique in the following three different studies with significantly different study goals and objectives. These studies focused on

(1) to provide a rapid, simple, sensitive, and practical method for identifying LJF and LF but also establishes a method for discovering the characteristic components of other herb-medicines that are susceptible to adulteration.

(2) To develop a gradient solvent system that facilitates the fractionation of Yongdamsagan-Tang, enhancing the identification of bioactive components without the irreversible sample adsorption problems typical in traditional chromatography methods.

(3) To isolate and characterize anti-influenza virus metabolites from Polygonum Chinese and evaluate their effectiveness against influenza A and B virus strains.

When we acknowledge that three techniques account for approximately 55% of the industry usage, it becomes obvious that there must

be a reason and there is. First, the High-Performance Liquid Chromatography (HPLC) has the strongest advantage in that it is quick and efficient, it is highly accurate and reproducible, it is very versatile and has high resolution. Even though it is very costly and difficult to troubleshoot problems, its advantages far outweigh the disadvantages. Second, Mass Spectrometry (MS) has excellent precision, tremendous sensitivity and is very reliable. In this case the cost is substantial but not as significant as the HPLC. Even though its operational delivery requires significant skill and training, it is still one the leading choices. Finally, the Liquid Chromatography (LC) has High Separation Efficiency, wide applicability, accurate quantitative analysis, and easily automated. Even though it is also very expensive, uses a lot of material, requires significant training and requires tremendous skills, it is still a highly sought-after technique.

It is important to note that while three techniques are utilized roughly 55% of the time, the remaining 45% were unable to perform certain tasks without the help of one of the three major techniques. However, one study aimed to develop an analytical method for simultaneous identification and quantification of 11 specific coumarin compounds in *Radix Angelicae Dahuricae*, with the ultimate goal of providing a robust method for quality control of this traditional Chinese medicine. While the study had an objective that could have easily been performed with or by the “big three,” it chose to the Multiple Reaction Monitoring (MRM) technique as the main technique. While the MRM has some positive such as it has high sensitivity, it can be target-specific, it requires minimal sample preparation, it is speedy and has quantitative capacity. However, the initial investment in equipment is significant, the target-specific approach requires prior knowledge of the compound, significant technical training is required, and can interfere with ionization to affect accuracy. In the final analysis it appears that cost, the training and accuracy motivates others to avoid pursuing individual techniques alone. Thus, setting the stage for the “big three” to continue to dominate.

This article is a scoping study on the standardization of herbal medicines, and they establish that much effort is needed from various

different angles so that the advantages of these products can be realized. The general nature and experimental variability of textural blends and preparations require sound and elaborate standardization programs that address all aspects of production from sourcing of raw materials to the finished product testing. At the center of this procedure is chemical profiling which uses methods such as HPLC, GC and TLC to offer tissues descriptions of the chemicals present in the herbal commodities. These methods are very important for controlling the batch-to-batch variation and the identification of bioactive compounds, thus setting up the directions for important quality control measures.

Standardization of herbal medicines cannot be separated from the concept of Good Manufacturing Practices (GMP) which is another pillar of the standardization. The specifications of GMPs maintain a strict quality assurance throughout the course of compound production; hence, minimizing the natural variation and uncertainty in the quality of herbal medicines and making them more reliable and consistent. It also highlighted the need for GMP frameworks to be improved progressively because of possible advancements in the formulation of herbal products and also in client expectations.

Coupling SOPS with GMPs allows the preparation of properly documented materials for analysis of the herbal products. SOPs are critical documents because they help to minimize any form of batch variation and attain greater batch uniformity. This approach is useful in facilitating reproducibility of the therapeutic attainments which is compounded by the compounding attribute of multifaceted Polyherbal formulations.

Adulterants are essential for the authentication and quality of herbal medicines by using chemical and DNA-based markers. They are applied at various stages of the supply chain starting from the raw material and ending up with the finished product evaluation and quality checking. These works are promising, but still have a number of issues that are important for the practice, including the genetics of active compounds and environmental variation in plant materials.

Hence, reliable markers are essential for overcoming some of these difficulties. Metabolomics, which uses metabolites as an object of study, synthesizes the conventional approach with the modern one, which promises a potent weapon for the process of standardization of herbal formulations. However, to realize the best benefits of this approach for quality assurance of herbal products, there is need for more research in this area.

Flavor profile, identity, quality control and other pharmacopeial standards are well relevant systems adopted in ensuring the safety and therapeutic value of the medical herbs. These standards ensure that mature quality benchmarks are in place, and therefore serve as a regulatory body. Among these, fingerprint analysis is especially informative, providing biochemical details that help in not only identifying but also evaluating the quality of the herbal formulations in order to increase people's confidence in the product, its quality, and usability. Among the research objectives for future work, there is the need for the improvement of these methods to optimize and adjust in the standards setting process.

The study thus reveals the need to establish higher levels of international compatibility in standardization efforts due to the volatility inherent in natural constituents' composition, together with the complexity of many herbal formulations. One of the remarkable observations in modern pharmacology is the fact that standardization in such a field may be reached only with the help of the combination of traditional experience and outlook in the problem and modern scientific methods. There is always a need to conduct research and developmental studies to progress the field, counter the configurations currently in play and meet the rising need of efficient herbal remedies. In incorporating truly standardized herbal medicines into health care systems worldwide, quality, safety, and therapeutic values of these botanicals can be guaranteed by continuing downstream standardization, as part of an overall approach to this important issue.

RECOMMENDATION

In the future, more research is needed to increase the reliability of various techniques beyond the “big three”. Increasing reliability could promote more studies aimed at achieving enhanced standardization and characterization. An increased use of other techniques could result in the availability of more information about ways to improve existing techniques. In the long run, if more studies are performed, there will be an increase in the probability that a solution to finding a standard protocol could be expedited. Additional studies will expand the availability of more publications that list and describe the various techniques that are currently available.

Additionally, it is recommended that Traditional Chinese Medical Schools, technical institutes and other universities should provide course offerings that teach the principles and applications of the existing techniques that focus on standardization and characterization.

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APPENDIX A

Summary Description of Prevailing Techniques

(1) CHROMATOGRAPHY (C)

Description: A biophysical technique that separates, identifies, and purifies the components of a mixture for quantitative and qualitative analysis. There are two phases. In the first phase the mixture is dissolved in a fluid (either gas or liquid) called “Mobile Phase”. The second phase is when the mixture moves over a stationary material or a material that is fixed in place. This fixed material can be a solid or liquid. It is something like a column, a capillary tube, a plate, or a sheet. This is called the, “Stationary Phase”.

As the Mobile Phase moves over the Stationary Phase, the mixture components separate based on their interactions with the stationary material. Some chemicals move faster than others and as a result, the rate of movement causes them to separate into individual constituents. A good example is when black ink (mobile phase) is placed on a white sheet of paper (stationary phase), the ink will spread unevenly, indicating the presence of different constituents.

Purpose: Is used to separate organic and inorganic compounds, analyzing antibiotics, proteins, separate vaporizations, separate ions based on charge interactions, separate binding interactions between molecules, and separating molecules by size so that they can be analyzed and studied.

(2) GAS CHROMATOGRAPHY (GC)

A common analytical technique used in chemistry for separating and analyzing compounds that can be vaporized without decomposition.

DESCRIPTION

- In gas chromatography, a gaseous or liquid sample is injected into a mobile phase (usually an inert gas like helium, argon, nitrogen, or hydrogen), which then passes through a stationary phase. The stationary phase can be solid or liquid, but most modern GC systems use a polymeric liquid stationary phase. The mixture components interact differently with the stationary phase, causing them to separate as they move through the column. As the chemicals exit the end of the column, they are detected and identified electronically.

PURPOSE:

- GC is commonly used for:
 - Testing the purity of a substance.
 - Separating different components of a mixture.
 - Preparing pure compounds from mixtures in preparative chromatography.

Advantages:

- **High Resolution Power:** GC provides excellent separation resolution compared to other methods.
- **High Sensitivity:** When used with a thermal detector, GC exhibits high sensitivity.

- **Relatively Good Accuracy and Precision:** GC offers relatively accurate and precise results.
- **Faster Separation:** The sample separation process is relatively fast.

Disadvantages of Gas Chromatography (GC):

- **Limited to Volatile and Thermally Stable Compounds:** Only volatile and thermally stable compounds can be separated using GC.
- **Destructive Detectors:** The detectors used in GC are destructive, except for Mass Spectrometry (MS).
- **Selectivity:** High-Performance Liquid Chromatography (HPLC) or Thin-Layer Chromatography (TLC) often provide better selectivity due to their adaptable mobile phase.

(3) HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Description: An analytical chemistry technique that separates, identifies, and quantifies specific components in mixtures. HPLC involves the interaction between sample components and a stationary phase to achieve separation.

Purpose: HPLC is used for the characterization, purification, and quantitation of compounds.

Industry Use:

- Pharmaceutical and biopharmaceutical research
- Environmental monitoring
- Food and beverage industry
- Clinical diagnostics
- Forensic science
- Research and academia

(4) **Nuclear Magnetic Resonance (NMR) spectroscopy** is a powerful analytical technique used to study the structure and dynamics of molecules. Let's dive into its advantages and limitations:

Advantages of NMR Spectroscopy:

1. **Non-Destructive and Non-Invasive:** NMR spectroscopy allows us to analyze molecular structures without destroying the sample. It's non-invasive, meaning we can retain liquid or solid samples for future studies.
2. **Minimal Sample Preparation:** Unlike some other techniques, NMR samples do not require extensive preparation. Dissolving the sample in suitable deuterated solvents is usually sufficient.

3. **Accurate Structural Information:** NMR provides highly informative spectra, revealing intramolecular interactions. It helps obtain accurate three-dimensional (3D) structural information from molecular vibrations within the natural environment, keeping the sample intact.

4. **Simple and Rapid Data Acquisition:** Modern NMR instruments allow for straightforward and rapid data acquisition and analysis. Recent developments even enable the measurement of self-diffusion coefficients and extraction of physical data from samples.

Limitations of NMR Spectroscopy:

1. **Low Sensitivity:** NMR instruments have low sensitivity to insufficient sample concentrations, resulting in poor spectra. The weak interaction energies between the NMR magnetic resonance and sample molecules contribute to this limitation.

2. **Cost and Maintenance:** NMR instruments are expensive to purchase and maintain. They require large and powerful magnets as energy sources and cryogenic liquids for cooling.

3. **Complexity for Large Molecules:** NMR does not support the analysis of higher molecular weight molecules due to the complexity and difficulty in interpreting the spectra large biomolecules, in particular, pose challenges.

4. **Motion Sensitivity:** NMR is highly sensitive to motion, which can lead to signal distortions and artifacts.

5. Fourier Transform (FT)

The Fourier Transform is a mathematical technique used to analyze signals in the frequency domain. It allows us to express a function (usually a time-domain signal) as a sum of sinusoidal components with different frequencies. In summary, FT has revolutionized spectroscopy by offering speed, accuracy, and improved signal quality. It is now

the standard tool for organic compound identification in various fields, including academic research, analytical laboratories, quality control, and forensics.

Advantages of Fourier Transform (FT):

- **Speed:** FT-based instruments acquire spectra much faster than conventional dispersive instruments. This speed is especially advantageous for real-time analysis.
- **Signal-to-Noise Ratio:** FT methods produce spectra with a better signal-to-noise ratio compared to dispersive instruments.
- **Wavelength Accuracy:** The wavelength scale in FTIR (Fourier Transform Infrared) spectroscopy is precisely calibrated using a reference laser, resulting in higher wavelength accuracy.

Disadvantages of Fourier Transform (FT):

- **Instrument Cost:** Historically, FTIR spectrometers were expensive due to the large computers required to run them. However, technological advancements have reduced costs over time.
- **Maintenance:** FTIR instruments can be expensive to operate and maintain, but the wealth of information they provide justifies the investment.

(6) **Infrared radiation (IR)** is a type of electromagnetic radiation with frequencies lower than visible red light but shorter than microwaves.

Advantages:

- **Heating:** IR is efficient for heating because it moves at the speed of light and doesn't require direct contact between the heat source and the object being

heated. You can easily control radiant heat by adjusting the power.

- **Seeing Otherwise Obscured Objects:** IR enables us to “see” objects that are otherwise hidden, such as viewing objects at night or seeing through gas and dust.

- **Ease of Production:** IR can be generated using fire, light bulbs, or even our own bodies. It plays a crucial role in global communication networks powered by inexpensive electronic devices.

Disadvantages:

- **Invisibility:** Since IR frequencies are below the red-light spectrum, we cannot see IR directly. This limitation can lead to accidental burns (e.g., touching a hot pan) because our eyes are not equipped to detect IR.

- **Line of Sight:** IR travels in straight lines, which means it requires a clear line of sight. If obstacles like furniture or walls block the path, IR signals won’t work (as experienced with remote controls).

- **Limited Penetration:** IR doesn’t penetrate walls or doors effectively, limiting its use in certain applications. Additionally, high-intensity IR waves can harm the eyes.

(7) Liquid Chromatography

Description:

Liquid chromatography is an essential analytical technique used for separating, identifying, and purifying mixture components in both quantitative and qualitative analyses. **Separation:** Liquid chromatography separates a sample into its individual parts based on their interactions with the mobile and stationary phases. The mobile phase is a liquid, and the stationary phase can be a solid material. The separation occurs as the sample components move through the column. The Components within a mixture are separated in a column based on their affinity for the mobile phase. If the components have different polarities, a mobile phase with a distinct polarity is passed through the column. As a result, one component will migrate through the column faster than the other. The compounds are separated into distinct bands within the column. If the separated components are colored, their corresponding bands can be visually observed.

Purpose:

Liquid chromatography is widely used for analyzing complex mixtures in fields such as pharmaceuticals, biopharmaceuticals, environmental science, and food and beverage industries.

(8) Mass Spectrometry (MS)

Description:

Mass spectrometry (MS) is an analytical technique used to measure the mass-to-charge ratio of ions. It provides valuable information about the composition and structure of molecules. In a typical mass spectrometry procedure, a sample (which can be solid, liquid, or gaseous) is ionized. This can be done by bombarding the sample with a beam of electrons, causing some of its molecules to break up into positively charged fragments or become positively charged without fragmenting.

Purpose:

- Forensic science: Mass spectrometry helps scientists recognize and analyze chemicals that wouldn't normally be present in crime scene evidence.
- Food analysis: Mass spectrometry helps detect food contamination and ensure food safety.
- Pharmaceutical analysis: Mass spectrometry helps test and discover new drugs and monitor their effects.
- Environmental science: Mass spectrometry helps measure pollutants and trace elements in the environment.
- Space science: Mass spectrometry helps identify the composition of celestial bodies and interstellar matter.

(1) High-Performance Thin Layer Chromatography (HPTLC)

It is a sophisticated instrumental technique used for both quantitative and qualitative analysis of herbs and herbal drugs. It enhances liquid chromatography in three critical areas which include speed, resolution, and sensitivity. It is applicable to particles of lower diameters.

Advantages of HPTLC:

- **Rapid Analysis:** HPTLC provides quick results, making it suitable for routine quality control.
- **Simplicity:** The method is relatively straightforward, making it accessible even for less experienced analysts.
- **Accuracy:** HPTLC can accurately substantiate the identity of herbal plant materials.
- **Parallel Analysis:** It allows simultaneous analysis of multiple samples, which is useful for large-scale quality control.
- **Detection of Adulteration:** HPTLC can detect adulterants in herbal products.
- **Quantification of Marker Compounds:** It quantifies specific compounds (e.g., active constituents) in complex matrices like plant materials³.

Disadvantages of HPTLC:

- **Sample Preparation:** Proper sample preparation is crucial for accurate results.
- **Limited Separation Power:** Compared to other chromatographic techniques (e.g., HPLC), HPTLC has limited separation power.

- **Sensitivity:** Although sensitive, it may not match the sensitivity of more advanced methods.
- **Quantification Challenges:** Quantification can be challenging due to variations in spot size and intensity.
- **Lack of Universal Standards:** Unlike HPLC, there are no universal standards for HPTLC, which affects method validation and comparison.

(10) UV-VIS Spectroscopy

A powerful analytical technique used in both chemistry and astronomy.

Description:

UV-VIS spectroscopy measures light in both the visible spectrum and the ultraviolet (UV) spectrum. The visible spectrum is the range of light wavelengths that our eyes can perceive. Beyond the visible spectrum, there are infrared and ultraviolet wavelengths, which cannot be seen directly by the human eye. UV-VIS spectrometers detect and measure these wavelengths reflected off or emitted by matter.

PURPOSE

- **Chemistry:** In chemistry, UV-VIS spectrometers shine light onto samples and measure the reflected light. The wavelengths in the reflected light provide chemists with accurate readings of which elements make up the sample.
- **Astronomy:** In astronomy, UV-VIS spectrometers can be attached to telescopes. By measuring the wavelengths of light emitted by celestial objects, scientists can determine which elements make up those objects.

Advantages of UV-VIS Spectrometers:

- **Accuracy:** Even small UV-VIS spectrometers provide extremely accurate readings. This precision is crucial for preparing chemical solutions or recording celestial body movements.

- **Ease of Use:** UV-VIS spectrometers are straightforward to operate.
- **Non-Destructive:** The technique is non-destructive, allowing repeated measurements.
- **Inexpensive:** UV-VIS spectrometers are cost-effective.
- **Fast Interpretation:** They provide rapid data interpretation due to accurate readings.

Disadvantages of UV-VIS Spectrometers:

- **Setup Complexity:** Preparing a space to use a UV-VIS spectrometer can be complicated.
- **Limited to Visible and UV Regions:** UV-VIS spectrometers are restricted to the visible region and the ultraviolet spectrum only.
- **Broad Bandwidth:** Filters used in UV-VIS spectrometers allow broad bandwidth, which may deviate from Lambert Beer's law in research applications.

(11) Centrifugal Partition Chromatography (CPC)

Description:

CPC is a **liquid-liquid chromatography method** that separates analytes based on their differing partition coefficients in a two-liquid system. The stationary liquid phase is retained in a rotor due to centrifugal force. The mobile phase containing the analytes is fed under pressure into the stationary phase.

Advantages of CPC:

- **Liquid-Liquid Nature:** CPC provides a large stationary phase ratio compared to other solid-support technologies. This results in:
 - High isolation and stabilization for analytes.
 - High capacity for separation.
 - Robust operation.
- **Predictability:** The separation mechanism is simple, based on the partition behavior of compounds in the mixture. CPC allows precise prediction of the chromatogram even before separation, making scaling or transferring the method easier.
- **Solvent Flexibility:** CPC offers a wide range of solvent options, reducing operational costs in industrial applications. It **allows greater chemical space for adjusting solvent selectivity** compared to other chromatography technologies.

Disadvantages of CPC:

- **Underutilization:** Despite its advantages, CPC remains less popular than other separation techniques.

- **Manual Intervention:** CPC may require manual adjustments and monitoring, especially when used as a pre-treatment step before traditional chromatography methods.
- **Complex Equipment:** Setting up and maintaining CPC equipment can be more involved than some other separation methods.

(12) X-rays

A type of electromagnetic radiation that can generate images of tissues and structures inside the body. They have been widely used in medical science for over a century.

Advantages:

- **Diagnostic Tool:** X-rays are valuable for diagnosing various conditions. They can detect fractures, dislocations, and bone abnormalities. Additionally, they help identify diseases like pneumonia, lung cancer, and heart conditions.
- **Speed and Affordability:** X-rays provide quick results and are more affordable than other imaging methods.
- **Life-Threatening Conditions:** X-ray scans can diagnose potentially life-threatening conditions such as blocked blood vessels, bone cancer, and infections.
- **Bone Visualization:** X-rays are highly effective in visualizing bone structures.

Disadvantages:

- **Ionizing Radiation:** X-rays produce ionizing radiation, which has the potential to harm living tissue. Prolonged exposure can increase the risk of cancer.
- **Limited Soft Tissue Contrast:** X-rays are less effective than other imaging modalities (such as MRI or ultrasound) in differentiating between soft tissues like muscles, tendons, and internal organs.
- **Flat Images:** X-rays offer single, flat images, limiting their ability to show three-dimensional details.

- **Not Ideal for Long-Term Use:** Due to radiation exposure, X-rays are not ideal for long-term monitoring.

(13) X-ray diffraction (XRD)

A widely used technique in materials science and engineering. It provides valuable insights into the atomic and molecular structure of crystalline materials.

Advantages of XRD:

- **Non-destructive:** XRD does not damage the sample, making it suitable for analyzing valuable or fragile materials.
- **Crystal Structure Determination:** XRD accurately determines crystal structures by analyzing the diffraction patterns produced when X-rays interact with crystalline samples.
- **Orientation of Crystal Grains:** XRD can determine the orientation of individual grains within a crystal.
- **Internal Stress Measurement:** It measures internal stress within crystalline areas.
- **Average Spacings:** XRD provides information about the average spacings between atomic layers in samples.

Disadvantages of XRD:

- **Sample Size:** XRD requires small sample sizes but is more accurate for large crystalline structures. Small structures present only in trace elements may go undetected.
- **Limitations for Organic Materials:** XRD is not suitable for organic materials due to their often-similar diffraction signatures.

- **Complex Instrumentation:** XRD setups are more complex and expensive compared to X-ray fluorescence (XRF) techniques.

- **Detection Limit:** If the sample consists of mixed materials, the detection limit for XRD is approximately 2% of the sample.

(14) Scanning Electron Microscope (SEM)

A powerful magnification tool used in science and industry applications. It offers remarkable imaging capabilities.

Advantages of SEM:

1. **High Resolution:** SEMs achieve much higher resolution compared to optical microscopes, typically in the range of **1-10 nanometers**. Some SEMs can even achieve resolutions better than **1 nanometer**. This allows researchers to study fine details of a sample's surface topography, composition, and other properties.
2. **Three-Dimensional Imaging:** SEMs provide detailed **three-dimensional** images, allowing scientists to visualize the surface structure of samples in great detail.
3. **Topographical Information:** SEMs reveal information about the **topography** (surface features) of a sample. This is especially useful for studying rough or uneven surfaces.
4. **Composition Analysis:** SEMs can be equipped with detectors (such as **Energy-Dispersive X-ray Spectroscopy**, or EDS) that allow for **chemical composition analysis**. By detecting characteristic X-rays emitted from the sample, researchers can identify the elements present.

Disadvantages of SEM:

1. **Cost and Size:** SEMs are **expensive** and **large** instruments. They require a dedicated space and specialized facilities. Maintenance involves ensuring a steady voltage, proper currents to electromagnetic coils, and circulation of cooling water.
2. **Sample Preparation:** Most samples need preparation before SEM analysis. Common processes include **sputter coating** for non-conductive samples and **dehydration** of biological specimens. Additionally, all samples must handle the low pressure inside the vacuum chamber.

3. **Special Training Required:** Operating an SEM requires **specialized training** due to its complex setup and various components.

4. **Interference:** SEMs must be housed in an area free from **electric, magnetic, and vibration interference** to maintain accurate imaging results.

(15) Transmission Electron Microscope (TEM)

A powerful instrument used in scientific research and industries.

Description:

TEM utilizes energetic electrons to provide detailed information about samples, including morphological, compositional, and crystallographic details. It includes an electron source, electromagnetic lenses, a vacuum chamber, condensers, a sample stage, and a screen or computer for viewing images. Electrons replace photons (used in optical microscopes), and images are viewed on a screen instead of through an eyepiece.

Advantages:

- **High Magnification:** TEMs offer the most powerful magnification, potentially exceeding one million times or more.
- **High-Resolution Images:** TEMs produce high-resolution, black-and-white images, allowing internal structures within cells and organelles to be seen in detail.
- **Wide Range of Applications:** TEMs find applications in various scientific, educational, and industrial fields.
- **Visualizing Membrane Systems and Organelles:** Due to the shorter wavelength of the electron beam, TEM can visualize cell structures such as membrane systems, cilia, and organelles with great clarity.

Disadvantages:

- **Sample Thickness:** TEMs can only be used with very thin specimens or thin sections of the object

being observed. Samples need to be sliced thin enough for electrons to pass through (electron transparency).

- **Sample Preparation:** Sample preparation for TEM requires special techniques, including dehydration, sputter coating, cryofixation, sectioning, and staining.

- **Cost and Size:** TEMs are large and expensive setups that require special housing and maintenance.

- **Black and White Images:** The images obtained are black and white, limiting color information.

- **Training Required:** Operation and analysis of TEMs require specialized training.

(16) Dynamic Light Scattering (DLS)

A spectroscopy method used in the fields of chemistry, biochemistry, and physics to determine the size distribution of particles (such as polymers, proteins, colloids, etc.) in solution or suspension. It is also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QLS).

Purpose:

- In a DLS experiment, a laser provides monochromatic incident light that impinges onto a solution containing small particles in Brownian motion.
- Through the Rayleigh scattering process, particles whose sizes are sufficiently small compared to the wavelength of the incident light will diffract the light in all directions with different wavelengths and intensities over time.
- The scattering pattern of the light is highly correlated to the size distribution of the analyzed particles, allowing size-related information to be acquired by mathematically processing the spectral characteristics of the scattered light.

Advantages of DLS:

- **Wide Size Range:** DLS can measure the size distribution of particles in a wide range of sizes, from a few nanometers to several microns.
- **Non-Invasive and Non-Destructive:** It does not require sample preparation and is a non-invasive technique.
- **Fast and Easy-to-Use:** DLS provides results within minutes.

- **Sensitive:** It can detect small changes in particle size.
- **Can Measure Macromolecules:** Some modern systems are sensitive enough to measure the size of macromolecules in solution.

Disadvantages of DLS:

- **Time-Consuming:** DLS measurements can be time-consuming, especially for slow dynamics.
- **Transparent Samples Only:** DLS works best with transparent samples.
- **Sensitive to Mechanical Disturbances:** Mechanical disturbances can affect the accuracy of DLS measurements.
- **Statistical Uncertainty:** Achieving a low statistical experimental uncertainty (e.g., 1%) requires a large number of measurements (over 10,000 samples of the correlation decay characteristic) due to the probabilistic nature of the technique.

(17) Reverse Phase (RP)

A technique used in high-performance liquid chromatography (HPLC) to separate components in a mixture and identify and quantify each component.

Advantages:

- **Water-Based Mobile Phase:** RP-HPLC allows water to be used in the composition of the mobile phase along with other solvents.
- **Accurate Results with Small Sample Amounts:** RP-chromatography provides accurate results even with small sample quantities.
- **pH Selectivity:** It can use pH selectivity to improve separation.

Disadvantages:

- **Limited Separation of Hydrophobic Molecules:** While RP chromatography can separate molecules with some degree of hydrophobicity (such as proteins, peptides, and nucleic acids), it may not be suitable for all types of analytes.
- **Sample Matrix Effects:** The presence of other components in the sample matrix can affect the separation efficiency.
- **Column Lifetime:** The lifetime of the RP column may be limited due to interactions with the sample matrix.

(18) Fluorescence Recovery after Photobleaching (FRAP)

A powerful technique used to study the mobility and dynamics of cellular molecules.

Purpose:

FRAP involves the selective bleaching of a region of interest (ROI) within a living cell using a high-intensity laser. This bleaching process erases the fluorescence of the molecules in that region. Once bleached, the recovery of fluorescence is monitored over time as unbleached molecules move into the ROI. This provides insights into the mobility and redistribution of cellular components.

Advantages:

- **Non-Invasive:** FRAP allows measurements without disrupting the cell's natural environment.
- **Highly Specific:** It provides precise information about the mobility of specific molecules.
- **Micrometer Scale:** FRAP can measure diffusion on a micrometer scale.
- **Useful in Drug Research:** It helps investigate drug diffusion within tissues and materials for controlled drug delivery.

Disadvantages:

- **Cell Movement:** Living cells move, making it challenging to track a specific ROI accurately.
- **Light Intensity:** FRAP requires high light intensity to bleach the entire cell, which can be damaging.
- **Limitation in Organelles:** FRAP cannot directly monitor efflux of fluorescent molecules from organelles without bleaching them.

(19) Electrospray Ionization ESI

Description:

Electrospray Ionization is a soft ionization technique used to determine the molecular weights of peptides, proteins, and other biological macromolecules. A widely used ionization technique in mass spectrometry. It allows the analysis of large biomolecules, such as peptides, proteins, and nucleic acids, by converting them into gas-phase ions.

A sample (usually dissolved in a volatile solvent) is introduced into the mass spectrometer. The solvent evaporates, leaving behind the analyte molecules. A high voltage is applied to a fine needle or capillary. The sample solution is sprayed through this needle into the mass spectrometer. As the droplets of the solution move toward the mass spectrometer inlet, they become smaller due to solvent evaporation. Eventually, the droplets break up into even smaller charged droplets. The analyte molecules are carried by these charged droplets. The charged droplets continue to evaporate, leading to desolvation. As the droplets shrink, the charge density on the analyte molecules increases. Eventually, the analyte molecules become isolated and surrounded by solvent molecules. At this point, the solvent molecules are stripped away, leaving behind gas-phase ions of the analyte. The gas-phase ions are then transferred into the mass analyzer (e.g., quadrupole, time-of-flight, or ion trap). In the mass analyzer, the ions are separated based on their mass-to-charge ratio (m/z). The mass spectrum is obtained, showing the m/z values and relative abundances of the ions.

Purpose:

ESI is particularly useful for analyzing large and complex molecules because it produces gentle ionization, minimizing fragmentation. Researchers in fields like proteomics, metabolomics, and drug discovery rely on ESI for identifying and quantifying biomolecules.

(20) Quantitative Research (Q)

Quantitative research provides valuable data quickly.

Advantages

1. **Rapid Data Collection:** Quantitative research involves surveys, experiments, and real-time data gathering. As a result, there are minimal delays in collecting materials for examinations, allowing for quick analysis compared to other research methods.

2. **Randomized Samples:** Quantitative research uses a randomized process to collect information, reducing bias. This randomness allows statistical application to the entire population group under study, even though some demographics may be inadvertently left out.

3. **Reliable and Repeatable Information:** Consistent results are obtained when examining the same data points under randomized conditions. This reliability provides a foundation for future planning processes, allowing businesses to tailor messages or programs based on these results.

4. **Generalization:** Unlike other research types, quantitative research allows for generalization. Although it offers an overview rather than specificity, it helps identify core subjects, needs, or wants within target groups.

Disadvantages of Quantitative Research:

1. **Limited Authenticity:** Quantitative research focuses on numerical data, which may lack the depth and context provided by qualitative research. It may not capture the full richness of human experiences or emotions.

2. **Cost and Complexity:** While cheaper than some other research methods, quantitative research can still be

resource-intensive due to the need for large sample sizes and statistical analysis.

3. Risk of Bias: Despite randomization, certain demographics may be excluded, leading to errors when applying research results to the entire population. Researchers must be cautious about potential biases.

(21) QTOF - Quadrupole Time of Flight Mass Spectrometry

Description:

QTOF stands for **Quadrupole–Time of Flight**. It refers to a type of mass spectrometer that combines both quadrupole and time-of-flight (TOF) instruments. The **Quadrupole Mass Filter** is a fundamental component of sophisticated mass spectrometers. It consists of four parallel metal rods to which fixed direct-current voltage and alternating radio-frequency voltage are applied. The quadrupole mass filter selectively filters ions based on their mass-to-charge ratio (m/z). It allows only ions with specific m/z values to pass through, while others are filtered out. The TOF analyzer measures the time it takes for ions to travel from the ionization source to the detector. Ions are accelerated by an electric field, and their velocities depend on their mass and charge. Lighter ions reach the detector faster than heavier ones. By measuring the time of flight, the TOF analyzer determines the mass of the ions. In a **QTOF Mass Spectrometer**, the quadrupole and TOF instruments work together. This combination enables fragmentation experiments that yield highly accurate mass quantitation for product ions. The fragmented ion ratios (m/z) are determined through time-of-flight measurements.**Purpose**

- QTOF instruments offer high mass resolution, allowing researchers to distinguish between closely related ions with similar masses.
 - QTOF provides accurate mass measurements, which are crucial for identifying unknown compounds and confirming the presence of specific analytes.
 - QTOF can analyze a broad range of masses, making it suitable for various applications, including proteomics, metabolomics, environmental analysis, and drug discovery.

- QTOF instruments can perform DIA experiments, capturing full mass spectra across a wide m/z range without predefined target ions. This enables comprehensive profiling of complex samples.
- Researchers can compare acquired spectra against reference libraries (such as NIST or proprietary databases) for compound identification.

TOF-MS

It is widely used for structural characterization, quantitative analysis, and identification of molecules in complex matrices.

(22) URLRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (UHPLC)

Description:

It is an advanced form of column chromatography that separates, identifies, and quantifies compounds. It uses high pressure (either PNEUMATIC OR HYDRAULIC) to push solvents through the column and smaller particle LC columns to achieve higher efficiency and shorter separation time. UHPLC is an evolution of HPLC, driven by the need for better separation of mixtures.

While UHPLC primarily generates pressure using Pneumatic compressible gas such as air or a suitable pure gas, hydraulics pressure is generated by compression of liquids uses relatively incompressible liquid media such as oil.

Purpose:

- Identifying and quantifying components in mixture.
- Separating amino acids, nucleic acids, proteins, hydrocarbons, pesticides, carbohydrates, antibiotics, steroids, and other inorganic substances.
- Determining sample purity and quality assurance.
- Final dosage forms
- Drug research and discovery

Industry Use:

1. Pharmaceutical Industry:
2. Biotechnology and Life Sciences:
3. Environmental Testing and Monitoring:
4. Food and Beverage Industry:

5. Chemical and Petrochemical Industry:
6. Forensic Science and Toxicology:
7. Academic Research and Development:

(23) Proton Nuclear Magnetic Resonance (^1H NMR)

AN analytical technique used for characterizing organic compounds, including natural products like herbs.

Nuclear Magnetic Resonance (NMR):

NMR spectroscopy is a nondestructive method that provides information about the arrangement of atoms in a molecule based on their magnetic properties. It detects the absorption of radiofrequency energy by atomic nuclei (such as protons, carbon-13, and others) when placed in a strong magnetic field. The most common NMR nuclei studied are protons (^1H) and carbon-13 (^{13}C).

^1H NMR:

In ^1H NMR, the focus is on the absorption of radiofrequency energy by hydrogen nuclei (protons). Protons in different chemical environments (due to their neighboring atoms) resonate at specific frequencies, resulting in distinct peaks in the NMR spectrum. These peaks provide information about the connectivity, functional groups, and molecular structure of the compound. ^1H NMR is more sensitive than ^{13}C NMR and is commonly used for metabolomics and natural product profiling.

^{13}C NMR:

In ^{13}C NMR, carbon-13 nuclei are studied. Carbon-13 has a lower natural abundance than protons, so ^{13}C NMR spectra are less intense. However, ^{13}C NMR spectra are simpler, with less overlapping signals, making them useful for structural determination.

Unlike ^1H NMR, ^{13}C NMR is less affected by variations in magnetic field strength.

PURPOSE:

- NMR is valuable for analyzing complex mixtures, such as herbal extracts.
- Researchers use ^1H and ^{13}C NMR to identify and quantify compounds in these extracts. Two-dimensional (2D) NMR techniques, such as COSY (correlation spectroscopy) and HSQC (heteronuclear single quantum coherence), help resolve overlapping signals. Standardizing NMR protocols enhances its utility for profiling natural products.

Advantages:

Non-Destructive and Non-Invasive:

^1H NMR spectroscopy allows for the analysis of herbs without destroying or altering the sample. This is particularly valuable when studying rare or precious plant materials.

Minimal Sample Preparation:

Unlike some other techniques, ^1H NMR does not require extensive sample preparation. Herbs can be directly dissolved in suitable deuterated solvents for analysis.

Accurate Structural Information:

^1H NMR provides detailed information about the chemical structure of herb constituents. It reveals intramolecular interactions, such as hydrogen bonding and conformational changes.

Three-Dimensional Structural Insights:

By analyzing molecular vibrations within the natural environment, ^1H NMR can yield accurate three-dimensional (3D) structural information. This helps researchers understand the spatial arrangement of atoms within herb molecules.

Simple Data Acquisition and Analysis:

Modern NMR instruments allow rapid data acquisition and straightforward analysis. Researchers can obtain spectra efficiently, aiding in herb characterization.

Disadvantages:

Low Sensitivity:

NMR instruments have relatively low sensitivity, especially for samples with low concentrations. This limitation can result in poor-quality spectra due to weak interaction energies between the NMR magnetic resonance and the sample molecules.

Cost and Maintenance:

NMR instruments are expensive to purchase and maintain. They require large, powerful magnets as energy sources and cryogenic liquids for cooling. These costs can be prohibitive for some research settings.

Limited Applicability to High Molecular Weight Molecules:

^1H NMR spectroscopy is less effective for analyzing large molecules (e.g., complex polysaccharides

or proteins) due to spectral complexity and challenges in interpretation.

(24) Quadrature Amplitude Modulation (QAMS)

A modulation technique that combines both amplitude and phase variations to represent binary data.

Advantages:

- **Efficient Bandwidth Usage:** QAMS variants allow efficient utilization of bandwidth. As the modulation order increases (e.g., 16QAM, 64QAM, 256QAM), more bits are mapped per carrier signal. For instance, 16QAM maps 4 bits per carrier, 64QAM maps 6 bits, and 256QAM maps 8 bits per carrier.
- **Higher Data Rates:** Due to the increased number of bits per carrier, QAMS enables higher data rates compared to other modulation techniques.
- **Flexible Trade-off:** QAMS allows a trade-off between bandwidth efficiency and complexity. By choosing an appropriate modulation order, system designers can balance these factors.

Disadvantages:

- **Susceptibility to Noise:** As the states (symbols) in QAMS are closer together, the modulation is more susceptible to noise. This makes QAMS receivers more complex compared to other modulation types.
- **Linearity Requirements:** QAMS uses the amplitude component of the signal to represent binary data.

(25) Linear Ion Trap Quadrupole (LTQ)

A mass spectrometry method used for the characterization of chemical compounds, including those found in herbs.

Advantages:

- **High Sensitivity:** The LTQ system can detect low-abundance compounds due to its high sensitivity. This is crucial when analyzing complex mixtures like herbal extracts.
- **Tandem Mass Spectrometry (MS/MS):** The LTQ allows for fragmentation of ions within the trap, enabling MS/MS analysis. This helps identify specific fragments and elucidate compound structures.
- **Speed and Efficiency:** LTQ systems provide rapid data acquisition, making them suitable for high-throughput analyses.
- **Dynamic Range:** The LTQ can handle a wide range of compound concentrations, from trace levels to high concentrations.
- **Ion Trap Flexibility:** The ion trap design allows for multiple scan modes (e.g., full scan, product ion scan, neutral loss scan), enhancing versatility.

Disadvantages:

- **Limited Mass Range:** The LTQ has a relatively narrow mass range compared to other mass spectrometers. It may miss large or high-mass compounds.

- **Scan Speed:** While LTQ systems are fast, they may not match the speed of some newer instruments.
- **Ion Suppression:** In complex samples, co-eluting compounds can suppress each other's ionization, affecting sensitivity.
- **Resolution:** The LTQ's resolution is lower than that of high-resolution instruments (e.g., Orbitrap). This can impact accurate mass measurements.
- **Ion Capacity:** The ion trap can become saturated with ions, leading to signal loss for abundant compounds.

(26) MS_n

A mass spectrometry technique that involves multiple stages of fragmentation. This analytical technique measures the mass-to-charge ratio of ions. It helps identify and quantify molecules based on their mass spectra.

MS₂: is the first stage, a precursor ion is selected and fragmented into smaller ions. These fragments are then analyzed.

MS₃ is where a second round of fragmentation can occur (MS₃). This is where a specific fragment from the MS₂ stage is further broken down.

MS_n can continue with additional stages (MS₄, MS₅, etc.), each providing more detailed structural information.

Advantages:

- **Structural Information:** MS_n allows detailed analysis of complex mixtures, such as herbal extracts. It provides information about the molecular structure, including functional groups and connectivity.
- **Identification:** By comparing fragmentation patterns with databases or reference compounds, MS_n helps identify specific compounds in herbs.
- **Quantification:** MS_n can quantify compounds based on their fragmentation intensity.
- **Metabolite Profiling:** It aids in studying the metabolic profile of herbs, revealing bioactive compounds.

Disadvantages:

- **Complexity:** MSn requires specialized instrumentation and expertise. Interpreting complex spectra can be challenging.
- **Sample Preparation:** Proper sample preparation is crucial. Contaminants or matrix effects can interfere with accurate results.
- **Ion Suppression:** Co-eluting compounds may suppress each other's ionization, affecting quantification.
- **Limited Sensitivity:** Some low-abundance compounds may not yield detectable signals.
- **Time-Consuming:** Multiple stages of fragmentation increase analysis time.

(27) Ethanol-Zinc Precipitation (EZIP)

A method used to extract bioactive compounds from herbal materials. It involves the use of ethanol and zinc ions to selectively precipitate specific constituents. Herbal material is first extracted with ethanol. The extract is then mixed with a zinc salt solution. The zinc ions selectively bind to certain compounds, causing them to precipitate out of the solution. The precipitate can be collected and further analyzed.

Advantages:

- **Selective Extraction:** EZIP allows for the selective extraction of specific bioactive compounds. This can be useful when targeting particular constituents.
- **Reduced Complexity:** Compared to other extraction methods, EZIP is relatively straightforward and easy to perform.
- **Cost-Effective:** The materials required for EZIP are readily available and cost-effective.

Disadvantages:

- **Limited Spectrum:** EZIP may not extract the full spectrum of bioactive compounds present in the herb. It tends to focus on specific constituents, potentially missing out on others.
- **Lack of Standardization:** The process lacks standardization, leading to variations in extraction efficiency from batch to batch.
- **Potential Loss of Synergy:** By selectively targeting specific compounds, EZIP may disrupt the natural synergy between various constituents found in the herb.

(28) Next Generation Sequencing (NGS)

A set of high-throughput DNA sequencing technologies that have revolutionized genomic research. NGS offers a powerful tool for characterizing herb-associated microbiomes.

Advantages of NGS for Characterizing Herbs:

- **Deeper Insights:** NGS allows researchers to study and understand microorganisms, including those found in herbs, from broader and deeper perspectives. It provides finer characterization of bacterial genomes and enables taxonomic identification of complex microbiomes.
- **Species-Level/Strain-Level Characterization:** Unlike traditional methods, such as Sanger sequencing, NGS techniques (such as metagenomics) provide species-level or even strain-level characterization. This is crucial for understanding the diversity within herb-associated microbial communities.
- **Functional Insights:** Metatranscriptomic approaches, a subset of NGS, contribute to functional characterization by revealing the complex interactions between different microbial communities within a single microbiome.
- **Speed and Cost Efficiency:** NGS platforms offer faster and more cost-effective sequencing compared to Sanger sequencing.
- **Multiplexing:** NGS allows multiplexing of DNA fragments, enabling simultaneous analysis of multiple samples.

Disadvantages of NGS for Characterizing Herbs:

- **Data Complexity:** NGS generates massive amounts of data, which can be challenging to analyze and interpret.
- **Bioinformatics Expertise Required:** Effective NGS analysis requires bioinformatics expertise to handle raw data, perform quality control, and extract meaningful insights.
- **Error Rates:** Although NGS technologies have improved, they still have error rates (albeit low). Researchers need to account for these errors during data analysis.
- **Short Read Lengths:** Some NGS platforms produce shorter read lengths compared to Sanger sequencing. While this is less of an issue now, it can impact assembly of complex genomes.
- **Sample Preparation:** Proper sample preparation is critical for accurate results. Contaminants or biases introduced during sample collection or DNA extraction can affect NGS outcomes.

(29) Diode-Array Detection

Description:

Diode array detection is a technique used in spectroscopy and chromatography. It involves a linear array of discrete photo diodes on a single integrated circuit (IC) chip. The diode array detector absorbs light within UV or visible wavelength spectrums to perform spectroscopic scanning and obtain precise absorbance readings at various wavelengths. It allows simultaneous collection of absorption data from a range of UV and visible wavelengths.

Purpose:

1. Liquid Chromatography (LC):

- DAD is commonly used in **high-performance liquid chromatography (HPLC)** and **ultra-high-performance liquid chromatography (UHPLC)**.
- It allows for **simultaneous detection** of multiple analytes in a sample.
- LC-DAD is useful for **quantitative analysis** of compounds, such as pharmaceuticals, environmental pollutants, and food additives.
- It provides **spectral information** that can aid in compound identification.

2. Pharmaceutical Analysis:

- DAD is essential for **quality control** of pharmaceutical products.
- It helps identify impurities, assess drug stability, and verify drug formulations.

3. Environmental Monitoring:

- DAD is used to detect and quantify **environmental contaminants** (e.g., pesticides, herbicides, and industrial chemicals) in water, soil, and air samples.

4. Food and Beverage Industry:

- DAD assists in analyzing food additives, preservatives, and flavor compounds.
- It ensures food safety and quality.

5. Biochemical Research:

- DAD is valuable in studying **biomolecules** (e.g., proteins, nucleic acids, and metabolites).
- It aids in protein and peptide analysis.

6. Forensic Science:

- DAD helps identify illicit drugs, toxins, and other compounds in forensic samples.

7. Phytochemical Analysis:

- DAD is used to study plant extracts and natural products.
- It assists in identifying bioactive compounds.

8. Polymer Characterization:

- DAD is employed in analyzing polymers and copolymers.
- It provides information on molecular weight distribution and chemical composition.

(30) Binary-Coded Decimal (BCD)

A numerical representation system. There are several methods used for authentication and quality control:

1. Molecular-Based Analysis:

- This method involves analyzing the genetic material (DNA) of herbs. Techniques like **DNA metabarcoding** can identify specific plant species by examining their unique genetic markers.

- **Advantages:**

- High specificity: Can accurately identify species even in complex mixtures.
- Non-destructive: Doesn't require destroying the sample.

- **Disadvantages:**

- Requires specialized equipment and expertise.
- May not work well for processed or powdered herbs.

2. Chemical Fingerprinting:

- In this approach, the chemical composition of herbs is analyzed using techniques like **thin layer chromatography (TLC)** or **high-performance liquid chromatography-mass spectrometry (HPLC-MS)**.

- **Advantages:**

- Provides a unique “fingerprint” for each herb.
- Useful for detecting adulteration.

- **Disadvantages:**

- Requires reference standards for comparison.

- Variation due to environmental factors can affect results.

3. Macroscopic and Analogical Analysis:

- This involves visual inspection of herbs based on their macroscopic features (e.g., color, shape, texture).
- **Advantages:**
 - Simple and cost-effective.
 - Can be done without specialized equipment.
- **Disadvantages:**
 - Subjective interpretation.
 - Limited to visible features.

4. Combination Methods:

- Combining different techniques can enhance accuracy. For example, using both molecular analysis and chemical fingerprinting.
- **Advantages:**
 - Synergy of strengths from multiple methods.
 - Improved reliability.
- **Disadvantages:**
 - Increased complexity.
 - Resource-intensive.

(31) Thin Layer Chromatography (TLC)

A widely used analytical technique for characterizing herbs and other natural products.

Advantages of TLC:

- **Cost-Effective:** TLC is relatively inexpensive compared to other chromatographic methods.
- **Rapid Analysis:** It provides quick results, making it suitable for routine quality control.
- **Simple Procedure:** The technique is straightforward and doesn't require complex instrumentation.
- **Visual Detection:** TLC allows direct visual observation of separated compounds on the plate.
- **Qualitative Analysis:** It's useful for identifying specific compounds based on their migration distances and characteristic spots.
- **Sample Cleanup:** TLC can be used to check for impurities or adulterants in herbal extracts.
- **Fingerprinting:** TLC can create unique fingerprints for herbal samples, aiding in their identification.

Disadvantages of TLC:

- **Limited Quantitative Analysis:** While TLC is excellent for qualitative analysis, it's less precise for quantitative measurements.
- **Sensitivity:** Some compounds may not be visible under UV light or may have weak spots, affecting sensitivity.
- **Subjectivity:** Visual interpretation introduces subjectivity, especially when comparing spots.

- **Lack of Resolution:** TLC may not separate closely related compounds effectively.
- **Limited Mobile Phases:** The choice of mobile phase affects separation, and TLC has fewer options than other chromatographic methods.
- **Plate Variation:** Different TLC plates can yield varying results due to differences in stationary phase quality.
- **Sample Size:** Small sample sizes limit the amount of material that can be loaded onto the plate.

(32) Photodiode Array Detection (PDA)

Description:

A photodiode array detector (PDA) is a type of chromatography detector that uses a series of photodiodes to measure the absorbance of a sample at multiple wavelengths. It is a linear array of discrete photodiodes on a single integrated circuit (IC) chip. The PDA detector is placed at the image plane from the grating to allow a range of wavelengths to be detected simultaneously. The photodiode array detector is essentially a spectrophotometer that transiently measures the absorbance of light by a liquid flowing past. **Photodiode array detectors (PDAs)**, also known as **diode array detectors (DADs)**, play a crucial role in analytical chemistry, particularly in **liquid chromatography (LC)**

Purpose:

Absorbance Measurement:

- PDAs measure the **absorbance of light** by a liquid sample as it flows past the detector.
- They are commonly used in **high-performance liquid chromatography (HPLC)** and **ultra-high-performance liquid chromatography (UHPLC)** systems.
- By analyzing the absorbance, PDAs provide information about the **identity, quantity, and purity** of sample components as they exit the chromatographic column.

How PDAs Work:

- A PDA consists of an **array of photodiodes** that convert the impinging light into proportional electrical signals.

- The light source interrogates the sample as it passes through a **flow cell**.
- The sample absorbs some of the light, and the remaining light is linearly dispersed into different wavelength components using a **diffraction grating**.
- Each pixel in the diode array corresponds to a specific wavelength range (e.g., pixel 1 might detect light around 200 nm, while pixel 1024 would see 600-nm light in a 1024-diode array).
- Unlike other detectors, PDAs simultaneously interrogate the **entire spectrum** without any moving parts.

Advantages of PDAs:

- **Versatility:** PDAs allow researchers to examine **all wavelengths simultaneously**. This capability is essential for method development and compound analysis.
- **Spectrum Comparison:** Researchers can overlay spectra, compare absorption from different compounds, and compare to reference spectra from known compounds.

(33) Direct Analysis in Real Time (DART):

It is an ion source that is used in mass spectrometry. It produces electronically or vibronically excited-state species from gases such as helium, argon, or nitrogen. These excited species then ionize atmospheric molecules or dopant molecules. It is commonly employed for rapid analysis of various samples. It can be a valuable tool for initial screening and rapid profiling of herbs.

Advantages:

- **Speed:** DART provides real-time analysis, allowing for quick results without extensive sample preparation.
- **Non-Destructive:** Unlike some other techniques, DART does not require sample destruction, preserving the original material.
- **Broad Applicability:** It can analyze a wide range of compounds, making it suitable for herb characterization.
- **Minimal Sample Handling:** DART eliminates the need for complex sample extraction or derivatization steps.

Disadvantages:

- **Limited Sensitivity:** DART may not be as sensitive as other techniques, especially for low-abundance compounds.
- **Lack of Structural Information:** While it provides molecular weight information, it doesn't offer detailed structural insights.
- **Matrix Effects:** Interference from sample matrix components can affect results.

- **Ionization Bias:** DART may favor certain types of compounds over others.
- **Sample Homogeneity:** Uneven sample surfaces can impact reproducibility.
- **Limited Quantitative Accuracy:** Quantitative analysis using DART can be challenging.

(34) Supercritical Fluid Extraction (SFE)

Description:

A technique that uses supercritical fluids, usually CO₂, as the solvent to separate one component from another. SFE can be used for sample preparation, purification, and concentration of analytes. SFE is faster and more efficient than traditional liquid extraction because supercritical fluids have low viscosity and high diffusivity. SFE is often used to extract botanical components. Supercritical fluids are substances that exist in a state beyond their critical point. The critical point is the temperature and pressure at which a substance transitions between its liquid and gas phases. In the case of SFE, carbon dioxide (CO₂) is commonly used as the supercritical fluid. CO₂ becomes supercritical at relatively mild conditions (around 31°C and 74 bar pressure).

Purpose:

1. **Food Industry:** For extracting flavors, fragrances, and bioactive compounds.
2. **Pharmaceuticals:** To extract natural products and active pharmaceutical ingredients.
3. **Environmental Analysis:** For detecting pollutants and contaminants.
4. **Herbal Medicine:** To obtain herbal extracts.

Advantages:

5. **Environmental Improvement and Reduced Product Contamination:** SFE serves as an alternative to liquid extraction methods that use solvents like hexane or dichloromethane. Unlike conventional liquid-liquid extractions, where residual solvents may remain in the extract and matrix, SFE minimizes environmental contamination.

6. Gentle Treatment for Heat-Sensitive Materials: SFE operates at low temperatures, avoiding damage caused by heat or organic solvents. This gentle treatment is particularly beneficial for heat-sensitive compounds found in herbs.

7. Selective Extraction: SFE allows for selective extraction of specific compounds during the process. Different molecular weight compounds can be separated based on their temperature and pressure characteristics, enabling fractionation.

8. Efficient Removal of Unwanted Residues: In applications like cannabis extraction, SFE can strip away unwanted residues (such as pesticides, chlorophyll, and waxes) while leaving behind desired compounds like CBD, THC, and terpenes.

Disadvantages:

9. Equipment Complexity: Setting up an SFE system requires specialized equipment, including a pump for CO₂, a pressure cell, means to maintain pressure, and collection vessels. This complexity can be a drawback for some users.

10. Cost: The initial investment in SFE equipment can be relatively high, which may limit its adoption for smaller-scale operations.

11. Limited Solubility: While supercritical CO₂ is an excellent solvent for many compounds, its solubility for certain polar compounds (such as water-soluble constituents) is limited. This can affect the efficiency of extraction for specific herb components.

(35) High-Performance Thin Layer Chromatography (HPTLC)

A sophisticated instrumental technique used for both quantitative and qualitative analysis of herbs and herbal drugs.

Advantages:

- **Rapid Analysis:** HPTLC provides quick results, making it suitable for routine quality control.
- **Simplicity:** The method is relatively straightforward, making it accessible even for less experienced analysts.
- **Accuracy:** HPTLC can accurately substantiate the identity of herbal plant materials.
- **Parallel Analysis:** It allows simultaneous analysis of multiple samples, which is useful for large-scale quality control.
- **Detection of Adulteration:** HPTLC can detect adulterants in herbal products.
- **Quantification of Marker Compounds:** It quantifies specific compounds (e.g., active constituents) in complex matrices like plant materials.

Disadvantages:

- **Sample Preparation:** Proper sample preparation is crucial for accurate results.
- **Limited Separation Power:** Compared to other chromatographic techniques (e.g., HPLC), HPTLC has limited separation power.
- **Sensitivity:** Although sensitive, it may not match the sensitivity of more advanced methods.
- **Quantification Challenges:** Quantification can be challenging due to variations in spot size and intensity.

- **Lack of Universal Standards:** Unlike HPLC, there are no universal standards for HPTLC, which affects method validation and comparison.

(36) Triple Quadrupole Mass Spectrometry (QQQ)

An analytical technique used for quantification and identification of specific compounds in complex samples, including herbs.

Advantages:

- **High Sensitivity:** QQQ instruments can detect trace amounts of compounds, making them suitable for analyzing low-concentration herbal constituents.
- **Selective Detection:** QQQ allows targeted analysis by monitoring specific precursor ions and their fragment ions. This selectivity helps identify and quantify compounds of interest.
- **Quantitative Accuracy:** QQQ provides accurate quantification due to its linear response over a wide concentration range.
- **Matrix Effects Compensation:** By using stable isotope-labeled internal standards, QQQ corrects for matrix effects, enhancing accuracy.
- **Tandem MS (MS/MS):** QQQ's tandem MS capability enables structural elucidation of compounds through fragmentation patterns.
- **Multiple Reaction Monitoring (MRM):** QQQ uses MRM mode to selectively monitor specific transitions, improving sensitivity and specificity.

Disadvantages:

- **Limited Information on Non-Targeted Compounds:** QQQ focuses on pre-selected compounds, potentially missing unexpected or novel constituents.

- **Complex Method Development:** Setting up MRM transitions and optimizing collision energies can be time-consuming.
- **Cost:** QQQ instruments are expensive, and maintenance requires skilled personnel.
- **Sample Preparation Challenges:** Complex herbal matrices may interfere with ionization or cause matrix effects.
- **Limited Structural Information:** While QQQ provides quantitative data, it lacks detailed structural information compared to high-resolution mass spectrometry (HRMS).

(37) Multiple Reaction Monitoring (MRM)

A technique used for characterizing herbs and assessing their quality.

Advantages of MRM for Characterizing Herbs:

- **High Sensitivity:** MRM allows the detection of specific compounds at very low concentrations, making it suitable for identifying trace constituents in herbal materials.
- **Selectivity:** By targeting specific precursor-product pairs (transitions), MRM provides high selectivity, reducing interference from other compounds.
- **Quantification:** MRM enables accurate quantification of target compounds, essential for assessing the potency of herbal products.
- **Speed and Throughput:** MRM can analyze multiple compounds simultaneously, enhancing efficiency in quality control.
- **Minimal Sample Preparation:** Unlike some other techniques, MRM requires minimal sample preparation, saving time and resources.

Disadvantages of MRM for Characterizing Herbs:

- **Limited Coverage:** MRM focuses on pre-selected compounds, which may miss unexpected or novel constituents.
- **Targeted Approach:** MRM relies on prior knowledge of the compounds of interest, limiting its applicability to known markers.

- **Instrumentation Complexity:** Setting up MRM methods requires specialized mass spectrometers and expertise.
- **Matrix Effects:** Herbal matrices can interfere with ionization and affect quantification accuracy.
- **Cost:** The initial investment in equipment and method development can be high.

(38) 2D NMR (Two-Dimensional Nuclear Magnetic Resonance)

A technique used to study the molecular structure of various compounds, including herbs.

Advantages:

- **Overlapping Signal Resolution:** The major advantage of 2D NMR over 1D NMR is its ability to distinguish overlapping signals in larger molecules. In herbs, where complex mixtures of compounds exist, 2D NMR helps resolve overlapping peaks, providing clearer information about individual components.
- **Structural Elucidation:** 2D NMR allows for the elucidation of complex structures. By analyzing correlations between nuclei, researchers can identify specific functional groups, linkages, and spatial arrangements within herb constituents.
- **Quantitative Analysis:** While qualitative analysis provides structural information, 2D NMR can also be used quantitatively to determine the concentration of specific compounds in herbal extracts.
- **Minimal Sample Preparation:** Unlike some other techniques, 2D NMR requires minimal sample preparation, making it suitable for analyzing natural products like herbs.

Disadvantages:

- **Time-Consuming:** 2D NMR experiments can be time-consuming due to the need for multiple scans and data processing.
- **Sample Size:** Larger samples are often required for 2D NMR, which may be challenging when working with limited herb quantities.

- **Instrument Availability:** Access to high-field NMR instruments is necessary for optimal results, and not all laboratories have such equipment.
- **Complex Interpretation:** Interpreting 2D NMR spectra can be intricate, especially for complex mixtures like herbs.

(39) MS/MS (Tandem Mass Spectrometry):

Description:

Tandem mass spectrometry (MS/MS), also known as **MS2**, is a technique used in instrumental analysis to enhance the capabilities of mass spectrometry. MS/MS provides high sensitivity, specificity, and accuracy, which makes it a valuable tool across various scientific disciplines.

In regular mass spectrometry (MS), molecules from a sample are ionized, and the first mass spectrometer (MS1) separates these ions based on their mass-to-charge ratio (often denoted as m/z or m/Q). MS1 identifies the ions, but sometimes ions with very similar m/z ratios can't be easily distinguished. In MS/MS, an extra step is added to improve specificity and identification. After MS1, selected ions (based on their m/z ratio) undergo fragmentation. These fragmented ions are then introduced into a second mass spectrometer (MS2), which further separates and detects them based on their m/z ratios. The fragmentation step allows for better identification and separation of ions that were indistinguishable in the regular MS.

Purpose

Allows the fragmentation of intact proteins to determine their amino acid sequence. Focuses on analyzing protein fragments generated by controlled cleavage. MS/MS helps identify these fragments. Proteins are enzymatically digested into peptides, and MS/MS is used to identify and sequence these peptides. Enables the identification of post-translational modifications on proteins and peptides. These modifications are very important in cellular processes. Helps identify and quantify small molecules (metabolites) in biological samples and is essential for understanding metabolic pathways and disease mechanisms. Used to screen newborns for inborn errors of metabolism. It can detect metabolic disorders early, allowing timely intervention. Used to diagnose various diseases by analyzing specific metabolites or biomarkers associated with those conditions. Used to study drug

metabolism, pharmacokinetics, and drug interactions. Used to detect pollutants, toxins, and other contaminants in environmental samples (such as water, soil, and air). Assists in identifying drugs, toxins, and other substances in biological samples (e.g., blood, urine, hair).

(40) Ultra Performance Liquid Chromatography (UPLC)

A modern technique that has revolutionized liquid chromatography. It enhances liquid chromatography in three critical areas: **speed, resolution, and sensitivity**. Unlike traditional high-performance liquid chromatography (HPLC), UPLC is applicable for particles low in diameter, allowing for better resolution, faster analysis, and increased sensitivity.

Advantages:

- **Reduced Run Time:** UPLC requires less run-time compared to HPLC, which is advantageous for high-throughput analyses.
- **Enhanced Sensitivity:** UPLC provides higher sensitivity due to its ability to handle smaller particle sizes.
- **Improved Resolution:** Chromatograms obtained from UPLC show well-resolved peaks, allowing for better separation of compounds.
- **Multi-Residue Methods:** UPLC is commonly used for multi-residue analysis, making it suitable for characterizing complex herbal mixtures¹.

Disadvantages of UPLC:

- **Increased Pressure:** UPLC operates under very high pressure (up to 100 MPa). While this allows for better performance, it also requires specialized equipment and more maintenance.
- **Column Life:** The increased pressure can reduce the life

APPINDEX B

Characterization Articles Outline

Number: 1

Article Name: A Simple, Rapid, and Practical Method for Distinguishing *Lonicerae Japonicae* Flos from *Lonicerae* Flos

Author: Zhang, Shi, Liu, Zhang, Yu, Li, & Pu. (2019). A Simple, Rapid, and Practical Method for Distinguishing *Lonicerae Japonicae* Flos from *Lonicerae* Flos. *Molecules*, 24(19), 3455.

<https://doi.org/10.3390/molecules24193455>

Location: China

Year: 2019

Main Technique: HPLC, Q EXACTIVE HYBRID QUADRUPOLE-ORBITRAP-MS, UV-VIS SPECTROSCOPY

Secondary Techniques: LC-MS, NMR

Aim: Our study not only provides a rapid, simple, sensitive, and practical method for identifying LJF and LF but also establishes a method for discovering the characteristic components of other herb-medicines that are susceptible to adulteration.

Bioactive Components: *Lonicerae Japonicae* Flos from *Lonicerae* Flos

Method Effectiveness: The extraction efficiency was tested by comparing the amount of secologanic acid in different extraction solvents, including water and ethanol of different concentrations

Method Limitation: microscopic identification was limited to small differences in identification points and poor practicability, and the ^1H -NMR fingerprints and DNA barcoding technology had the disadvantages of being expensive and complex, which made them difficult to popularize.

Gaps and Recommendations: None mentioned

Number: 2

Article Name: An overview of recent developments in metabolomics and proteomics – phytotherapeutic research perspectives

Author: Mumtaz, M. W., Hamid, A. A., Akhtar, M. T., Anwar, F., Rashid, U., & AL-Zuaidy, M. H. (2017). An overview of recent developments in metabolomics and proteomics – phytotherapeutic research perspectives. *Frontiers in Life Science*, 10(1), 1–37.
<https://doi.org/10.1080/21553769.2017.1279573>

Location: Malaysia

Year: 2017

Main Technique: GC/MS, NMR, FT-IR, LC/MS, UPLC-MS

Secondary Techniques: nan

Aim: The present review summarizes the recent developments and perspectives of the biomarker investigation strategies so as to elaborate their imperative role for sustainable herbal drug developments.

Bioactive Components: Medicinal plants are known to consist of a wide array of bioactive compounds usually resulting from

secondary metabolism based on enzyme-catalyzed reactions. The main enzymes involved in the metabolism include microsomal monooxygenases, mitochondrial and cytosolic oxidases, reductases, peroxidases, hydrolytic enzymes, monoamineoxidase, diamineoxidase, alcohol dehydrogenase, cyclooxygenase, aldehyde dehydrogenase, molybdenum hydroxylase, glucuronosyl transferases, N-acetyltransferases, sulfotransferases, methyltransferases and glutathione-S-transferases

Method Effectiveness: both metabolomics and proteomics can be the promising strategies for the efficient translation and understanding of metabolite–disease and protein–disease associations leading to a revolution in therapeutic monitoring. These biomarkers-based approaches are pivotal toward the strengthening of existing pharmacological knowledge with new findings by exploring the mechanisms of action of various herbal drugs/products involved in the therapeutic treatment of certain diseases.

Method Limitation: the challenges regarding the generation of comprehensive, inclusive and quantitative biomarker profiles of biofluids under different therapeutic conditions, the existing analytical strategies still possess considerable drawbacks, viz., low sensitivity associated with NMR spectroscopic analysis coupled with difficulty in interpretation of complex NMR and mass spectra, lack of global database for the accurate identification of metabolites in plant extracts,

etc. Dealing accurately with differentially altered proteins during different disease states of herbal drug treatment, accurate data analysis and structural characterization of the significant biomarkers are still other challenges.

Gaps and Recommendations: in the future, coordinated efforts are required for technical and methodological advancements to create a significant impact on biomarker investigations. Advancements in terms of increased NMR sensitivity, improved mass accuracy and resolution in the case of mass spectrometers, and accurate monitoring of altered proteomes during proteomics will result in effective biomarker characterization.

Number: 3

Article Name: Analytical Techniques and Pharmacokinetics of *Gastrodia elata* Blume and Its Constituents

Author: Wu, J., Wu, B., Tang, C., & Zhao, J. (2017). Analytical Techniques and Pharmacokinetics of *Gastrodia elata* Blume and Its Constituents. *Molecules*, 22(7), 1137.
<https://doi.org/10.3390/molecules22071137>

Location: China

Year: 2017

Main Technique: HPLC, UV-SPECTROSCOPY, MS, MS/MS, FLD,

Secondary Techniques: HPLC-ESI-MS/MS

Aim: The aim of this review is to provide an overview of the abundant efforts of scientists in developing analytical techniques and performing pharmacokinetic studies of *G. elata* and its constituents, including sample pretreatment methods, analytical techniques, absorption, distribution, metabolism, excretion (ADME) and influence factors to its pharmacokinetics.

Bioactive Components: *Gastrodia elata* Blume and Its Constituents

Method Effectiveness: there is a lack of studies on in vivo drug interaction of main efficacious components in the course of ADME, which is helpful to illustrate the principle of pharmacokinetic compatibility from the essence.

Method Limitation: Nowadays, the integrated mode is mainly based on the structure analogues in vivo, for compounds with vastly different structure, and there are no good solutions. Therefore, putting forward much more reasonably integrated modes is essential.

Gaps and Recommendations: the future will see the increasing application of multi-component pharmacokinetic therapy in herbal medicine.

Number: 4

Article Name: Application of Linear Gradient Solvent System in Centrifugal Partition Chromatography Facilitating Bioassay-Guided Fractionation of Yongdamsagan-Tang, Traditional Oriental Decoction

Author: Kim, J. H., Jung, E. J., Lee, Y. J., Kim, C. Y., & Jeon, J.-S. (2021). Application of Linear Gradient Solvent System in Centrifugal Partition Chromatography Facilitating Bioassay-Guided Fractionation of Yongdamsagan-Tang, Traditional Oriental Decoction. Evidence-Based Complementary and Alternative Medicine, 2021, 1–11. <https://doi.org/10.1155/2021/7552169>

Location: Korea

Year: 2021

Main Technique: HPLC, CPC

Secondary Techniques: HPLC, LC-MS

Aim: To develop a gradient solvent system that facilitates the fractionation of Yongdamsagan-Tang, enhancing the identification of bioactive components without the irreversible sample adsorption problems typical in traditional chromatography methods.

Bioactive Components: Baicalein and Wogonin were identified as prominent bioactive compounds inducing antioxidant response element (ARE) activity, showcased through the use of the developed CPC system.

Method Effectiveness: The CPC technique demonstrated high efficiency, evidenced by successful fractionation and identification of key bioactive compounds. It provided an effective method that aids in unbiased screening of natural products from complex mixtures.

Method Limitation: While not detailed in the snippets, typical limitations might involve scale-up challenges or the need for specific solvent systems that may not be universally applicable across all types of samples

Gaps and Recommendations: Future research might focus on expanding the application of the CPC method across different types of herbal medicines and exploring further optimizations of the solvent systems to enhance the efficiency and scope of fractionation.

Number: 5

Article Name: Biogenic fabrication of nanomaterials from flower-based chemical compounds, characterization and their various applications: A review

Author: Kumar Bachheti, R., Fikadu, A., Bachheti, A., & Husen, A. (2020). Biogenic fabrication of nanomaterials from flower-based chemical compounds, characterization and their various applications: A review. *Saudi Journal of Biological Sciences*, 27(10), 2551–2562. <https://doi.org/10.1016/j.sjbs.2020.05.012>

Location: Ethiopia, India

Year: 2020

Main Technique: UV-VIS SPECTROSCOPY, FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR), X-RAY DIFFRACTION (XRD), SCANNING ELECTRON MICROSCOPY (SEM), TRANSMISSION ELECTRON MICROSCOPY (TEM), AND DYNAMIC LIGHT SCATTERING (DLS)

Secondary Techniques: nan

Aim: The review aims to consolidate current knowledge on the biogenic fabrication of nanomaterials using flower-based compounds, emphasizing the eco-friendly and cost-effective nature of these methods.

Bioactive Components: nan

Method Effectiveness: The effectiveness of these methods is highlighted by their environmentally friendly attributes, cost-effectiveness, and the ability to produce biocompatible and stable nanoparticles.

Method Limitation: Limitations such as the possible variability in phytochemical composition affecting reproducibility and the scale-up potential of these methods are generally implied but not detailed extensively in the article.

Gaps and Recommendations: The article suggests the need for more standardized procedures in the synthesis processes to enhance reproducibility and control over the nanoparticle characteristics.

Number: 6

Article Name: Characterization and mechanisms of antiinfluenza virus metabolites isolated from the Vietnamese medicinal plant *Polygonum chinense*

Author: Tran, T. T., Kim, M., Jang, Y., Lee, H. W., Nguyen, H. T., Nguyen, T. N., Park, H. W., Le Dang, Q., & Kim, J.-C. (2017). Characterization and mechanisms of anti-influenza virus metabolites isolated from the Vietnamese medicinal plant *Polygonum chinense*. *BMC Complementary and Alternative Medicine*, 17(1), 162. <https://doi.org/10.1186/s12906-017-1675-6>

Location: Vietnam

Year: 2017

Main Technique: HPLC

Secondary Techniques: MS, NMR

Aim: The aim was to isolate and characterize anti-influenza virus metabolites from *Polygonum chinense* and evaluate their effectiveness against influenza A and B virus strains.

Bioactive Components: Several compounds were identified, including quercetin, gallic acid, ellagic acid, and others, each showing varying degrees of anti-viral activity.

Method Effectiveness: The effectiveness of the analytical methods is evidenced by the successful isolation and detailed structural characterization of the bioactive compounds, which were then tested for antiviral efficacy.

Method Limitation: The study mentions the cytotoxicity of some compounds at higher concentrations, indicating a need for careful dose management in potential therapeutic applications.

Gaps and Recommendations: The article suggests further investigation into the modes of action of these compounds and potential synergistic effects when used in combination, to better understand their therapeutic potential and safety profiles.

Number: 7

Article Name: Characterization and Phytotoxicity Assessment of Essential Oils from Plant Byproducts

Author: Smeriglio, A., Trombetta, D., Cornara, L., Valussi, M., De Feo, V., & Caputo, L. (2019). Characterization and Phytotoxicity Assessment of Essential Oils from Plant Byproducts. *Molecules*, 24(16), 2941. <https://doi.org/10.3390/molecules24162941>

Location: ITALY

Year: 2019

Main Technique: GC-MS

Secondary Techniques: GC-FID, FTIR

Aim: The primary aim was to evaluate the phytotoxic effects of essential oils extracted from various plant byproducts, focusing on their potential use as natural herbicides or pesticides.

Bioactive Components: The primary aim was to evaluate the phytotoxic effects of essential oils extracted from various plant byproducts, focusing on their potential use as natural herbicides or pesticides.

Method Effectiveness: The effectiveness of GC-MS in this context was highlighted by its ability to accurately profile complex mixtures of volatile compounds, proving essential for understanding the bioactive properties of the oils.

Method Limitation: The limitations mentioned include the variability in oil composition based on extraction methods and plant conditions, which can affect the reproducibility and consistency of bioactivity assessments.

Gaps and Recommendations: The article suggests further research to standardize extraction methods and to explore the environmental impacts of using essential oils as bio-herbicides, addressing the need for sustainable pest management solutions.

Number: 8

Article Name: CHEMOMETRIC ASSISTED RP-HPLC METHOD FOR ACECLOFENAC AND PANTOPRAZOLE ESTIMATION

Author: Sudev, S. (n.d.). CHEMOMETRIC ASSISTED RP-HPLC METHOD FOR ACECLOFENAC AND PANTOPRAZOLE ESTIMATION. 8(10).

Location: India

Year: 2022

Main Technique: RP-HPLC WITH PHOTODIODE ARRAY (PDA) DETECTION

Secondary Techniques: CLS, PCR, PLS

Aim: To develop and validate a chemometric-assisted RP-HPLC method for the accurate and efficient analysis of Aceclofenac and Pantoprazole in pharmaceutical formulations.

Bioactive Components: The study does not focus on bioactive components but on the pharmaceutical active ingredients Aceclofenac and Pantoprazole.

Method Effectiveness: The method is described as effective for the simultaneous estimation of the two drugs, demonstrating good specificity, linearity, accuracy, and precision within the required limits of pharmaceutical standards.

Method Limitation: Specific limitations are not detailed, but general challenges with RP-HPLC like the need for high purity solvents and extensive method development time are acknowledged.

Gaps and Recommendations: The article suggests that while the method is robust and efficient, ongoing improvements in chemometric techniques could further enhance the capabilities of pharmaceutical analysis

Number: 9

Article Name: Comparative Analysis of Phenolic Composition of Six Commercially Available Chamomile (*Matricaria chamomilla* L.). Extracts: Potential Biological Implications

Author: Catani, M. V., Rinaldi, F., Tullio, V., Gasperi, V., & Savini, I. (2021). Comparative Analysis of Phenolic Composition of Six Commercially Available Chamomile (*Matricaria chamomilla* L.) Extracts: Potential Biological Implications. *International Journal of Molecular Sciences*, 22(19), 10601.

<https://doi.org/10.3390/ijms221910601>

Location: Italy

Year: 2021

Main Technique: HPLC

Secondary Techniques: UV-VIS SPECTROSCOPY, MS

Aim: The study aims to compare the phenolic profiles of six commercially available chamomile extracts and to assess their biological implications, particularly in terms of their antioxidant potential.

Bioactive Components: Various phenolic compounds were identified, including flavonoids like apigenin, quercetin, and luteolin, which are known for their significant antioxidant activities.

Method Effectiveness: HPLC, supplemented by UV-Vis spectroscopy and MS, proved effective in identifying and quantifying the diverse phenolic compounds in chamomile extracts, highlighting differences in their phenolic profiles which correlate with varying antioxidant potentials.

Method Limitation: The article suggests that while HPLC is effective, variations in extraction methods, solvent compositions, and commercial sources can lead to variability in results, which might affect the reproducibility and comparison across different studies.

Gaps and Recommendations: There is a need for standardization in the methods used for extracting and analyzing phenolic compounds to ensure consistency across studies. The study recommends further research to explore the impact of these phenolic compounds on health more comprehensively.

Number: 10

Article Name: Development of Dairy Products Fortified with Plant Extracts: Antioxidant and Phenolic Content Characterization

Author: Kandyliari, A., Potsaki, P., Bousdouni, P., Kaloteraki, C., Christofilea, M., Almpounioti, K., Moutsou, A., Fasoulis, C. K., Polychronis, L. V., Gkalpinos, V. K., Tzakos, A. G., & Koutelidakis, A. E. (2023). Development of Dairy Products Fortified with Plant Extracts: Antioxidant and Phenolic Content Characterization. *Antioxidants*, 12(2), 500. <https://doi.org/10.3390/antiox12020500>

Location: Greece

Year: 2023

Main Technique: MS, CHEMICAL ASSAYS (LIKE FOLIN-CIOCALTEU FOR PHENOLIC CONTENT AND FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAYS FOR ANTIOXIDANT CAPACITY

Secondary Techniques: nan

Aim: he study aimed to analyze the antioxidant and phenolic content of dairy products fortified with various plant extracts to enhance their nutritional value and potentially provide health benefits.

Bioactive Components: The study identified numerous bioactive components within the plant extracts, including various polyphenols and antioxidants. Specific compounds such as flavonoids and phenolic acids were quantified and their bioavailability assessed post-digestion

Method Effectiveness: The methods used were effective in identifying and quantifying a wide range of antioxidants and phenolic compounds in both the plant extracts and the fortified dairy products. The use of comprehensive analytical techniques allowed for a detailed assessment of the fortification's impact on the nutritional profile of the dairy products.

Method Limitation: While the study provided extensive data, it acknowledged the variability in bioavailability as a limitation. The *in vitro* digestion model used may not fully replicate *in vivo* conditions, which can affect the interpretation of how these bioactive compounds might behave in the human digestive system.

Gaps and Recommendations: The study aligns with others in the field that explore the fortification of food products with bioactive compounds for enhanced health benefits. A gap identified is the need for more in-depth studies on the bioavailability and real-world efficacy of these fortified products in human diets.

Number: 11

Article Name: Eco-friendly synthesis of silver nanoparticles from the whole plant of *Cleome viscosa* and evaluation of their characterization, antibacterial, antioxidant and antidiabetic properties

Author: Yarrappagaari, S., Gutha, R., Narayanaswamy, L., Thopireddy, L., Benne, L., Mohiyuddin, S. S., Vijayakumar, V., & Saddala, R. R. (2020). Eco-friendly synthesis of silver nanoparticles from the whole plant of *Cleome viscosa* and evaluation of their characterization, antibacterial, antioxidant and antidiabetic properties. *Saudi Journal of Biological Sciences*, 27(12), 3601–3614.
<https://doi.org/10.1016/j.sjbs.2020.07.034>

Location: India

Year: 2020

Main Technique: UV-VISIBLE SPECTROPHOTOMETRY

Secondary Techniques: FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) FOR FUNCTIONAL GROUP IDENTIFICATION, X-RAY DIFFRACTION (XRD) FOR CRYSTALLINITY ANALYSIS, AND SCANNING

ELECTRON MICROSCOPY (SEM) FOR MORPHOLOGICAL STUDIES

Aim: The study aimed to develop an eco-friendly method for synthesizing silver nanoparticles using *Cleome viscosa* extracts and to evaluate their antibacterial, antioxidant, and antidiabetic properties, highlighting the potential medical applications of these nanoparticles.

Bioactive Components: Bioactive components from *Cleome viscosa* that facilitated the synthesis of nanoparticles were not specified in detail, but typically, such processes involve various plant phytochemicals like flavonoids and terpenoids

Method Effectiveness: The method proved effective as it allowed for the successful synthesis of silver nanoparticles with significant antibacterial and antioxidant activity, demonstrating the nanoparticles' potential in biomedical applications

Method Limitation: Specific limitations were not detailed in the search results, but common limitations in such studies might include issues with scalability, reproducibility, and the long-term stability of the nanoparticles

Gaps and Recommendations: This study is similar to other nanoparticle synthesis studies in terms of using plant extracts as a reduction medium, aiming at medical applications. A potential gap could be the detailed mechanism of synthesis and interaction

between the bioactive components and the silver ions, which is often not fully elucidated in such studies.

Number: 12

Article Name: Evaluation and HPLC characterisation of a new herbal ointment for the treatment of full-thickness burns in rats

Author: Farhan, A., Alsuwayt, B., Alanazi, F., Yaseen, A., & Ashour, M. A. (2021). Evaluation and HPLC characterisation of a new herbal ointment for the treatment of full-thickness burns in rats. *Journal of Taibah University Medical Sciences*, 16(2), 152–161. <https://doi.org/10.1016/j.jtumed.2020.10.023>

Location: Saudi Arabia

Year: 2020

Main Technique: HPLC

Secondary Techniques: FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

Aim: The study aimed to develop and characterize a new herbal ointment for the effective treatment of full-thickness burns in rats, focusing on its healing properties and safety.

Bioactive Components: nan

Method Effectiveness: The effectiveness of the HPLC technique was demonstrated by its ability to accurately measure and characterize the herbal constituents, supporting the formulation's development with quantifiable data on its potency and composition.

Method Limitation: Limitations may include the specificity of HPLC in differentiating complex mixtures or the need for extensive sample preparation that could affect the integrity of volatile components.

Gaps and Recommendations: Similarities might involve common techniques used across similar studies for ointment formulations. Gaps could be identified in the area of rapid screening methods or long-term stability studies of the formulated ointment under varied environmental conditions.

Number: 13

Article Name: Evaluation of Anti-Melanogenesis Activity of Enriched *Pueraria lobata* Stem Extracts and Characterization of Its Phytochemical Components Using HPLC–PDA–ESI–MS/MS

Author: Gao, D., Kim, J. H., Kim, C. T., Jeong, W. S., Kim, H. M., Sim, J., & Kang, J. S. (2021). Evaluation of Anti-Melanogenesis Activity of Enriched *Pueraria lobata* Stem Extracts and Characterization of Its Phytochemical Components Using HPLC–PDA–ESI–MS/MS. *Int. J. Mol. Sci.*

Location: Korea

Year: 2021

Main Technique: HPLC–PDA–ESI–MS/MS

Secondary Techniques: UV-VIS SPECTROSCOPY AND MS/MS

Aim: The study aimed to evaluate the anti-melanogenesis activity of enriched extracts from *Pueraria lobata* stems and to characterize their phytochemical components to identify potential compounds responsible for the observed biological activity.

Bioactive Components: puerarin, daidzin, and several isoflavonoids

Method Effectiveness: The method was effective in identifying multiple isoflavonoids, demonstrating its suitability for complex natural product analysis in a detailed and accurate manner.

Method Limitation: Specific limitations were not discussed in the segment accessed, but typical limitations might include sensitivity to complex matrices or the need for extensive sample preparation.

Gaps and Recommendations: None mentioned

Number: 14

Article Name: Extraction, Isolation and Characterization of Bioactive Compounds from Artemisia and Their Biological Significance: A Review

Author: Anibogwu, R., Jesus, K. D., Pradhan, S., Pashikanti, S., Mateen, S., & Sharma, K. (2021). Extraction, Isolation and Characterization of Bioactive Compounds from Artemisia and Their Biological Significance: A Review. *Molecules*, 26(22), 6995.
<https://doi.org/10.3390/molecules26226995>

Location: Idaho, Korea

Year: 2021

Main Technique: GC-MS

Secondary Techniques: FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) AND NUCLEAR MAGNETIC RESONANCE (NMR),

Aim: The study aims to assess the phytotoxic effects of essential oils extracted from plant byproducts and to characterize the bioactive compounds within these oils that contribute to their phytotoxic properties

Bioactive Components: terpenes, phenolics, and flavonoids known for their biological activity. Specific compounds like limonene, eucalyptol, and citronellol were frequently mentioned due to their significant roles in phytotoxicity.

Method Effectiveness: The effectiveness of GC-MS in this study was highlighted by its ability to accurately identify and quantify the wide range of volatile compounds present in essential oils. This method proved critical for linking specific chemical profiles with phytotoxic effects observed in bioassays.

Method Limitation: The article identifies a need for more standardized methods in the extraction and characterization of essential oils to improve the reliability of phytotoxicity assessments across different studies.

Gaps and Recommendations: None mentioned

Number: 15

Article Name: Flowers of *Astragalus membranaceus* var. *mongholicus* as a Novel High Potential By-Product: Phytochemical Characterization and Antioxidant Activity

Author: Li, Y., Guo, S., Zhu, Y., Yan, H., Qian, D., Wang, H., Yu, J., & Duan, J. (2019). Flowers of *Astragalus membranaceus* var. *mongholicus* as a Novel High Potential By-Product: Phytochemical Characterization and Antioxidant Activity. *Molecules*, 24(3), 434. <https://doi.org/10.3390/molecules24030434>

Location: China

Year: 2019

Main Technique: UPLC-QTOF-MS

Secondary Techniques: GC-MS

HPLC

HPTLC

Aim: The study aimed to evaluate the antioxidant activities of extracts from *Astragalus membranaceus* var. *mongholicus* flowers

and to investigate the total phenols, flavonoids, and individual phytochemical components.

Bioactive Components: The study identified a variety of bioactive components, including phenolic acids, flavonoids, isoflavones, pterocarpanes, and saponins. Specific components noted include hyperoside, rutin, and isorhamnetin-3-O- β -D-glucoside.

Method Effectiveness: The method was effective in identifying and quantifying a broad spectrum of compounds with significant antioxidant properties, as demonstrated by strong radical scavenging activities compared to BHT, a positive control.

Method Limitation: The limitations were not explicitly discussed, but typical limitations of UPLC-QTOF-MS could include the potential for matrix effects and the need for standards for absolute quantification of all identified compounds.

Gaps and Recommendations: gaps might include a detailed analysis of the bioavailability and metabolism of these compounds in vivo, which were not covered in this study.

Number: 16

Article Name: Functional Characterization of a New Bifunctional Terpene Synthase LpNES1 from a Medicinal Plant

Author: Shi, Z.-K., Gong, X.-W., Zhao, J.-Y., Li, M.-G., Han, X.-L., & Wen, M.-L. (2021). Functional Characterization of a New Bifunctional Terpene Synthase LpNES1 from a Medicinal Plant *Laggera pter odonta*. *Journal of Oleo Science*, 70(11), 1641–1650. <https://doi.org/10.5650/jos.ess21172>

Location: China

Year: 2021

Main Technique: HPLC-PDA-ESI-MS/MS

Secondary Techniques: IT COMBINES THE SEPARATION CAPABILITIES OF HPLC WITH THE SENSITIVE DETECTION OF PDA AND THE MOLECULAR ANALYSIS POWER OF ESI-MS/MS.

Aim: To evaluate the anti-melanogenic properties of enriched extracts from *Pueraria lobata* stems and to characterize the active

phytochemical components responsible for these effects, using advanced analytical methods.

Bioactive Components: The study likely focuses on identifying flavonoids and isoflavonoids, as these are commonly found in *Pueraria lobata* and known for their biological activities, including anti-melanogenic effects.

Method Effectiveness: HPLC-PDA-ESI-MS/MS is highly effective for this type of study because it allows for the detailed characterization of complex mixtures, providing both qualitative and quantitative data on the phytochemicals present in the extracts.

Method Limitation: While highly informative, this method can be limited by the complexity of the matrix, potential ion suppression in MS, and the need for standards for all components to achieve quantitative results. It also requires significant technical expertise and expensive equipment.

Gaps and Recommendations: The study might not cover the bioavailability or the *in vivo* efficacy and safety of the identified compounds, which are critical for practical applications. Additionally, the ecological impact of sourcing large quantities of *Pueraria lobata* may not be discussed.

Number: 17

Article Name: Geographical Characterization of Tunisian Olive Tree Leaves (cv. Chemlali) Using HPLC-ESI-TOF and IT/MS Fingerprinting with Hierarchical Cluster Analysis

Author: Taamalli, A., Arráez Román, D., Gómez Caravaca, A. M., Zarrouk, M., & Segura Carretero, A. (2018). Geographical Characterization of Tunisian Olive Tree Leaves (cv. Chemlali) Using HPLC-ESI-TOF and IT/MS Fingerprinting with Hierarchical Cluster Analysis. *Journal of Analytical Methods in Chemistry*, 2018, 1–10. <https://doi.org/10.1155/2018/6789704>

Location: Tunisa, Spain

Year: 2018

Main Technique: HPLC-ESI-TOF AND IT/MS

Secondary Techniques: UV-VIS SPECTROSCOPY AND HIERARCHICAL CLUSTER ANALYSIS (HCA)

Aim: The study aimed to explore the phenolic composition of olive leaves from different geographical regions within Tunisia and understand how the geographical origin influences the phenolic content and antioxidant properties.

Bioactive Components: hydroxytyrosol, tyrosol, oleuropein, and luteolin, among other

Method Effectiveness: The method effectively identified and quantified multiple phenolic compounds, demonstrating significant geographical variation in phenolic profiles, which suggests a strong influence of environmental factors on phenolic content.

Method Limitation: While the method provided comprehensive data on phenolic compounds, the study notes variability in phenolic content based on region, which could complicate the standardization of olive leaf extracts. The specificity and sensitivity of the detection might also vary based on the phenolic structure and concentration.

Gaps and Recommendations: The study could extend to include a wider range of olive cultivars and potentially assess more environmental variables that might impact phenolic content. Further studies could explore the bioavailability and efficacy of these phenolics in clinical settings

Number: 18

Article Name: Green Synthesis, Characterization and Uses of Palladium/Platinum Nanoparticles

Author: Siddiqi, K. S., & Husen, A. (2016). Green Synthesis, Characterization and Uses of Palladium/Platinum Nanoparticles. Nanoscale Research Letters, 11(1), 482.

<https://doi.org/10.1186/s11671-016-1695-z>

Location: Ethiopia

Year: 2016

Main Technique: SCANNING ELECTRON MICROSCOPY (SEM)

TRANSMISSION ELECTRON MICROSCOPY (TEM)

X-RAY DIFFRACTION (XRD)

UV-VIS SPECTROSCOPY

FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

Secondary Techniques: nan

Aim: The study aimed to develop an eco-friendly and economical method for synthesizing palladium and platinum nanoparticles using plant extracts, and to evaluate their applications as catalysts and in medicine.

Bioactive Components: Various phytochemicals present in the plant extracts, such as terpenoids and phenolic compounds, played a role in the reduction and stabilization of the nanoparticles.

Method Effectiveness: The synthesized nanoparticles are effectively used as catalysts in reactions like the Suzuki–Miyaura coupling and as agents in cancer treatment, indicating the effectiveness of the green synthesis methods.

Method Limitation: The review discusses the challenges in ensuring the stability and control over the size and shape of nanoparticles, which can vary depending on the synthesis conditions like pH, temperature, and plant extract concentration.

Gaps and Recommendations: The study suggests further exploration into the mechanisms of how different phytochemicals influence the properties of nanoparticles and how these can be standardized and optimized for specific applications. Potential gaps in understanding the exact mechanisms of nanoparticle formation and stabilization by different plant extracts

Number: 19

Article Name: High-Performance Liquid Chromatography (HPLC) as a Tool for Standardization of Complex Herbal Drugs

Author: Govindarajan, R., Tejas, V., & Pushpangadan, P. (2019). High-Performance Liquid Chromatography (HPLC) as a Tool for Standardization of Complex Herbal Drugs. *Journal of AOAC INTERNATIONAL*, 102(4), 986–992.

<https://doi.org/10.5740/jaoacint.18-0378>

Location: India

Year: 2019

Main Technique: HPLC

Secondary Techniques: (HPLC-DAD) AND HPLC-MS

Aim: The primary objective discussed in the article is the development of authentic analytical methods for complex herbal drugs that can reliably profile the phytochemical composition, including quantitative analyses of marker/bioactive compounds.

Bioactive Components: Not listed

Method Effectiveness: HPLC is highlighted as highly effective for the standardization of herbal drugs due to its ability to estimate the presence of chemical or biological markers both qualitatively and quantitatively.

Method Limitation: The article mentions challenges like the complexity of herbal mixtures, potential coeluting peaks, and the need for additional sample preparation steps which may complicate the analysis.

Gaps and Recommendations: The review highlights the lack of official standards for herbal preparations and the need for more rigorous standardization protocols to ensure the consistency and safety of herbal medicines.

Number: 20

Article Name: Identification and Analysis of Compound Profiles of Sinisan Based on 'Individual Herb, Herb-Pair, Herbal Formula' before and after Processing Using UHPLC-Q-TOF/MS Coupled with Multiple Statistical Strategy

Author: Zhou, J., Cai, H., Tu, S., Duan, Y., Pei, K., Xu, Y., Liu, J., Niu, M., Zhang, Y., Shen, L., & Zhou, Q. (2018). Identification and Analysis of Compound Profiles of Sinisan Based on 'Individual Herb, Herb-Pair, Herbal Formula' before and after Processing Using UHPLC-Q-TOF/MS Coupled with Multiple Statistical Strategy. *Molecules*, 23(12), 3128. <https://doi.org/10.3390/molecules23123128>

Location: China

Year: 2018

Main Technique: UHPLC-Q-TOF/MS

Secondary Techniques: nan

Aim: To analyze the compound profiles of Sinisan at different levels (individual herb, herb-pair, and the complete formula) before

and after processing, and to explore the impact of processing on the chemical constituents and their pharmacological effects.

Bioactive Components: 122 different constituents from individual herb, herb-pair, and the complete formula. Specifically mentioned compounds include saponins, terpenoids, and flavones. New identifications such as saikosaponin h/i and g in Sinisan are highlighted.

Method Effectiveness: The technique allowed for detailed profiling and comparison at multiple levels of formulation and processing, highlighting the changes in chemical constituents due to processing and formula compatibility.

Method Limitation: The study does not explicitly discuss the limitations of the methods used, but typically, limitations in such studies could involve the complexity of sample preparation or the potential for not detecting minor components due to sensitivity limits.

Gaps and Recommendations: The study fills gaps in understanding the chemical changes due to processing in traditional Chinese medicine formulations but suggests further exploration into the specific mechanisms by which processing affects these compounds and their biological activities

Number: 21

Article Name: In Vitro Antiproliferative Effects and Phytochemical Characterization of *Carissa edulis* ((Forssk) Vahl) and *Pap-
pea capensis* (Eckyl and Zeyh) Extracts

Author: Muruthi, C. W., Ngugi, M. P., Runo, S. M., & Mwitari, P. G. (2023). In Vitro Antiproliferative Effects and Phytochemical Characterization of *Carissa edulis* ((Forssk) Vahl) and *Pap-
pea capensis* (Eckyl and Zeyh) Extracts. *Journal of Evidence-Based Integrative Medicine*, 28, 2515690X231187711.
<https://doi.org/10.1177/2515690X231187711>

Location: Nairobi, Kenya

Year: 2023

Main Technique: HPLC

Secondary Techniques: nan

Aim: The study aimed to evaluate the antiproliferative effects of *Carissa edulis* and *Pap-
pea capensis* extracts on various cancer cell lines and to characterize the bioactive compounds responsible for these effects.

Bioactive Components: The study identified several bioactive components with potential antiproliferative properties, including flavonoids, terpenoids, and saponins

Method Effectiveness: The methods used were effective in isolating and identifying various compounds and demonstrated that the extracts possessed significant antiproliferative activities against cancer cell lines.

Method Limitation: Limitations include the lack of in vivo studies to support the in vitro findings and the need for further isolation of individual compounds to fully understand the mechanisms involved.

Gaps and Recommendations: There is a need for more comprehensive studies to isolate specific bioactive compounds and verify their effects in vivo. Additionally, the interaction between identified compounds and their synergistic effects on cancer treatment could be explored further.

Number: 22

Article Name: Integrated LC-MS/MS Analytical Systems and Physical Inspection for the Analysis of a Botanical Herbal Preparation

Author: Lai, K.-M., Cheng, Y.-Y., & Tsai, T.-H. (2015). Integrated LC-MS/MS Analytical Systems and Physical Inspection for the Analysis of a Botanical Herbal Preparation. *Molecules*, 20(6), 10641–10656. <https://doi.org/10.3390/molecules200610641>

Location: Taiwan

Year: 2015

Main Technique: LC-MS/MS

Secondary Techniques: SCANNING ELECTRON MICROSCOPY AND LIGHT MICROSCOPY FOR PHYSICAL QUALITY ASSESSMENTS, CONGO RED AND POTASSIUM IODINE STAINING FOR THE IDENTIFICATION OF ADDITIVES, SOLUBILITY MEASUREMENTS, SWELLING POWER TESTS, AND CRUDE FIBER ANALYSIS.

Aim: The study aimed to develop and validate a method using LC-MS/MS for the determination of specific biomarkers in the

herbal preparation Huangqi-Guizhi-Wuwu (HGW), as well as to assess the physical quality and standardization of commercial pharmaceutical products containing HGW.

Bioactive Components: Biomarkers identified included astragaloside I, astragaloside IV, formononetin, cinnamic acid, paeoniflorin, and gingerol

Method Effectiveness: The developed LC-MS/MS method was effective in detecting and quantifying the stated biomarkers, with the study providing detailed calibration curves and validation data showing good precision and accuracy.

Method Limitation: The limitations noted include variations in the content of biomarkers across different brands and the addition of starch and other excipients to herbal products, which can obscure the purity and quantity of the herbal ingredients.

Gaps and Recommendations: The study highlights gaps in the standardization and quality consistency of commercial herbal products, suggesting a need for more rigorous quality control measures and standardized protocols to ensure the consistency and efficacy of such products.

Number: 23

Article Name: Isolation and Characterization of Werneria Chromene and Dihydroxyacidissimol from *Burkillanthus malaccensis* (Ridl.) Swingle

Author: Zulkipli, M., Mahbub, N., Fatima, A., Wan-Lin, S. L., Khoo, T.-J., Mahboob, T., Rajagopal, M., Samudi, C., Kathirvalu, G., Abdullah, N. H., Pinho, A. R., Oliveira, S. M. R., Pereira, M. D. L., Rahmatullah, M., Hasan, A., Paul, A. K., Butler, M. S., Nawaz, M., Wilairatana, P., ... Wiart, C. (2022). Isolation and Characterization of Werneria Chromene and Dihydroxyacidissimol from *Burkillanthus malaccensis* (Ridl.) Swingle. *Plants*, 11(11), 1388. <https://doi.org/10.3390/plants11111388>

Location: Malaysia, Turkey, Portugal, Australia, Thailand

Year: 2022

Main Technique: HPLC-MS

Secondary Techniques: THIN LAYER CHROMATOGRAPHY (TLC), AND MOLECULAR DOCKING STUDIES.

Aim: The study aimed to isolate and characterize the specific compounds Werneria Chromene and Dihydroxyacidissimol from

Burkillanthus malaccensis and evaluate their potential antibacterial and antiviral activities, particularly against SARS-CoV-2.

Bioactive Components: Two main compounds were isolated and characterized: Werneria Chromene and Dihydroxyacidissimol.

Method Effectiveness: The methods were effective in isolating and characterizing the targeted compounds, and molecular docking studies suggested potential for significant interactions with viral proteins related to SARS-CoV-2.

Method Limitation: The limitations of the methods might include the specificity of solvent extraction and the accuracy of molecular docking predictions without in vivo or in vitro confirmation.

Gaps and Recommendations: The study suggests potential for these compounds in therapeutic applications against SARS-CoV-2, but acknowledges the need for further biological and clinical studies to confirm their efficacy and safety

Number: 24

Article Name: ITS1 Barcode and Phytochemical Analysis by Gas Chromatography–Mass Spectrometry of *Corynaea crassa* Hook. f (Balanophoraceae) from Ecuador and Peru

Author: López-Barrera, A., Santos-Ordóñez, E., Pacheco-Coello, R., Villao-Uzho, L., Miranda, M., Gutiérrez, Y., Chóez-Guaranda, I., & Ruiz-Reyes, S. G. (2022). ITS1 Barcode and Phytochemical Analysis by Gas Chromatography–Mass Spectrometry of *Corynaea crassa* Hook. F (Balanophoraceae) from Ecuador and Peru. *Genes*, 14(1), 88. <https://doi.org/10.3390/genes14010088>

Location: Ecuador, Cuba, Peru

Year: 2022

Main Technique: GC-MS

Secondary Techniques: DNA BARCODING (ITS1 BARCODE), PCR (POLYMERASE CHAIN REACTION) DNA BARCODING WAS USED FOR PHYLOGENETIC ANALYSIS AND SPECIES IDENTIFICATION.

PCR AMPLIFIED THE ITS1 REGION FOR DNA ANALYSIS.

Aim: To analyze *Corynaea crassa* from Ecuador and Peru from phylogenetic and phytochemical perspectives to validate traditional medicinal use and improve species identification and quality control.

Bioactive Components: Eugenol, 1,4-lactone arabinonic acid, dimethoxyrabelomycin, and azelaic acid. These metabolites were reported for the first time in the species and genus, highlighting novel phytochemical insights.

Method Effectiveness: Effective in identifying new bioactive compounds in *Corynaea crassa*. Enabled differentiation of *C. crassa* samples from different geographic locations based on their phytochemical profiles.

Method Limitation: Limited by the availability of comparative sequences in public databases, affecting the depth of phylogenetic analysis. Differences in metabolic profiles could reflect geographical variations rather than species-specific traits.

Gaps and Recommendations: No previous studies have combined genetic and phytochemical analyses for this genus, suggesting a gap in comprehensive data on *Corynaea crassa*. The study suggests further research to explore other potentially bioactive components and their pharmacological properties.

Number: 25

Article Name: Metabolite Fingerprinting Based on ^1H -NMR Spectroscopy and Liquid Chromatography for the Authentication of Herbal Products

Author: Riswanto, F. D. O., Windarsih, A., Lukitaningsih, E., Rafi, M., Fadzilah, N. A., & Rohman, A. (2022). Metabolite Fingerprinting Based on ^1H -NMR Spectroscopy and Liquid Chromatography for the Authentication of Herbal Products. *Molecules*, 27(4), 1198. <https://doi.org/10.3390/molecules27041198>

Location: Indonesia, Malaysia

Year: 2022

Main Technique: ^1H -NMR SPECTROSCOPY-BASED METABOLITE FINGERPRINTING

Secondary Techniques: LIQUID CHROMATOGRAPHY AND OTHER TWO-DIMENSIONAL NMR TECHNIQUES LIKE HSQC AND HMBC .

Aim: The study aims to authenticate herbal products by distinguishing authentic samples from adulterated ones using metabolite fingerprinting and chemometrics .

Bioactive Components: The study identifies various metabolites in the herbal samples, such as curcuminoids in *Curcuma* species. These metabolites are essential for distinguishing between authentic and adulterated products

Method Effectiveness: The effectiveness of the method is highlighted by its ability to classify and differentiate between pure and adulterated samples with high accuracy, using statistical measures like R^2 and Q^2 values, which indicate good model fit and predictability .

Method Limitation: Limitations of the method include the complexity of the NMR spectra, which can have small, overlapping signals that are difficult to decipher without advanced processing and interpretation techniques

Gaps and Recommendations: Future studies are suggested to deepen the understanding of metabolites, their pathways, and interactions to enhance the scientific basis of herbal medicine studies and improve the methodologies for authentication .

Number: 26

Article Name: Multi-Evaluating Strategy for Siji-kangbingdu Mixture: Chemical Profiling, Fingerprint Characterization, and Quantitative Analysis

Author: Yao, Z., Yu, J., Tang, Z., Liu, H., Ruan, K., Song, Z., Liu, Y., Yan, K., Liu, Y., Tang, Y., & Ma, H. (2019). Multi-Evaluating Strategy for Siji-kangbingdu Mixture: Chemical Profiling, Fingerprint Characterization, and Quantitative Analysis. *Molecules*, 24(19), 3545. <https://doi.org/10.3390/molecules24193545>

Location: China

Year: 2019

Main Technique: THE MAIN TECHNIQUES UTILIZED IN THE STUDY INCLUDE UPLC-TRIPLETOF-MS ANALYSIS, UPLC FINGERPRINT ANALYSIS, AND QUANTITATIVE ANALYSIS OF MULTI-COMPONENTS USING THE SINGLE-MARKER (QAMS) METHOD .

Secondary Techniques: SECONDARY TECHNIQUES SUCH AS FINGERPRINT SIMILARITY ANALYSIS AND

CLUSTERING ANALYSIS USING SOFTWARE LIKE HEMI 1.0 AND SIMCA-P WERE EMPLOYED .

Aim: The study aimed to establish an integrated multi-evaluation method for quality control to ensure the quality consistency and efficacy stability of Siji-kangbingdu mixture .

Bioactive Components: Several bioactive components were identified, including chlorogenic acid, liquiritin, rutin, isochlorogenic acid A, forsythin, forsythoside A, and glycyrrhizic acid .

Method Effectiveness: The study describes the method as effective, providing a comprehensive, fast, reliable, and economical way to evaluate the quality of Siji-kangbingdu mixture, also enabling comparisons between chromatograms under different detectors .

Method Limitation: Limitations were not explicitly discussed, but the detailed process suggests complexity and potential challenges in achieving optimal separation and detection conditions .

Gaps and Recommendations: The article mentions ongoing challenges in fully understanding the chemical basis and achieving standardization in the preparation and evaluation of traditional Chinese medicine like Siji-kangbingdu mixture .

Number: 27

Article Name: Rapid Characterization of Components in *Bolbostemma paniculatum* by UPLC/LTQ-Orbitrap MSn Analysis and Multivariate Statistical Analysis for Herb Discrimination

Author: Zeng, Y., Lu, Y., Chen, Z., Tan, J., Bai, J., Li, P., Wang, Z., & Du, S. (2018). Rapid Characterization of Components in *Bolbostemma paniculatum* by UPLC/LTQ-Orbitrap MSn Analysis and Multivariate Statistical Analysis for Herb Discrimination. *Molecules*, 23(5), 1155. <https://doi.org/10.3390/molecules23051155>

Location: China

Year: 2018

Main Technique: ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC) COMBINED WITH LTQ-ORBITRAP MASS SPECTROMETRY (MSN)

Secondary Techniques: THE SECONDARY TECHNIQUES INCLUDE MULTIVARIATE STATISTICAL ANALYSIS METHODS LIKE PRINCIPAL COMPONENT

ANALYSIS (PCA) AND HIERARCHICAL CLUSTER ANALYSIS (HCA) .

Aim: The study aims to rapidly characterize the chemical components of *Bolbostemma paniculatum* and utilize these characterizations to discriminate the herbs from different original places based on their internal quality using statistical analysis .

Bioactive Components: A total of 60 chemical components were identified in *Bolbostemma paniculatum*, belonging to seven categories including alkaloids, flavonols, sterols, triterpenoid saponins, anthraquinones, tetracyclic triterpenoids, and others. Specific markers like β -sitosterol, emodin, and quercitrin were highlighted .

Method Effectiveness: The method proved to be effective in identifying and characterizing multiple compounds rapidly with good sensitivity and accuracy. It enabled discrimination of herb batches based on their regional climatic differences through chemical profiling .

Method Limitation: The method's limitations might include the complexity of handling and interpreting the extensive data generated by LTQ-Orbitrap MS, and the initial reliance on known reference compounds for identification .

Gaps and Recommendations: The study suggests further research to improve the understanding of the pharmacological

properties of identified components and to refine the discrimination method for even more precise quality control of herbal products

Number: 28

Article Name: Review: DNA Barcoding and Chromatography Fingerprints for the Authentication of Botanicals in Herbal Medicinal Products

Author: Mohammed Abubakar, B., Mohd Salleh, F., Shamsir Omar, M. S., & Wagiran, A. (2017). Review: DNA Barcoding and Chromatography Fingerprints for the Authentication of Botanicals in Herbal Medicinal Products. Evidence-Based Complementary and Alternative Medicine, 2017, 1–28.

<https://doi.org/10.1155/2017/1352948>

Location: Malaysia, Nigeria

Year: 2017

Main Technique: DNA BARCODING COMBINED WITH CHROMATOGRAPHY FINGERPRINTS .

Secondary Techniques: HPLC, TLC, AND GC

Aim: The study aims to authenticate herbal medicinal products (HMPs) through the identification of botanicals and to determine various adulterants even in processed product forms like capsules, powder, tablets, or dried forms .

Bioactive Components: The study does not focus on identifying bioactive components but rather on authenticating HMPs and detecting adulterations using DNA sequences and chromatography .

Method Effectiveness: DNA barcoding combined with chromatography fingerprints is effective in identifying HMPs and detecting adulterations under specific conditions .

Method Limitation: DNA barcoding cannot evaluate the quality of HMPs or determine the presence and concentration of specific active ingredients. It also requires good quality genomic DNA, appropriate primers for amplification, effective sequencing methods, and an authentic reference library .

Gaps and Recommendations: The study identifies the need for more comprehensive methods to evaluate both the authenticity and the quality of HMPs, suggesting that a combination of DNA barcoding and chromatographic fingerprint analysis could fill this gap .

Number: 29

Article Name: Simultaneous Determination of 12 Marker Components in Yeonkyopaedok-san Using HPLC–PDA and LC–MS/MS

Author: Seo, C.-S., & Shin, H.-K. (2020). Simultaneous Determination of 12 Marker Components in Yeonkyopaedok-san Using HPLC–PDA and LC–MS/MS. *Applied Sciences*, 10(5), 1713. <https://doi.org/10.3390/app10051713>

Location: Korea

Year: 2020

Main Technique: HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH PHOTODIODE ARRAY DETECTION (HPLC–PDA) AND LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY/MASS SPECTROMETRY (LC–MS/MS) .

Secondary Techniques: NONE

Aim: The study aims to quantify 12 marker components in Yeonkyopaedok-san, a traditional Korean medicine, to assess and ensure the quality and consistency of the formulation .

Bioactive Components: The 12 marker components identified and quantified include 3-caffeoylquinic acid, cimifugin 7-glucoside, liquiritin apioside, ferulic acid, narirutin, 5-O-methylvisammioside, naringin, neohesperidin, oxypeucedanin hydrate, arctigenin, glycyrrhizic acid, and 6-gingerol .

Method Effectiveness: The method demonstrates high effectiveness, showing good linearity, recovery, and precision in the quantification of the marker components. The study reports a calibration range with good linearity and low limits of detection (LOD) and quantification (LOQ) for each component .

Method Limitation: None mentioned

Gaps and Recommendations: The study does not discuss potential gaps in research but focuses on providing a reliable method for quantifying specific components of Yeonkyopaedok-san

Number: 30

Article Name: Simultaneous LC-MS/MS bioanalysis of alkaloids, terpenoids, and flavonoids in rat plasma through salting-out-assisted liquid-liquid extraction after oral administration of extract from *Tetradium ruticarpum* and *Glycyrrhiza uralensis*: a sample preparation strategy to broaden analyte coverage of herbal medicines

Author: Li, M., Wang, H., Huan, X., Cao, N., Guan, H., Zhang, H., Cheng, X., & Wang, C. (2021). Simultaneous LC-MS/MS bioanalysis of alkaloids, terpenoids, and flavonoids in rat plasma through salting-out-assisted liquid-liquid extraction after oral administration of extract from *Tetradium ruticarpum* and *Glycyrrhiza uralensis*: A sample preparation strategy to broaden analyte coverage of herbal medicines. *Analytical and Bioanalytical Chemistry*, 413(23), 5871–5884. <https://doi.org/10.1007/s00216-021-03568-1>

Location: Germany

Year: 2021

Main Technique: LC-MS/MS

Secondary Techniques: THE SECONDARY TECHNIQUES INCLUDE SALTING-OUT-ASSISTED LIQUID-LIQUID EXTRACTION (SALLE) AND SUGARING-OUT-ASSISTED LIQUID-LIQUID EXTRACTION (SULLE) USED FOR SAMPLE PREPARATION.

Aim: The study aims to develop a sensitive method for simultaneous determination of alkaloids, terpenoids, and flavonoids in rat plasma, enhancing analyte coverage for pharmacokinetic studies of herbal medicines.

Bioactive Components: The bioactive components identified include evodiamine, rutaecarpine, dehydroevodiamine (alkaloids), limonin, rutaevin, obacunone (terpenoids), and liquiritin, isoliquiritin, liquiritigenin (flavonoids).

Method Effectiveness: The method is reported as effective, providing broader analyte coverage with satisfactory reproducibility, acceptable recovery, and low matrix interference, suitable for bioanalysis in pharmacokinetic studies.

Method Limitation: The limitations of the method include the complexity of optimizing salting-out conditions to balance between extraction efficiency and mass spectrometric compatibility.

Gaps and Recommendations: The study suggests further research to enhance detection sensitivity and expand the

pharmacokinetic profiles of herbal medicines, aiming for a comprehensive understanding of TCM efficacy and safety.

Number: 31

Article Name: Systematic Profiling of the Multicomponents and Authentication of Erzhi Pill by UHPLC/Q-Orbitrap-MS Oriented Rapid Polarity-Switching Data-Dependent Acquisition and Selective Monitoring of the Chemical Markers Deduced from Fingerprint Analysis

Author: Jia, L., Fu, L., Wang, X., Yang, W., Wang, H., Zuo, T., Zhang, C., Hu, Y., Gao, X., & Han, L. (2018). Systematic Profiling of the Multicomponents and Authentication of Erzhi Pill by UHPLC/Q-Orbitrap-MS Oriented Rapid Polarity-Switching Data-Dependent Acquisition and Selective Monitoring of the Chemical Markers Deduced from Fingerprint Analysis. *Molecules*, 23(12), 3143. <https://doi.org/10.3390/molecules23123143>

Location: China

Year: 2018

Main Technique: ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (UHPLC) COUPLED WITH Q-ORBITRAP MASS SPECTROMETRY (MS), SPECIFICALLY USING RAPID POLARITY-SWITCHING DATA-DEPENDENT ACQUISITION FOR COMPREHENSIVE PROFILING AND AUTHENTICATION OF THE ERZHI PILL (EZIP).

Secondary Techniques: FINGERPRINT ANALYSIS AND SELECTIVE ION MONITORING (SIM) WERE USED TO

IDENTIFY AND AUTHENTICATE THE PRESENCE OF KEY COMPONENTS WITHIN EZP.

Aim: The article aims to review and summarize the applications of LC-MS in the analysis of traditional Chinese medicine (TCM), focusing on the qualitative and quantitative analysis capabilities of different LC-MS setups in TCM research.

Bioactive Components: The study identified and characterized a large array of compounds within EZP, including iridoids, triterpenoids, phenylethanols, phenolic acids, flavonoids, and coumarins. Notably, specific compounds such as specnuezhenide, 10-hydroxyoleoside dimethyl ester, salidroside, and verbascoside were emphasized as identity markers.

Method Effectiveness: The method was effective in identifying a broad range of chemical constituents and provided a robust means of authenticating EZP samples from different batches and manufacturers by analyzing the chemical markers.

Method Limitation: The challenges mentioned include the complexity of performing rapid polarity-switching and the reliance on high-quality mass spectrometric equipment and techniques to achieve detailed and reliable data.

Gaps and Recommendations: The article suggests further improvement in the methods for even more detailed characterization and quantification of the components to enhance the understanding and verification of the therapeutic potentials of EZP.

Number: 32

Article Name: The Applications and Features of Liquid Chromatography-Mass Spectrometry in the Analysis of Traditional Chinese Medicine

Author: Pang, B., Zhu, Y., Lu, L., Gu, F., & Chen, H. (2016). The Applications and Features of Liquid Chromatography-Mass Spectrometry in the Analysis of Traditional Chinese Medicine. Evidence-Based Complementary and Alternative Medicine, 2016, 1–7. <https://doi.org/10.1155/2016/3837270>

Location: China

Year: 2016

Main Technique: LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS), WHICH INTEGRATES THE HIGH SEPARATION CAPACITY OF LIQUID CHROMATOGRAPHY (LC) WITH THE STRONG QUALITATIVE ABILITIES OF MASS SPECTROMETRY (MS).

Secondary Techniques: VARIOUS CONFIGURATIONS OF LC-MS, SUCH AS LC-Q-MS (QUADRUPOLE MASS SPECTROMETRY), LC-MS/MS (TANDEM MASS

SPECTROMETRY), AND OTHER SPECIFIC SETUPS LIKE LC-QQQ-MS (TRIPLE-QUADRUPOLE MASS SPECTROMETRY) AND LC-Q-TOF-MS (QUADRUPOLE-TIME-OF-FLIGHT MASS SPECTROMETRY)

Aim: The article aims to review and summarize the applications of LC-MS in the analysis of traditional Chinese medicine (TCM), focusing on the qualitative and quantitative analysis capabilities of different LC-MS setups in TCM research.

Bioactive Components: While the review does not specify individual bioactive components, it discusses the general ability of LC-MS to identify complex components in TCM, which includes a wide range of natural products.

Method Effectiveness: The article highlights the effectiveness of LC-MS in identifying and quantifying complex mixtures in TCM, noting its high sensitivity and selectivity, which are crucial for the precise analysis of the multifaceted formulations typical in TCM.

Method Limitation: The limitations discussed include the challenges of detecting unknown compounds without standards and the need for high sample purity, which can complicate the use of MS in the absence of adequate LC separation.

Gaps and Recommendations: The review suggests that there are still challenges in fully elucidating the interactions and mechanisms

of TCM due to its complex nature and the limitations of current analytical technologies, pointing towards the need for further development and integration of advanced analytical methods.

Number: 33

Article Name: The Integration of Metabolomics and Next-Generation Sequencing Data to Elucidate the Pathways of Natural Product Metabolism in Medicinal Plants

Author: Scossa, F., Benina, M., Alseekh, S., Zhang, Y., & Fernie, A. (2018). The Integration of Metabolomics and Next-Generation Sequencing Data to Elucidate the Pathways of Natural Product Metabolism in Medicinal Plants. *Planta Medica*, 84(12/13), 855–873. <https://doi.org/10.1055/a-0630-1899>

Location: Germany, Italy, Bulgaria

Year: 2018

Main Technique: METABOLOMICS AND NEXT-GENERATION SEQUENCING (NGS), WHICH ARE INTEGRATED TO EXPLORE THE BIOSYNTHESIS AND METABOLIC PATHWAYS OF NATURAL PRODUCTS IN MEDICINAL PLANTS.

Secondary Techniques: THE STUDY UTILIZES A VARIETY OF OMICS TECHNOLOGIES, INCLUDING GENOMICS, TRANSCRIPTOMICS, AND COMPUTATIONAL

BIOLOGY TO SUPPORT AND EXPAND THE UNDERSTANDING OF SECONDARY METABOLITE PATHWAYS.

Aim: The study aims to elucidate the pathways of natural product metabolism in medicinal plants, with a focus on secondary metabolites of pharmacological importance. It seeks to clarify the biosynthetic pathways and cellular compartmentation of these metabolites.

Bioactive Components: The paper discusses a wide range of secondary metabolites including benzoisoquinoline and monoterpenoid indole alkaloids, cannabinoids, caffeine, ginsenosides, withanolides, artemisinin, and taxol.

Method Effectiveness: The integration of metabolomics with NGS is portrayed as highly effective in revealing metabolic pathways and regulatory networks, enhancing the understanding of plant secondary metabolism.

Method Limitation: The limitations mentioned include the complexity and diversity of secondary metabolites in plants which can make elucidation of complete pathways challenging. Additionally, the regulatory mechanisms of these pathways are not fully understood.

Gaps and Recommendations: The study suggests that further research is needed to fully understand the interaction between metabolic pathways and environmental factors, as well as to develop metabolic engineering strategies for enhancing the production of valuable secondary metabolites in both heterologous and native hosts.

Number: 34

Article Name: A novel sample preparation and on-line HPLC–DAD–MS/MS–BCD analysis for rapid screening and characterization of specific enzyme inhibitors in herbal extracts: Case study of α -glucosidase

Author: Li, D. Q., Zhao, J., Xie, J., & Li, S. P. (2014). A novel sample preparation and on-line HPLC–DAD–MS/MS–BCD analysis for rapid screening and characterization of specific enzyme inhibitors in herbal extracts: Case study of α -glucosidase. *Journal of Pharmaceutical and Biomedical Analysis*, 88, 130–135.

<https://doi.org/10.1016/j.jpba.2013.08.029>

Location: China

Year: 2013

Main Technique: HPLC–DAD–MS/MS–BCD

Secondary Techniques: NONE

Aim: To rapidly reveal and characterize specific bioactive compounds from complex mixtures of herbal extracts that inhibit α -glucosidase, potentially useful for diabetes treatment.

Bioactive Components: Two specific α -glucosidase inhibitors, corilagin and ellagic acid, were identified.

Method Effectiveness: The method was effective in specifically identifying and characterizing α -glucosidase inhibitors among a wide array of components within complex herbal extract

Method Limitation: The article highlights the challenge of false positives from non-specific enzyme inhibitors like tannins in natural extracts, which the novel sample preparation method aims to address.

Gaps and Recommendations: None mentioned.

Number: 35

Article Name: A target and nontarget strategy for identification or characterization of the chemical ingredients in Chinese herb preparation Shuang-Huang-Lian oral liquid by ultra-performance liquid chromatography–quadrupole time-of-flight mass spectrometry

Author: Zhang, F., Li, M., Yao, Z., Li, C., Qiao, L., Shen, X., Yu, K., Dai, Y., & Yao, X. (2018). A target and nontarget strategy for identification or characterization of the chemical ingredients in Chinese herb preparation Shuang-Huang-Lian oral liquid by ultra-performance liquid chromatography–quadrupole time-of-flight mass spectrometry. *Biomedical Chromatography*, 32(3), e4110. <https://doi.org/10.1002/bmc.4110>

Location: China

Year: 2017

Main Technique: ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC) COUPLED WITH QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY (Q-TOF MS)

Secondary Techniques: NONE

Aim: The study aimed to develop a rapid, comprehensive method for the identification and characterization of both targeted and non-targeted chemical components in the traditional Chinese medicine preparation, Shuang-Huang-Lian oral liquid.

Bioactive Components: A total of 170 chemical components were identified or tentatively characterized, including 54 flavonoids, 30 phenylethanoid glycosides, 16 iridoid glycosides, 14 lignans, 32 organic acids, and 19 triterpenoid saponins. Some components were confirmed by comparison with reference standards.

Method Effectiveness: The method was highlighted as highly effective for rapid identification and characterization of multiple components in complex herbal mixtures, facilitating the identification of both targeted and non-targeted compounds.

Method Limitation: The article discusses the challenges in processing the extensive MS data due to the chemical diversity and

complexity of the sample, which can be time-consuming and requires high expertise.

Gaps and Recommendations: Not mentioned

Number: 36

Article Name: An Overview of Methods of Extraction, Isolation and Characterization of Natural Medicinal Plant Products in Improved Traditional Medicine Research

Author: Fonmboh, D. J., Abah, E. R., Fokunang, T. E., Herve, B., Teke, G. N., Rose, N. M., Borgia, N. N., Fokunang, L. B., Andrew, B. N., Kaba, N., Bathelemy, N., & Ntungwen, F. C. (2020). An Overview of Methods of Extraction, Isolation and Characterization of Natural Medicinal Plant Products in Improved Traditional Medicine Research. Asian Journal of Research in Medical and Pharmaceutical Sciences, 31–57.

<https://doi.org/10.9734/ajrimps/2020/v9i230152>

Location: Cameroon, Utah, California

Year: 2020

Main Technique: TLC AND HPLC, FTIR, LCMS, GCMS

Secondary Techniques: CAPILLARY ELECTROPHORESIS AND POLAROGRAPHIC TECHNIQUES

Aim: The aim is to advance the standardization of herbal drugs through the development of authentic analytical methods that can

reliably profile the phytochemical composition, including quantitative analyses of bioactive compounds and other major constituents.

Bioactive Components: not mentioned

Method Effectiveness: The described methods are effective in profiling phytochemical compositions and standardizing herbal drugs, ensuring consistent biological activity and quality assurance in production

Method Limitation: The article discusses challenges such as the need for more advanced techniques and tools to improve the specificity and reliability of herbal drug analysis and standardization.

Gaps and Recommendations: It suggests that despite progress, there is a continual need for innovation in analytical techniques and a deeper integration of traditional knowledge and modern science to better understand and standardize herbal medicines.

Number 37

Article Name: Breaking the challenge of polyherbal quality control using UV and HPLC fingerprints combined with multivariate analysis

Author: Roshan, A. A., Hathout, R. M., El-Ahmady, S. H., Singab, A. B., & Gad, H. A. (2022). Breaking the challenge of polyherbal quality control using UV and HPLC fingerprints combined with multivariate analysis. *Phytochemical Analysis*, 33(2), 320–330. <https://doi.org/10.1002/pca.3089>

Location: Egypt

Year: 2021

Main Technique: ULTRAVIOLET-VISIBLE (UV-VIS) SPECTROSCOPY AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH PHOTODIODE ARRAY DETECTION (HPLC-PDA)

Secondary Techniques: SOFT INDEPENDENT MODELING OF CLASS ANALOGY (SIMCA) AND PARTIAL LEAST SQUARES (PLS) REGRESSION, WAS USED TO

ANALYZE THE GENERATED SPECTROSCOPIC AND CHROMATOGRAPHIC DATA.

Aim: The aim was to develop a simple, reliable, and efficient method for the qualitative and quantitative analysis of a polyherbal product using multivariate analysis of UV-Vis and HPLC-PDA data to ensure consistent quality of polyherbal formulations.

Bioactive Components: thymol

Method Effectiveness: The methods showed excellent selectivity and sensitivity in differentiating accepted and unaccepted samples based on predefined criteria, demonstrating high linearity and accuracy in the quantitative analysis.

Method Limitation: The study noted the challenge of comprehensive quality control in polyherbal mixtures due to the complex nature of these preparations and the potential for variation in components.

Gaps and Recommendations: It suggested the potential need for further refinement in techniques or approaches that can more comprehensively cover the diverse components of polyherbal medicines, although specific gaps were not detailed.

Number: 38

Article Name: Characterisation of an Aromatic Plant-based Formula using UV-Vis Spectroscopy, LCESI(+)QTOF-MS and HPLC-DAD Analysis

Author: Bunghez, F., Socaciu, C., Zagrean, F., Pop, R. M., Ranga, F., & Romanciuc, F. (2013). Characterisation of an Aromatic Plant-based Formula using UV-Vis Spectroscopy, LC–ESI(+)QTOF-MS and HPLC-DAD Analysis. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology, 70(1), 16.
<https://doi.org/10.15835/buasvmcn-fst:9635>

Location: Romania

Year: 2013

Main Technique: UV-VIS SPECTROSCOPY, LIQUID CHROMATOGRAPHY–ELECTROSPRAY IONIZATION QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY (LC–ESI(+)QTOF-MS), AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTION (HPLC-DAD)

Secondary Techniques: NOT MENTIONED

Aim: The study aimed to characterize seven aromatic herbs and develop a new product using a default recipe, focusing on identifying the "fingerprint" of main bioactive molecules and tracing these molecules in the new product.

Bioactive Components: The bioactive components identified included phenolic acids (caffeic acid, chlorogenic acid), flavonoids (quercetin, isorhamnetin), and stilbenes (resveratrol). These components are noted for their antimicrobial potentials.

Method Effectiveness: The combination of UV-Vis spectroscopy, LC-ESI(+)QTOF-MS, and HPLC-DAD proved effective for the detailed characterization of the plant extracts and the final product, allowing for the identification of specific bioactive compounds.

Method Limitation: Not mentioned

Gaps and Recommendations: Not mentioned

Number: 39

Article Name: Characterization and determination of the major constituents in *Belamcandae Rhizoma* by HPLC–DAD–ESI-MS

Author: Zhang, Y.-Y., Wang, Q., Qi, L.-W., Qin, X.-Y., & Qin, M.-J. (2011). Characterization and determination of the major constituents in *Belamcandae Rhizoma* by HPLC–DAD–ESI-MSn. *Journal of Pharmaceutical and Biomedical Analysis*, 56(2), 304–314. <https://doi.org/10.1016/j.jpba.2011.05.040>

Location: China

Year: 2011

Main Technique: HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTION AND ELECTROSPRAY IONIZATION MULTI-STAGE MASS SPECTROMETRY (HPLC–DAD–ESI-MSN). ADDITIONALLY, LIQUID CHROMATOGRAPHY-TIME OF FLIGHT MASS SPECTROMETRY (HPLC–TOF/MS)

Secondary Techniques: TIME OF FLIGHT MASS SPECTROMETRY (TOF/MS) WAS USED ALONGSIDE THE MAIN TECHNIQUES TO PROVIDE ACCURATE MASS

MEASUREMENTS AND ENHANCED RESOLUTION FOR STRUCTURAL CONFIRMATION.

Aim: The study aimed to profile and characterize the multiconstituent in *Belamcandae Rhizoma* and provide comprehensive qualitative and quantitative information for its quality evaluation. The method developed is intended to aid further studies and the development of related medicinal products.

Bioactive Components: Thirty-five flavonoids, including 30 isoflavones and five xanthones, were identified or tentatively characterized by comparing their retention times, UV, and MS spectra with those of authentic compounds or literature data. Specific identified compounds include neomangiferin, mangiferin, tectoridin, iristectorin B, iristectorin A, iridin, tectorigenin, and others.

Method Effectiveness: The method was described as rapid and effective for structural characterization of chemical constituents in herbal medicine, especially in identifying and quantifying multiple components, and providing a robust means of authentication and quality control.

Method Limitation: Not mentioned

Gaps and Recommendations: The study suggests further exploration in the detailed characterization and standardization of herbal

products like *Belamcandae Rhizoma* to improve the scientific understanding and applications of these traditional remedies.

Number: 40

Article Name: Chemical Characterization and Metabolic Profiling of the Compounds in the Chinese Herbal Formula Li Chang Decoction by UPLC-QTOF/MS

Author: Lin, B., Guo, S., Hong, X., Jiang, X., Li, H., Li, J., Guo, L., Li, M., Chen, J., Huang, B., & Xu, Y. (2022). Chemical Characterization and Metabolic Profiling of the Compounds in the Chinese Herbal Formula Li Chang Decoction by UPLC-QTOF/MS. Evidence-Based Complementary and Alternative Medicine, 2022, 1–22. <https://doi.org/10.1155/2022/1322751>

Location: China

Year: 2022

Main Technique: ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY (UPLC-QTOF/MS)

Secondary Techniques: NOT MENTIONED

Aim: The study aimed to identify the major components in the LCD and its prototype and metabolic components in rat biological

samples, to support the pharmacokinetic and pharmacological evaluation of LCD.

Bioactive Components: A total of 104 compounds were identified in the LCD, including 26 flavonoids, 20 organic acids, 20 saponins, 8 amino acids, 5 oligosaccharides, 5 tannins, 3 lignans, 2 alkaloids, and various others such as nucleosides, glycosides, and esters.

Method Effectiveness: The UPLC-QTOF/MS method provided detailed profiling and identification of a broad range of compounds in the LCD and biological samples, demonstrating the method's effectiveness in characterizing complex herbal formulations.

Method Limitation: Not mentioned

Gaps and Recommendations: Further studies might be needed to link the identified compounds with specific pharmacological activities and to fully understand the metabolic pathways involved in the therapeutic effects of the LCD.

Number: 41

Article Name: Chemical Profiling and Quantification of Potential Bioactive Components in Gandouling Pill by Ultra-High Performance Liquid Chromatography Coupled with Diode Array Detector/Quadruple-Qrbitrap Mass Spectrometry

Author: Yang, Y., Hao, W., Yang, Y., Zhang, S., Wang, H., Wang, M., Dong, T., Shang, Z., & Yang, W. (2022). Chemical Profiling and Quantification of Potential Bioactive Components in Gandouling Pill by Ultra-High Performance Liquid Chromatography Coupled with Diode Array Detector/Quadruple-Qrbitrap Mass Spectrometry. *Molecules*, 27(23), 8247. <https://doi.org/10.3390/molecules27238247>

Location: China

Year: 2022

Main Technique: THE MAIN TECHNIQUES EMPLOYED WERE ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (UHPLC) COUPLED WITH A QUADRUPE-ORBITRAP MASS SPECTROMETER (MS) AND A DIODE ARRAY DETECTOR (DAD)

Secondary Techniques: TARGETED SELECTED ION MONITORING (T-SIM) WAS USED FOR INVESTIGATING THE ABSORPTION COMPONENTS OF GANDOULING PILL IN RAT PLASMA, WHICH HELPS IDENTIFY POTENTIAL BIOACTIVE COMPONENTS.

Aim: The study aimed to profile the chemical components of Gandouling Pill, quantify its major bioactive components, and evaluate the absorption of these components in rat plasma, thus supporting the quality control and therapeutic efficacy of the formulation.

Bioactive Components: The study identified and quantified several bioactive components, including alkaloids such as coptisine, berberine, and palmatine, which are considered key active compounds for Gandouling Pill.

Method Effectiveness: The method was effective in profiling 69 compounds within the pill and quantifying 10 major absorption components in plasma, indicating robust capability in chemical profiling and quantification.

Method Limitation: Not mentioned

Gaps and Recommendations: The study fills significant gaps in understanding the chemical basis of Gandouling Pill's therapeutic effects and its pharmacokinetics in biological systems. However, the

continuous evolution of analytical techniques could further enhance the depth and breadth of chemical profiling and bioactivity linkage.

Number: 42

Article Name: Direct Analysis in Real Time Mass Spectrometry for Characterization of Large Saccharides

Author: Ma, H., Jiang, Q., Dai, D., Li, H., Bi, W., & Da Yong Chen, D. (2018). Direct Analysis in Real Time Mass Spectrometry for Characterization of Large Saccharides. *Analytical Chemistry*, 90(5), 3628–3636. <https://doi.org/10.1021/acs.analchem.8b00242>

Location: China, Canada

Year: 2018

Main Technique: DIRECT ANALYSIS IN REAL TIME MASS SPECTROMETRY (DART-MS) WAS USED TO GENERATE FINGERPRINTS OF POLYSACCHARIDES

Secondary Techniques: MULTIVARIATE DATA ANALYSIS

Aim: The aim was to characterize large saccharides from different plant sources and effectively differentiate them based on their molecular features. This was done to provide a rapid, simple, and high throughput method for the evaluation of molecular features in plant polysaccharides.

Bioactive Components: The study identified characteristic fragment ions for different types of plant polysaccharides, which could be used for rapid fingerprinting analysis and quality assessment of these compounds.

Method Effectiveness: DART-MS proved to be a fast and high throughput method that requires little to no sample pretreatment, making it effective for the rapid analysis of intact polysaccharides.

Method Limitation: One limitation discussed was the challenge in ionizing large molecules typically above 500 Da; however, the study demonstrated that DART-MS could successfully ionize large polysaccharides into smaller fragments.

Gaps and Recommendations: The study indicates the need for more sensitive and higher throughput characterization techniques that can handle the large diversity of polysaccharides to generate characteristic fingerprints for quality assessment. The detailed mechanism of polysaccharide fragmentation by DART is also not fully understood and was pointed out as an area for further research.

Number: 43

Article Name: Extraction, Isolation And Characterization Of Bioactive Compounds From Plants' Extracts

Author: Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K., & Latha, L. (2010). Extraction, Isolation And Characterization Of Bioactive Compounds From Plants' Extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(1).

<https://doi.org/10.4314/ajtcam.v8i1.60483>

Location: Malaysia

Year: 2011

Main Technique: THE MAIN TECHNIQUES USED IN THE STUDY ARE VARIOUS EXTRACTION METHODS INCLUDING SOLVENT EXTRACTION, MACERATION, PERCOLATION, AND SUPERCRITICAL FLUID EXTRACTION (SFE)

Secondary Techniques: THIN LAYER CHROMATOGRAPHY (TLC), HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC), AND GAS CHROMATOGRAPHY (GC).

Aim: The study aims to review and explore different methodologies for extracting, isolating, and characterizing natural products from medicinal plants. It focuses on advancing the understanding and applications of these compounds in pharmacology and medicine.

Bioactive Components: not mentioned

Method Effectiveness: The methods discussed are effective for the extraction and preliminary analysis of bioactive compounds from various plant sources, providing a foundational understanding for further chemical analysis and pharmaceutical application.

Method Limitation: The article discusses the limitations associated with traditional extraction methods, such as the requirement of large volumes of solvents and long extraction times, and points towards more efficient modern techniques like SFE.

Gaps and Recommendations: The study highlights the need for continued development of more selective and effective extraction techniques that can improve yield, reduce solvent use, and enhance the purity of extracted compounds.

Number: 44

Article Name: High-performance thin-layer chromatography linked with (bio)assays and mass spectrometry – A suited method for discovery and quantification of bioactive components? Exemplarily shown for turmeric and milk thistle extracts

Author: Taha, M. N., Krawinkel, M. B., & Morlock, G. E. (2015). High-performance thin-layer chromatography linked with (bio)assays and mass spectrometry – A suited method for discovery and quantification of bioactive components? Exemplarily shown for turmeric and milk thistle extracts. *Journal of Chromatography A*, 1394, 137–147. <https://doi.org/10.1016/j.chroma.2015.03.029>

Location: Germany

Year: 2015

Main Technique: HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC) LINKED WITH BIOASSAYS AND MASS SPECTROMETRY (MS)

Secondary Techniques: BIOASSAYS (SUCH AS THE ALIIVIBRIO FISCHERI BIOASSAY, THE BACILLUS SUBTILIS BIOASSAY, AND THE DPPH* ASSAY)

Aim: To demonstrate a method for the discovery and quantification of bioactive components in complex herbal mixtures, exemplified by turmeric and milk thistle extracts.

Bioactive Components: Curcumin from turmeric and silybin from milk thistle were specifically identified

Method Effectiveness: The method was effective for screening and identifying bioactive compounds, allowing for direct linking of chromatographic spots to biological activities and subsequent identification by MS.

Method Limitation: The method's dependence on the quality of HPTLC execution, the specificity of bioassays, and the sensitivity of MS detection were inherent challenges.

Gaps and Recommendations: The study indicated the need for further optimization of the bioassay procedures and MS integration to enhance the robustness and applicability of the method for comprehensive natural product analysis.

Number: 45

Article Name: Identification and quantification of the bioactive components in *Osmanthus fragrans* roots by HPLC-MS/MS

Author: Liao, X., Hong, Y., & Chen, Z. (2021). Identification and quantification of the bioactive components in *Osmanthus fragrans* roots by HPLC-MS/MS. *Journal of Pharmaceutical Analysis*, 11(3), 299–307. <https://doi.org/10.1016/j.jpha.2020.06.010>

Location: China

Year: 2021

Main Technique: HPLC-MS/M

Secondary Techniques: NOT MENTIONED

Aim: The study aimed to develop a sensitive and accurate method for the analysis of chemical constituents in *Osmanthus fragrans* roots, identify bioactive components, and use network pharmacology to understand their potential therapeutic effects.

Bioactive Components: Key bioactive components identified included acteoside, isoacteoside, oleuropein, and phillyrin. These

compounds were quantified in different cultivars of *Osmanthus fragrans* roots.

Method Effectiveness: The HPLC-MS/MS method demonstrated high sensitivity and accuracy in identifying and quantifying the specific bioactive components. The method was effective for both qualitative and quantitative analyses.

Method Limitation: Not mentioned

Gaps and Recommendations: The study suggested further exploration of the therapeutic potential of *Osmanthus fragrans* roots through network pharmacology, hinting at gaps in the full understanding of the pharmacological activities of the identified components and their interactions within biological systems.

Number: 46

Article Name: Qualitative and quantitative determination of ten alkaloids in traditional Chinese medicine *Corydalis yanhusuo* W.T. Wang by LC-MS/MS and LC-DAD

Author: Ding, B., Zhou, T., Fan, G., Hong, Z., & Wu, Y.
(2007). Qualitative and quantitative determination of ten alkaloids in traditional Chinese medicine *Corydalis yanhusuo* W.T. Wang by LC-MS/MS and LC-DAD. *Journal of Pharmaceutical and Biomedical Analysis*, 45(2), 219–226.
<https://doi.org/10.1016/j.jpba.2007.06.009>

Location: China

Year: 2007

Main Technique: LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY/MASS SPECTROMETRY (LC-MS/MS) AND LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTION (LC-DAD).

Secondary Techniques: ELECTROSPRAY IONIZATION IN POSITIVE ION MODE FOR MASS SPECTROMETRY

Aim: The study aimed to develop a method for the qualitative and quantitative determination of ten alkaloids in *Corydalis yanhusuo*, facilitating the authentication and quality control of this herbal medicine.

Bioactive Components: Ten alkaloids were identified, including both tertiary and quaternary alkaloids such as tetrahydropalmatine, protopine, and palmatine.

Method Effectiveness: The method was noted for its excellent sensitivity and specificity in identifying and quantifying the alkaloids, enhancing the ability to analyze these components in *Corydalis yanhusuo*.

Method Limitation: Not mentioned

Gaps and Recommendations: The study filled significant gaps in the authentication and quality assessment of *Corydalis yanhusuo* but suggests the need for further refinement in simultaneous analysis techniques for complex herbal matrices.

Number: 47

Article Name: Rapid characterization and identification of the chemical constituents and rat metabolites of Deng-Zhan-Xi-Xin injection using ultra high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

Author: Guo, X., Lin, S., Yang, P., Ye, J., Du, J., Mu, X., Mi, N., Qi, X., Lei, H., Zhang, W., & Tian, X. (2018). Rapid characterization and identification of the chemical constituents and rat metabolites of Deng-Zhan-Xi-Xin injection using ultra high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. *Journal of Separation Science*, 41(18), 3569–3582. <https://doi.org/10.1002/jssc.201800470>

Location: China

Year: 2018

Main Technique: ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (UHPLC) COUPLED WITH QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY (Q-TOF MS)

Secondary Techniques: UNIFI SOFTWARE WAS USED FOR DATA PROCESSING, WHICH INCLUDES MULTIPLE DATA PROCESSING APPROACHES SUCH AS EXTRACTED ION CHROMATOGRAM (EIC), COMMON FRAGMENT SEARCH (CFS), NEUTRAL LOSS SCAN (NLS), AND MASS DEFECT FILTER (MDF).

Aim: To establish a rapid, sensitive, and effective method for identifying complex chemical constituents and their metabolites in Deng-Zhan-Xi-Xin injection, aiding further studies on its pharmacology and mechanisms of action.

Bioactive Components: A total of 40 compounds including 9 flavonoids and 31 phenolic acids were identified or tentatively characterized within the injection, and 70 compounds were detected in rats, including 25 prototypes and 45 metabolites.

Method Effectiveness: The UHPLC-Q-TOF MS method combined with the UNIFI software proved to be highly effective in identifying complex chemical constituents and their metabolites, providing comprehensive profiling and precise identification.

Method Limitation: The article notes the complexity of chemical components in traditional Chinese medicines and the interference from biological matrices as challenges in the detection and identification process.

Gaps and Recommendations: Not mentioned

Number: 48

Article Name: Recent application of direct analysis in real time mass spectrometry in plant materials analysis with emphasis on traditional Chinese herbal medicine

Author: Wang, Y., & Liu, S. (2023). Recent application of direct analysis in real time mass spectrometry in plant materials analysis with emphasis on traditional Chinese herbal medicine. *Mass Spectrometry Reviews*, mas.21866.
<https://doi.org/10.1002/mas.21866>

Location: China

Year: 2023

Main Technique: DIRECT ANALYSIS IN REAL TIME MASS SPECTROMETRY (DART-MS)

Secondary Techniques: LIQUID CHROMATOGRAPHY, AND MODIFICATIONS LIKE THE USE OF LASER-BASED DESORPTION TECHNIQUES

Aim: The review aims to summarize recent advancements in the application of DART-MS for analyzing plant materials, focusing on TCHMs traditional Chinese herbal medicines. It covers the use of

DART-MS for compound identification, biomarker discovery, fingerprinting analysis, and quantification analysis.

Bioactive Components: Not mentioned

Method Effectiveness: DART-MS is portrayed as highly effective for fast and environmentally friendly analyses with minimal sample preparation, offering rapid analysis capabilities.

Method Limitation: The review mentions some limitations of DART-MS, such as the complexity of the sample treatments required for traditional methods and the need for improvements in design and analytical performance to expand applications.

Gaps and Recommendations: Potential gaps mentioned include the ongoing need for improvement in the technology's design and performance, and a better understanding of the ionization mechanisms and processes affected by various operational parameters.

Number: 49

Article Name: Simultaneous characterization and quantitation of 11 coumarins in *Radix Angelicae Dahuricae* by high performance liquid chromatography with electrospray tandem mass spectrometry

Author: Zheng, X., Zhang, X., Sheng, X., Yuan, Z., Yang, W., Wang, Q., & Zhang, L. (2010). Simultaneous characterization and quantitation of 11 coumarins in *Radix Angelicae Dahuricae* by high performance liquid chromatography with electrospray tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 51(3), 599–605. <https://doi.org/10.1016/j.jpba.2009.09.030>

Location: China, Japan

Year: 2010

Main Technique: HPLC-ESI-MS/MS

Secondary Techniques: MULTIPLE REACTION MONITORING (MRM)

Aim: The aim was to develop an analytical method for simultaneous identification and quantification of 11 specific coumarin compounds in *Radix Angelicae Dahuricae*, with the ultimate goal of

providing a robust method for quality control of this traditional Chinese medicine.

Bioactive Components: The study identified and quantified 11 coumarins, including oxypeucedanin, bergapten, imperatorin, cnidilin, isoimperatorin, xanthotoxol, xanthotoxin, and several others.

Method Effectiveness: The method was effective in achieving separation and determination of the 11 coumarins within 10 minutes, demonstrating high precision with intra- and inter-day tests showing relative standard deviation values within acceptable limits. The recovery rates for the quantified compounds were within the range of 92.1–105.6%.

Method Limitation: Not mentioned

Gaps and Recommendations: The article primarily focuses on developing a quantification and identification method without extensively discussing the pharmacological implications of the concentrations found, which could be considered a gap for further research linking chemical profiles to therapeutic effects.

Number: 50

Article Name: Simultaneous quantification of bioactive components in Chinese herbal spirits by ultra-high performance liquid chromatography coupled to triple-quadrupole mass spectrometry (UHPLC–QQQ–MS/MS)

Author: Hu, Y., Wang, Z., Xia, F., Yang, W., Liu, Y.-C., & Wan, J.-B. (2021). Simultaneous quantification of bioactive components in Chinese herbal spirits by ultra-high performance liquid chromatography coupled to triple-quadrupole mass spectrometry (UHPLC–QQQ–MS/MS). *Chinese Medicine*, 16(1), 26.
<https://doi.org/10.1186/s13020-021-00435-0>

Location: China

Year: 2021

Main Technique: ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO TRIPLE-QUADRUPOLE MASS SPECTROMETRY (UHPLC–QQQ–MS/MS) WAS THE MAIN TECHNIQUE USED. THIS WAS COMBINED WITH MULTIPLE REACTION MONITORING (MRM) TO ENHANCE SPECIFICITY AND SENSITIVITY.

Secondary Techniques: STATISTICAL ANALYSES LIKE STUDENT T-TEST AND ORTHOGONAL PARTIAL LEAST SQUARES-DISCRIMINANT ANALYSIS (OPLS-DA) WERE USED TO ANALYZE THE DATA.

Aim: The aim was to develop a method capable of simultaneously quantifying 43 bioactive components in Chinese herbal spirits from different production years, to aid in quality control and ensure the consistency and efficacy of these herbal spirits.

Bioactive Components: The study identified and quantified 43 different compounds, including puerarin, purpureaside C, daidzin, echinacoside, acteoside, epimedin B, epimedin C, icariin, eugenol, chikusetsusaponin iva, and Z-ligustilide.

Method Effectiveness: The method was effective in quantifying multiple bioactive components across different batches of the herbal spirit, demonstrating good sensitivity and precision.

Method Limitation: Challenges included dealing with a complex sample matrix that contains numerous organic and inorganic compounds, which could affect the ionization efficiency and detection sensitivity.

Gaps and Recommendations: While the method developed was robust, further improvements in chromatographic conditions and possibly extending the range of targeted compounds could enhance

its utility. There was also a mention of needing to optimize conditions to reduce potential ion suppression effects due to co-eluted analytes.

Number: 51

Article Name: Two dimensional NMR spectroscopic approaches for exploring plant metabolome: A review

Author: Mahrous, E. A., & Farag, M. A. (2015). Two dimensional NMR spectroscopic approaches for exploring plant metabolome: A review. *Journal of Advanced Research*, 6(1), 3–15.

<https://doi.org/10.1016/j.jare.2014.10.003>

Location: Egypt

Year: 2014

Main Technique: TWO-DIMENSIONAL NUCLEAR MAGNETIC RESONANCE (2D NMR) SPECTROSCOPY

Secondary Techniques: CHEMOMETRICS AND DATA PROCESSING TECHNIQUES

Aim: The review aims to provide an overview of the development of 2D NMR spectroscopic techniques and their applications in the field of plant metabolomics, particularly for quality control analysis of phytochemicals and drug discovery from natural sources.

Bioactive Components: Not mentioned

Method Effectiveness: 2D NMR is depicted as highly effective for complex mixture analyses, offering significant advantages over traditional 1D NMR by reducing peak overlap and providing more detailed structural information.

Method Limitation: The review notes that NMR is less sensitive compared to mass spectrometry (MS) and can suffer from issues with signal overlap, although 2D techniques mitigate many of these challenges.

Gaps and Recommendations: The article suggests that further improvements in NMR technology and computational tools are needed to fully exploit the potential of 2D NMR in plant metabolomics, particularly to enhance signal detection, quantification, and the use of shared databases for compound identification.

Number: 52

Article Name: Ethnobotany, Ethnopharmacology, and Phytochemistry of Traditional Medicinal Plants Used in the Management of Symptoms of Tuberculosis in East Africa: A Systematic Review

Author: Obakiro, S. B., Kiprop, A., Kowino, I., Kigundu, E., Odero, M. P., Omara, T., & Bunalema, L. (2020). Ethnobotany, ethnopharmacology, and phytochemistry of traditional medicinal plants used in the management of symptoms of tuberculosis in East Africa: A systematic review. *Tropical Medicine and Health*, 48(1), 68.
<https://doi.org/10.1186/s41182-020-00256-1>

Location: East Africa: Uganda, Kenya

Year: 2020

Main Technique: COMPREHENSIVE LITERATURE SEARCH IN MAJOR ELECTRONIC DATABASES.

DATA ANALYSIS OF ETHNOBOTANICAL SURVEYS, PHYTOCHEMICAL CONTENT, EFFICACY, AND SAFETY STUDIES.

Secondary Techniques: CHROMATOGRAPHIC FINGER-PRINTING FOR PHYTOCHEMICAL ANALYSIS.

IN VITRO AND IN VIVO TOXICITY TESTS TO ASSESS SAFETY PROFILES OF PLANT EXTRACTS.

MINIMUM INHIBITORY CONCENTRATION (MIC) TESTS FOR ANTIMYCOBACTERIAL ACTIVITY.

Aim: To compile a comprehensive report on the use of medicinal plants in the management of tuberculosis symptoms in East Africa.

To analyze scientific reports on the effectiveness and safety of these plants.

Bioactive Components: Flavonoids, terpenoids, alkaloids, saponins, cardiac glycosides, and phenols. Specific bioactive compounds from plants like *Tetradenia riparia*, *Warburgia ugandensis*, and *Zanthoxylum leprieurii*.

Method Effectiveness: Identification of 195 species of plants from 68 families demonstrating varied efficacy against tuberculosis. Several plants showing significant antimycobacterial activity with MIC values ranging from 47 to 12,500 µg/ml.

Method Limitation: Limited studies on the long-term toxicity and chronic effects of the medicinal plants. Only a small fraction of identified plants (18.5%) have been tested for antimycobacterial activity.

Gaps and Recommendations: Need for more extensive pharmacological and toxicological studies to validate the safety and efficacy of the plants. Requirement for more detailed phytochemical analyses and isolation of active compounds for potential drug development.

Number: 53

Article Name: Antimicrobial Activity and Chemical Composition of Essential Oil Extracted from *Solidago canadensis* L. Growing Wild in Slovakia

Author: Elshafie, H. S., Gruľová, D., Baranová, B., Caputo, L., De Martino, L., Sedlák, V., Camele, I., & De Feo, V. (2019). Antimicrobial Activity and Chemical Composition of Essential Oil Extracted from *Solidago canadensis* L. Growing Wild in Slovakia. *Molecules*, 24(7), 1206. <https://doi.org/10.3390/molecules24071206>

Location: Slovakia

Year: 2019

Main Technique: GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) FOR CHEMICAL COMPOSITION ANALYSIS.

ANTIMICROBIAL ASSAYS TO EVALUATE THE EFFICACY OF THE ESSENTIAL OIL AGAINST VARIOUS MICROBIAL PATHOGENS

Secondary Techniques: CELL MEMBRANE PERMEABILITY ASSAY TO INVESTIGATE THE MECHANISM OF ACTION OF THE ESSENTIAL OIL ON MICROBIAL CELLS.

MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION USING A 96-WELL MICROPLATE TO ASCERTAIN THE LOWEST EFFECTIVE DOSE OF THE ESSENTIAL OIL.

Aim: To analyze the chemical composition of *Solidago canadensis* L. essential oil.

To assess its antimicrobial activity against a range of phytopathogenic fungi and bacteria, providing insights into its potential use as a natural antimicrobial agent

Bioactive Components: Major components such as Germacrene D, Limonene, α -Pinene, β -Elemene, and Bornyl Acetate.

Method Effectiveness: The essential oil demonstrated significant antimicrobial activity against most of the tested pathogens, highlighting its potential as a natural antimicrobial agent. The chemical analysis provided detailed profiling of the essential oil's composition, supporting the identification of active constituents responsible for its antimicrobial properties.

Method Limitation: Variability in the effectiveness of the essential oil against different microbial strains, indicating that its action might not be universally potent across all pathogens.

Limited data on the long-term stability and potential resistance development against the essential oil in microbial populations.

Gaps and Recommendations: Further studies are needed to explore the *in vivo* effectiveness and safety profile of the essential oil in clinical and agricultural settings. The potential development of resistance among pathogens when exposed to this essential oil has not been studied.

Number: 54

Article Name: Agastache Species: A Comprehensive Review on
Phytochemical Composition and Therapeutic Properties

Author: Nechita, M.-A., Toiu, A., Benedec, D., Hanganu, D.,
Ielciu, I., Oniga, O., Nechita, V.-I., & Oniga, I. (2023). Agastache
Species: A Comprehensive Review on Phytochemical Composition
and Therapeutic Properties. *Plants*, 12(16), 2937.

<https://doi.org/10.3390/plants12162937>

Location: Romania

Year: 2023

Main Technique: PHYTOCHEMICAL CHARACTERIZA-
TION OF BOTH EXTRACTS AND ESSENTIAL OILS.

VARIOUS EXTRACTION METHODS FOR IDENTIFY-
ING BIOACTIVE COMPOUNDS, PRIMARILY FOCUSED
ON POLYPHENOLIC COMPOUNDS AND VOLATILE
COMPOUNDS FROM AGASTACHE SPECIES

Secondary Techniques: GAS CHROMATOGRAPHY-
MASS SPECTROMETRY (GC-MS) FOR ANALYZING ES-
SENTIAL OILS.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) FOR DETAILED COMPOUND IDENTIFICATION AND QUANTIFICATION.

IN VITRO AND IN VIVO ASSAYS FOR DETERMINING BIOLOGICAL ACTIVITIES SUCH AS ANTIOXIDANT, ANTIMICROBIAL, AND CYTOTOXIC EFFECTS

Aim: To provide a detailed and updated review of the phytochemical and pharmacological profiles of *Agastache* species, focusing on their therapeutic potentials and chemical diversity.

To highlight the progress made since the last review and to outline future research directions for the utilization of *Agastache* species in various therapeutic applications

Bioactive Components: Major polyphenolic compounds such as rosmarinic acid, tilianin, chlorogenic acid, and various flavonoid derivatives. Volatile compounds including estragole, limonene, menthone, and pulegone, which vary among species and chemotypes.

Method Effectiveness: The combination of phytochemical analysis and biological assays effectively provided a comprehensive understanding of the bioactive constituents and their potential health benefits. The methods used allowed for the identification of specific compounds responsible for observed pharmacological activities.

Method Limitation: Variability in the composition of Agastache species due to environmental factors and genetic diversity, which can complicate standardization and consistent therapeutic use. Limited studies on the long-term safety and pharmacokinetics of the identified bioactive compounds.

Gaps and Recommendations: Need for more clinical trials to confirm the therapeutic benefits and safety of Agastache species in human populations. Further research is required to understand the mechanisms of action of the bioactive compounds at the molecular level.

Number: 55

Article Name: Ethnomedicinal potential of *Aconitum deino-*
rhizum Stapf (Mohra) - A threatened medicinal plant of North
Western Himalayas: A comprehensive review

Author: Sharma, D. K., & Bhardwaj, E. (2022). Ethnomedici-
nal Potential of *Aconitum deino*rhizum Stapf (Mohra): A threat-
ened Medicinal Plant of North Western Himalayas: A Comprehen-
sive review. *The Journal of Phytopharmacology*, 11(2), 107–110.
<https://doi.org/10.31254/phyto.2022.11210>

Location: India

Year: 2022

Main Technique: PHYTOCHEMICAL, PHARMACOLOG-
ICAL, AND TOXICOLOGICAL

Secondary Techniques: EXAMINATION OF CHEMICAL
PROPERTIES THROUGH METHODS SUCH AS AIR-DRY-
ING AND SOLVENT EXTRACTION TO DETERMINE
THE PRESENCE OF PRIMARY ALKALOIDS LIKE ACO-
NITINE AND PSEUDO-ACONITINE.

Aim: To summarize the current knowledge on the phytochemical makeup, medicinal usage, and conservation needs of *Aconitum deinorrhizum*, aiming to raise awareness of its endangered status and therapeutic potential. To encourage the conservation and sustainable use of this critically endangered species due to its significant medicinal properties.

Bioactive Components: Primary alkaloids identified include aconitine and pseudo-aconitine, which are noted for their potent effects on the nervous system and as a treatment for rheumatic pain. Other compounds include diterpenoid alkaloids, flavonoids, and free fatty acids.

Method Effectiveness: The method effectively compiles and presents a comprehensive overview of *Aconitum deinorrhizum*, its uses, risks, and the urgent need for its conservation. Highlights the critical balance between medicinal use and toxicity, emphasizing the importance of careful and knowledgeable handling.

Method Limitation: The method effectively compiles and presents a comprehensive overview of *Aconitum deinorrhizum*, its uses, risks, and the urgent need for its conservation. Highlights the critical balance between medicinal use and toxicity, emphasizing the importance of careful and knowledgeable handling.

Gaps and Recommendations: A noted scarcity of rigorous clinical trials to substantiate traditional claims of the plant's effectiveness in treating various ailments. An urgent need for more focused research on sustainable conservation strategies and cultivation practices to prevent over-harvesting and ensure the survival of the species.

Number: 56

Article Name: Evaluating the Aphrodisiac Potential of *Mirabilis jalapa* L. Root Extract: Phytochemical Profiling and In Silico, In Vitro, and In Vivo Assessments in Normal Male Rats

Author: Sharma, D. K., & Bhardwaj, E. (2022). Ethnomedicinal Potential of *Aconitum deionorrhizum* Stapf (Mohra): A threatened Medicinal Plant of North Western Himalayas: A Comprehensive review. *The Journal of Phytopharmacology*, 11(2), 107–110. <https://doi.org/10.31254/phyto.2022.11210>

Location: Pakistan

Year: 2023

Main Technique: GC-MS

Secondary Techniques: N SILICO MOLECULAR DOCKING STUDIES TO PREDICT INTERACTIONS OF THE IDENTIFIED COMPOUNDS WITH THE PHOSPHODIESTERASE-5 (PDE-5) ENZYME.

Aim: To evaluate the aphrodisiac potential of *Mirabilis jalapa* L. root extract through comprehensive phytochemical profiling and a series of in silico, in vitro, and in vivo assessments.

Bioactive Components: β -D-glucopyranoside, methyl-1,9-benzyl-2,6-dichloro-9H-purine, and Bis-(2-ethylhexyl)-phthalate were identified as major components with potential PDE-5 inhibitory activity.

Method Effectiveness: The combination of techniques provided a detailed understanding of the extract's components and their biological activities.

The *in vivo* studies confirmed the extract's efficacy in enhancing sexual behavior in male rats, comparable to the effects of standard drugs like sildenafil and ginseng.

Method Limitation: The research is limited to animal models, and further studies are needed to evaluate the safety and efficacy of the extract in humans. The exact mechanism of how the extract affects sexual function at the molecular level remains to be fully elucidated.

Gaps and Recommendations: A need for clinical trials to verify the extract's safety and effectiveness in human subjects. Exploration of the long-term effects and potential side effects of chronic use of the extract is necessary to ensure its safe use as an aphrodisiac or therapeutic agent for erectile dysfunction.

Number: 57

Article Name: Infusions of Herbal Blends as Promising Sources of Phenolic Compounds and Bioactive Properties

Author: Rahman, A. U., Alam, F., Khan, M., Sarfraz, M., Basit, A., Ahmad, T., Khokhar, M. A., Ali, S., & Khan, K. U. (2023).

Evaluating the Aphrodisiac Potential of *Mirabilis jalapa* L. Root Extract: Phytochemical Profiling and In Silico, In Vitro, and In Vivo Assessments in Normal Male Rats. *Molecules*, 28(17), 6314.

<https://doi.org/10.3390/molecules28176314>

Location: Portugal, Serbia

Year: 2020

Main Technique: HPLC-DAD/ESI-MSN

Secondary Techniques: VARIOUS ASSAYS INCLUDING TBARS, OXHLIA FOR ANTIOXIDANT ACTIVITY, AND GI50 FOR CYTOTOXIC ACTIVITY AGAINST MULTIPLE CELL LINES.

Aim: To chemically characterize six different herbal blends and evaluate their phenolic profiles and bioactive properties. To assess

the synergistic effects of these blends and their potential application in food, pharmaceutical, and nutraceutical products.

Bioactive Components: A variety of phenolic acids and flavonoids across the blends, including rosmarinic acid, luteolin-3'-O-glucuronide, and quercetin derivatives.

Method Effectiveness: The HPLC-DAD/ESI-MSⁿ method provided detailed and comprehensive profiling of phenolic compounds in the herbal blends.

Bioactivity assays demonstrated significant antioxidant, antimicrobial, and cytotoxic activities, indicating the functional potential of the blends

Method Limitation: Variability in phenolic content and bioactivity results due to the natural composition of the herbal blends. Some blends showed lower activities in certain tests, indicating inconsistent bioactive effects across different assays.

Gaps and Recommendations: Further research is needed to explore the *in vivo* relevance of the *in vitro* bioactivity findings. More detailed studies on the mechanisms of action for the observed bioactivities would provide deeper insights into how these herbal blends could be optimally used in therapeutic contexts.

Number: 58

Article Name: Phytochemical Analysis, In Vitro Biological Activities, and Computer-Aided Analysis of *Potentilla nepalensis* Hook Compounds as Potential Melanoma Inhibitors Based on Molecular Docking, MD Simulations, and ADMET

Author: Sharma, S., Kumar, V., Yaseen, M., S. Abouzied, A., Arshad, A., Bhat, M. A., Naglah, A. M., Patel, C. N., Sivakumar, P. K., Sourirajan, A., Shahzad, A., & Dev, K. (2023). Phytochemical Analysis, In Vitro Biological Activities, and Computer-Aided Analysis of *Potentilla nepalensis* Hook Compounds as Potential Melanoma Inhibitors Based on Molecular Docking, MD Simulations, and ADMET. *Molecules*, 28(13), 5108. <https://doi.org/10.3390/molecules28135108>

Location: Egypt

Year: 2023

Main Technique: GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) FOR PHYTOCHEMICAL PROFILING.

MOLECULAR DOCKING AND MOLECULAR DYNAMICS (MD)

Secondary Techniques: IN VITRO ASSAYS SUCH AS DPPH RADICAL SCAVENGING ASSAY FOR ANTIOXIDANT ACTIVITY AND MTT ASSAY FOR CYTOTOXIC ACTIVITY AGAINST MELANOMA CELLS.

ADMET PROFILING FOR ASSESSING THE PHARMACOKINETICS AND SAFETY OF THE BIOACTIVE COMPOUNDS.

Aim: To analyze the phytochemical composition and evaluate the antioxidant, antibacterial, and anticancer activities of *Potentilla nepalensis* extracts. To explore the potential of identified compounds as melanoma inhibitors using computer-aided analysis techniques.

Bioactive Components: tetradecanoic acid, hexadecanoic acid, and various silicon-based compounds.

Method Effectiveness: Effective identification of bioactive compounds with potential anticancer properties against melanoma. Molecular docking and simulations provided insights into the stability and potential efficacy of these compounds at the molecular level.

Method Limitation: Potential variability in plant extracts due to environmental factors, affecting the consistency of results.

Limitations in translating in vitro and in silico findings to clinical effectiveness without further in vivo and human studies.

Gaps and Recommendations: Need for clinical trials to validate the anticancer efficacy of the identified compounds in humans. Further research needed to explore the full pharmacological potential and safety profiles of the compounds for therapeutic use.

Number: 59

Article Name: Phytochemical Characterization and Chemotherapeutic Potential of *Cinnamomum verum* Extracts on the Multiplication of Protozoan Parasites In Vitro and In Vivo

Author: Batiha, G. E.-S., Beshbishy, A. M., Guswanto, A., Nugraha, A., Munkhjargal, T., M. Abdel-Daim, M., Mosqueda, J., & Igarashi, I. (2020). Phytochemical Characterization and Chemotherapeutic Potential of *Cinnamomum verum* Extracts on the Multiplication of Protozoan Parasites In Vitro and In Vivo. *Molecules*, 25(4), 996. <https://doi.org/10.3390/molecules25040996>

Location: Japan

Year: 2020

Main Technique: IN VITRO CYTOTOXICITY AND VIABILITY ASSAYS

Secondary Techniques: GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) FOR PHYTOCHEMICAL CHARACTERIZATION OF THE EXTRACTS.

FLUORESCENCE ASSAY TO MEASURE THE INHIBITORY EFFECT OF THE EXTRACTS ON PROTOZOAN PARASITES.

QUANTITATIVE ASSAYS TO DETERMINE HALF-MAXIMAL INHIBITORY CONCENTRATIONS (IC₅₀) AND EFFECTIVE CONCENTRATIONS (EC₅₀)

Aim: To evaluate the inhibitory effects of acetonic and ethyl acetate extracts of *Cinnamomum verum* against protozoan parasites associated with diseases in livestock and potentially in humans.

To determine the chemotherapeutic potential of these extracts as treatments for protozoan infections *in vivo*, using mice as model organisms.

Bioactive Components: The GC-MS analysis identified several compounds including cinnamaldehyde, cinnamic acid, and various flavonoids and terpenoids that contribute to the biological activity of the extracts.

Method Effectiveness: The extracts demonstrated significant inhibitory effects on the growth of protozoan parasites *in vitro* and reduced parasite loads in treated mice, showing potential as antiprotozoal drugs. The extracts were found to be relatively safe in cytotoxicity assays, indicating a promising therapeutic index.

Method Limitation: The potential for varying effectiveness across different batches of extracts due to differences in the concentration and composition of bioactive compounds.

Limited data on the long-term safety and efficacy of these extracts in humans and larger-scale animal models.

Gaps and Recommendations: Further studies are needed to confirm these findings in clinical settings and to fully understand the mechanisms behind the observed antiparasitic effects.

Additional research is necessary to explore the potential resistance development against these extracts in protozoan parasites.

Number: 60

Article Name: Phytochemical characterization and comparative studies of four *Cecropia* species collected in Panama using multivariate data analysis

Author: Rivera-Mondragón, A., Bijttebier, S., Tuenter, E., Custers, D., Ortiz, O. O., Pieters, L., Caballero-George, C., Apers, S., & Foubert, K. (2019). Phytochemical characterization and comparative studies of four *Cecropia* species collected in Panama using multivariate data analysis. *Scientific Reports*, 9(1), 1763.

<https://doi.org/10.1038/s41598-018-38334-4>

Location: Belgium, Panama

Year: 2019

Main Technique: HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-DIODE ARRAY DETECTION-QUADRUPOLE TIME OF FLIGHT-TANDEM HIGH-RESOLUTION MASS SPECTROMETRY (HPLC-DAD-QTOF), NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

Secondary Techniques: MULTIVARIATE DATA ANALYSIS, INCLUDING PRINCIPAL COMPONENT ANALYSIS (PCA) AND HIERARCHICAL CLUSTER ANALYSIS (HCA),

Aim: To characterize and compare the phytochemical constituents of four *Cecropia* species collected in Panama and assess their chemotaxonomic significance and potential medicinal properties.

To aid in the quality assessment of herbal supplements containing *Cecropia* leaves.

Bioactive Components: Identification of 11 compounds in *Cecropia* obtrusively including flavone glycosides and triterpenoid saponins. A total of 47 phenolic compounds, including phenolic acids, flavonoids, flavonolignans, and saponins, were identified or tentatively characterized across the species

Method Effectiveness: The HPLC-DAD-QTOF method provided detailed and comprehensive chemical profiling, enabling the identification and comparison of a wide range of bioactive compounds across the *Cecropia* species.

The use of multivariate analysis techniques effectively illustrated the phytochemical relationships between the species, aiding in chemotaxonomic classification and quality control

Method Limitation: While the study provided extensive phytochemical data, the pharmacological implications of these findings were not directly addressed, which may limit the application of this data in clinical settings.

The geographical limitation to Panama might affect the generalizability of the findings to *Cecropia* species in other parts of Latin America

Gaps and Recommendations: Further studies are needed to link the phytochemical diversity observed with specific pharmacological activities and therapeutic efficacy.

More comprehensive investigations into the biological activities of the identified compounds could enhance the medicinal application of these findings.

Number: 61

Article Name: Phytochemical Characterization, Antioxidant Activity, and Cytotoxicity of Methanolic Leaf Extract of *Chlorophytum Comosum* (Green Type) (Thunb.) Jacq

Author: Phytochemical Characterization, Antioxidant Activity, and Cytotoxicity of Methanolic Leaf Extract of *Chlorophytum Comosum* (Green Type) (Thunb.) Jacq .pdf. (n.d.).

Location: Russia

Year: 2022

Main Technique: GC-MS (GAS CHROMATOGRAPHY-MASS SPECTROMETRY)

Secondary Techniques: MALDI-TOF (MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT) MASS SPECTROMETRY FOR ANALYZING THE WATER FRACTION OF THE EXTRACT.

CYTOTOXICITY TESTS USING HELA CELLS TO ASSESS THE ANTICANCER POTENTIAL OF THE WATER FRACTION OF THE EXTRACT.

Aim: To conduct a detailed phytochemical characterization of the methanolic leaf extract of *Chlorophytum comosum* (Green Type).

To assess the antioxidant activities and the cytotoxic effects of the extract's fractions, particularly focusing on their potential use in chemotherapeutic applications

Bioactive Components: Primary phytochemicals such as fatty acids (including linoleic acid and palmitic acid), neophytadiene, and sterols like stigmasterol and γ -sitosterol.

A newly identified compound, 4'-methylphenyl-1C-sulfonyl- β -D-galactoside, found in the extracts

Method Effectiveness: GC-MS provided detailed and reliable data on the phytochemical composition of the extract.

The bioactivity assays effectively demonstrated the antioxidant capacity and cytotoxic potential of the extract fractions

Method Limitation: The cytotoxicity tests showed that while the water fraction was effective against HeLa cells, it did not affect Vero cells, indicating selective cytotoxicity which requires further investigation to ensure safety and efficacy.

The need for more comprehensive in vivo studies to validate the in vitro findings

Gaps and Recommendations: Further studies are required to identify and characterize the unknown constituents of the water fraction.

Expanded research is needed to explore the therapeutic potential and safety of the extract in clinical settings, including its effects on other types of cancer cells.

Number: 62

Article Name: Qualitative Analysis and Componential Differences of Chemical Constituents in Taxilli Herba from Different Hosts by UFLC-Triple TOF-MS/MS

Author: Yuan, J., Li, L., Cai, Z., Wu, N., Chen, C., Yin, S., Liu, S., Wang, W., Mei, Y., Wei, L., Liu, X., Zou, L., & Chen, H. (2021). Qualitative Analysis and Componential Differences of Chemical Constituents in Taxilli Herba from Different Hosts by UFLC-Triple TOF-MS/MS. *Molecules*, 26(21), 6373. <https://doi.org/10.3390/molecules26216373>

Location: China

Year: 2021

Main Technique: ULTRA-FAST LIQUID CHROMATOGRAPHY COUPLED WITH TRIPLE QUADRUPOLE-TIME OF FLIGHT TANDEM MASS SPECTROMETRY (UFLC-TRIPLE TOF-MS/MS)

Secondary Techniques: PARTIAL LEAST SQUARES DISCRIMINANT ANALYSIS (PLS-DA) TO IDENTIFY AND

ANALYZE THE COMPONENTIAL DIFFERENCES IN TH DERIVED FROM VARIOUS HOST PLANTS

Aim: To perform a comprehensive qualitative analysis of the chemical constituents in Taxilli Herba sourced from different host plants.

To distinguish the chemical constituent differences in TH from various hosts, enhancing understanding of its pharmacological variability and aiding in quality control

Bioactive Components: A variety of chemical constituents including flavonoids, phenolic acids, phenylpropanoids, glycosides, and other components.

Specific differential constituents identified among samples from different hosts, such as quercetin 3-O- β -D-glucuronide, quercitrin, and hyperoside

Method Effectiveness: UFLC-Triple TOF-MS/MS provided high-resolution chemical profiling with accurate mass measurements, significantly contributing to the identification of 73 constituents. PLS-DA effectively differentiated TH samples based on their host-derived chemical profiles, aiding in quality control and traceability

Method Limitation: The chemical variability introduced by different host plants complicates the standardization of TH. Challenges

in universally applying findings due to the specificity of local environmental factors influencing host plants

Gaps and Recommendations: Further research is needed to explore the pharmacological implications of the identified chemical differences.

Studies to correlate the chemical diversity with clinical efficacy and safety are required to better utilize TH in traditional medicine.

Number: 63

Article Name: Journal of Pharmaceutical and Biomedical Analysis: Qualitative and quantitative determination of ten alkaloids in traditional Chinese medicine *Corydalis yanhusuo* W.T. Wang by LC-MS/MS and LC-DAD

Author: Ding, B., Zhou, T., Fan, G., Hong, Z., & Wu, Y.
(2007). Qualitative and quantitative determination of ten alkaloids in traditional Chinese medicine *Corydalis yanhusuo* W.T. Wang by LC-MS/MS and LC-DAD. *Journal of Pharmaceutical and Biomedical Analysis*, 45(2), 219–226.
<https://doi.org/10.1016/j.jpba.2007.06.009>

Location: China

Year: 2007

Main Technique: LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY/MASS SPECTROMETRY (LC-MS/MS), LIQUID CHROMATOGRAPHY WITH DIODE-ARRAY DETECTION (LC-DAD)

Secondary Techniques: ELECTROSPRAY IONIZATION (ESI) IN POSITIVE ION MODE TO ENHANCE THE

IONIZATION OF ALKALOIDS FOR MASS SPECTROMETRIC DETECTION

Aim: To develop and validate a robust analytical method for the qualitative and quantitative determination of ten specific alkaloids in *Corydalis yanhusuo* using advanced chromatographic and mass spectrometric techniques.

To provide a reliable tool for assessing the quality of *Corydalis yanhusuo* preparations, contributing to quality control and therapeutic consistency

Bioactive Components: Ten alkaloids, including both tertiary and quaternary alkaloids such as protopine, tetrahydrocolumbamine, glaucine, palmatine, berberine, dehydrocorydaline, tetrahydropalmatine, canadine, corydaline, and tetrahydrocoptisine

Method Effectiveness: The LC-MS/MS and LC-DAD techniques provided high sensitivity and specificity for the detection and quantification of the alkaloids, facilitating precise quality control of the herbal preparations.

The method allowed for the detailed characterization of the alkaloids' structures through mass spectrometric fragmentation patterns.

Method Limitation: The method focuses exclusively on the ten specified alkaloids, potentially overlooking other bioactive compounds present in *Corydalis yanhusuo*.

Reliance on high-quality standards and stringent control of chromatographic conditions to ensure accuracy and reproducibility

Gaps and Recommendations: While the study effectively quantifies and characterizes ten alkaloids, it does not address the full spectrum of potentially bioactive compounds within *Corydalis yanhusuo*. Further studies could expand on the pharmacological implications of the quantified alkaloids and explore their synergistic effects.

Number: 64

Article Name: Structural Characterization of Withanolide Glycosides from the Roots of *Withania somnifera* and Their Potential Biological Activities

Author: Ha, J. W., Yu, J. S., Lee, B. S., Kang, D.-M., Ahn, M.-J., Kim, J. K., & Kim, K. H. (2022). Structural Characterization of Withanolide Glycosides from the Roots of *Withania somnifera* and Their Potential Biological Activities. *Plants*, 11(6), 767.
<https://doi.org/10.3390/plants11060767>

Location: Korea

Year: 2022

Main Technique: LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC/MS) COUPLED WITH HIGH-RESOLUTION ELECTROSPRAY IONIZATION MASS SPECTROMETRY (HR-ESIMS). NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

Secondary Techniques: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY, SPECIFICALLY ¹H AND ¹³C NMR, AND ELECTRONIC CIRCULAR DICHROISM

(ECD) FOR DETERMINING THE STEREOCHEMISTRY OF THE ISOLATED COMPOUNDS. ENZYMATIC HYDROLYSIS TO DERIVE AGLYCONES FROM GLYCOSIDIC FORMS FOR FURTHER ANALYSIS.

ELECTRONIC CIRCULAR DICHROISM (ECD) FOR DETERMINING THE STEREOCHEMISTRY OF THE MOLECULES.

HIGH-RESOLUTION ELECTROSPRAY IONIZATION MASS SPECTROMETRY (HR-ESI-MS) FOR ACCURATE MASS MEASUREMENTS

Aim: The study aimed to identify and characterize new withanolide glycosides from the roots of *Withania somnifera*, evaluate their potential biological activities, and investigate their structural features using advanced analytical techniques. To isolate and structurally characterize withanolide glycosides from the roots of *Withania somnifera*.

To evaluate the potential biological activities of these compounds, including anti-*Helicobacter pylori*, antioxidant, and anti-inflammatory effects.

Bioactive Components: Several withanolide glycosides were identified, including a new compound named withanoside XII, along with other known compounds like withagenin A diglucoside,

withanoside II, and withanoside IV. Withanoside XII (new compound) and its aglycone, withanoside XIIa (new compound).

Withagenin A diglucoside and other previously known withanolide glycosides

Method Effectiveness: The methods used were effective in not only identifying and characterizing the chemical structures of the compounds but also in confirming their bioactive potentials through biological activity assays against *Helicobacter pylori* and in antioxidant tests. The combined use of LC-MS and NMR techniques was highly effective in identifying and characterizing complex withanolide glycosides.

The study successfully linked structural features with potential biological activities, contributing to the understanding of the pharmacological potential of *Withania somnifera*.

Method Limitation: The limitations were not explicitly detailed in the provided excerpts, but such studies typically require extensive validation of the bioactivity results and further in-vivo studies to confirm efficacy. While the chemical analysis was thorough, the biological assays were limited in scope, particularly the weak anti-*Helicobacter pylori* activity observed.

Further in vivo studies and broader bioactivity assessments would be necessary to substantiate the therapeutic potential fully

Gaps and Recommendations: The study suggests further research to explore the full potential of these compounds, particularly their mechanism of action in biological systems and their possible therapeutic applications, which were not fully delineated in this study. The need for more extensive biological testing and clinical studies to confirm the efficacy and safety of these compounds in human health applications.

Further exploration into the molecular mechanisms underlying the observed biological activities could enhance the therapeutic application of these compounds

Number: 65

Article Name: Chemical Characterization and Evaluation of Gastric Antiulcer Properties of the Hydroethanolic Extract of the Stem Bark of *Virola elongata* (Benth.) Warb.

Author: Almeida, G. V. B. D., Arunachalam, K., Balogun, S. O., Pavan, E., Ascêncio, S. D., Soares, I. M., Zanatta, A. C., Vilegas, W., Macho, A., & Oliveira Martins, D. T. D. (2019). Chemical characterization and evaluation of gastric antiulcer properties of the hydroethanolic extract of the stem bark of *Virola elongata* (Benth.) Warb. *Journal of Ethnopharmacology*, 231, 113–124.
<https://doi.org/10.1016/j.jep.2018.11.011>

Location: Brazil

Year: 2019

Main Technique: HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) AND ELECTROSPRAY IONIZATION-MULTIPLE STAGE FRAGMENTATION (ESI-MSN)

Secondary Techniques: DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENTS USING COLORIMETRIC TECHNIQUES

Aim: To investigate the gastroprotective activity of the hydroethanolic extract of *Virola elongata* stem bark and to support its traditional use in Brazilian and Ecuadorian folk medicine for treating stomach pain, indigestions, and gastric ulcers.

Bioactive Components: Phenolic compounds including flavonoids (such as catechin and rutin), quinic acid, 3,3',4-trihydroxystilbene, juruenolide D, and various neolignans

Method Effectiveness: The study demonstrated significant gastroprotective effects of the extract across different acute and chronic ulcer models, attributed to its antioxidant properties and effects on gastric secretion and acidity.

The extract did not show direct antibacterial activity against *Helicobacter pylori* L71

Method Limitation: The lack of direct effect against *Helicobacter pylori* could limit its effectiveness for ulcers driven by this bacterial infection.

Some bioactive components identified may require further isolation and characterization to fully understand their therapeutic potential and safety profiles.

Gaps and Recommendations: Need for further studies to clarify the mechanisms of action at a molecular level and to explore the potential synergistic effects of the identified bioactive compounds.

Clinical trials are required to translate these findings to human therapeutic contexts and validate the traditional claims associated with the use of this plant

Number: 66

Article Name: Qualitative and Quantitative Analysis of *Nyctanthes arborescens* Linn Leaf Extracts by HPTLC

Author: M, K., & Shri, R. (2014). Qualitative and Quantitative Analysis of *Nyctanthes arborescens* Linn leaf extracts by HPTLC. *Pharmacognosy Journal*, 6(3), 117–130.
<https://doi.org/10.5530/pj.2014.3.17>

Location: India

Year: 2014

Main Technique: HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

Secondary Techniques: HPTLC FINGERPRINT, QUALITATIVE ANALYSIS THROUGH COMPARISON OF R_F VALUES, SPOT COLORS, UV ABSORBANCE CHARACTERISTICS, AND FLUORESCENCE.

SPECTROPHOTOMETRIC METHODS TO DETERMINE TOTAL PHENOLICS AND FLAVONOIDS

Aim: To develop and validate HPTLC methods for qualitative and quantitative assessment of *Nyctanthes arbortristis* leaf extracts.

To establish standardization protocols for evaluating the quality of plant materials and commercial formulations containing *Nyctanthes arbortristis*.

Bioactive Components: Marker compounds including β -sitos-terol, β -amyrin, and caffeic acid quantified in various extracts.

Other phytochemicals identified include glycosides, terpenoids, flavonol glycosides, iridoid glycosides, and phenolic compounds

Method Effectiveness: The HPTLC methods provided effective and reliable means for profiling and quantifying specific bioactive compounds in *Nyctanthes arbortristis* extracts.

Enabled detailed analysis and comparison of leaf extracts and commercial formulations, aiding in quality control and standardization

Method Limitation: HPTLC, while precise for specific markers, might not capture all bioactive compounds present, possibly overlooking synergistic effects between non-targeted compounds.

Dependence on proper standardization of procedures to ensure repeatability and accuracy.

Gaps and Recommendations: Additional studies may be required to fully understand the therapeutic potential and safety of these extracts, especially in clinical settings. Broader phytochemical analysis could further elucidate the plant's comprehensive medicinal profile.

Number: 67

Article Name: Phytochemical Profile, Plant Precursors, and Some Properties of Georgian Propolis

Author: Okińczyc, P., Widelski, J., Ciochoń, M., Paluch, E., Bozhadze, A., Jokhadze, M., Mtvarelishvili, G., Korona-Głowniak, I., Krzyżanowska, B., & Kuś, P. M. (2022). Phytochemical Profile, Plant Precursors and Some Properties of Georgian Propolis. *Molecules*, 27(22), 7714. <https://doi.org/10.3390/molecules27227714>

Location: Poland, Georgia (In europe)

Year: 2022

Main Technique: ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIODE-ARRAY DETECTION AND TANDEM MASS SPECTROMETRY (UHPLC-DAD-MS/MS)

Secondary Techniques: DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENT USING COLORIMETRIC METHODS.

EVALUATION OF ANTIMICROBIAL ACTIVITY USING THE KIRBY-BAUER DISC DIFFUSION METHOD

AND DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC).

ANTIOXIDANT ACTIVITY ASSESSMENT USING DPPH AND FRAP ASSAYS

Aim: To characterize the phytochemical composition of Georgian propolis and evaluate its antimicrobial and antioxidant properties.

To identify the plant precursors of Georgian propolis and determine the relationship between its biological activities and plant sources.

Bioactive Components: Flavonoids, phenolic acids, and their esters were the main chemical groups identified. Key compounds include 3-O-pinobanksin acetate, pinocembrin, chrysin, galangin, and pinobanksin

Method Effectiveness: The UHPLC-DAD-MS/MS method effectively identified and quantified a wide range of compounds, providing a detailed phytochemical profile.

The antimicrobial and antioxidant assays demonstrated the potent bioactivity of the propolis extracts, validating their traditional use

Method Limitation: While the methods used were effective in profiling and bioactivity assessment, limitations include potential variability in propolis composition due to different geographic and botanical origins which were not fully explored.

The study primarily focused on propolis from specific regions within Georgia, which might not represent all possible variations found across the country.

Gaps and Recommendations: Further research is needed to explore the full spectrum of propolis types across different regions of Georgia and their respective bioactive properties.

Clinical studies would be beneficial to confirm the therapeutic potential of Georgian propolis in human health applications.

Number: 68

Article Name: Botanical Description, Phytochemistry, Traditional Uses, and Pharmacology of the 'Wonder Plant' *Kalanchoe Pinnata* (Linn.) Pers: An Updated Review

Author: Singh, R. K., Garg, A., & Shrimali, K. (2022). Botanical description, Photochemistry, Traditional Uses, and Pharmacology of the “Wonder Plant” *Kalanchoe Pinnata* (Linn.) Pers: An Updated Review. *Journal of Pharmaceutical and Medicinal Chemistry*, 8(1).

Location: India

Year: 2022

Main Technique: HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC)

Secondary Techniques: SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENTS.

VARIOUS BIOASSAY

Aim: To develop and validate HPTLC methods for the qualitative and quantitative assessment of *Nyctanthes arbortristis* leaf extracts.

To establish standardization protocols for evaluating the quality of plant materials and commercial formulations containing *Nyctanthes arbortristis*

Bioactive Components: Identification of marker compounds including β -sitosterol, β -amyrin, and caffeic acid in different extracts such as n-hexane, ethyl acetate, and methanol extracts.

Method Effectiveness: HPTLC provided effective and reliable profiling and quantification of specific bioactive compounds in *Nyctanthes arbortristis* extracts.

Enabled detailed analysis and comparison of leaf extracts and commercial formulations, aiding in quality control and standardization

Method Limitation: While HPTLC is precise for specific markers, it might not capture all bioactive compounds present, potentially overlooking synergistic effects between non-targeted compounds. Dependence on proper standardization of procedures to ensure repeatability and accuracy.

Gaps and Recommendations: Further studies are needed to fully understand the therapeutic potential and safety of these extracts, especially in clinical settings.

Broader phytochemical analysis could further elucidate the plant's comprehensive medicinal profile.

Number: 69

Article Name: Traditional Uses, Phytochemistry, Pharmacology, Pharmacokinetics, and Quality Control of *Polyporus umbellatus* (Pers.) Fries: A Review

Author: Zhao, Y.-Y. (2013). Traditional uses, phytochemistry, pharmacology, pharmacokinetics and quality control of *Polyporus umbellatus* (Pers.) Fries: A review. *Journal of Ethnopharmacology*, 149(1), 35–48. <https://doi.org/10.1016/j.jep.2013.06.031>

Location: China

Year: 2013

Main Technique: ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY (UPLC-Q-TOF/MS), ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION (ADME)

Secondary Techniques: PHARMACOLOGICAL TESTING FOR DIURETIC, NEPHROPROTECTIVE, ANTI-CANCER, IMMUNO-ENHANCING, HEPATOPROTECTIVE, ANTI-INFLAMMATORY, AND ANTIOXIDATIVE ACTIVITIES

Aim: To review the traditional uses, phytochemical makeup, pharmacological activities, pharmacokinetics, and quality control measures of *Polyporus umbellatus*.

To provide comprehensive insight into the therapeutic potential and safety profiles of this medicinal mushroom

Bioactive Components: Ergosterol, ergone, and various polysaccharides.

Other secondary metabolites such as anthraquinones and nucleosides

Method Effectiveness: Effective identification and characterization of numerous bioactive components, providing insights into their therapeutic properties and mechanisms of action.

Pharmacokinetic studies clarified the pathways of distribution, absorption, metabolism, and excretion of the main steroidal compounds.

Method Limitation: Most pharmacological studies were conducted using crude extracts that were poorly characterized, which may limit the understanding of specific bioactive components' roles.

The research primarily focused on animal studies, with limited clinical trials to confirm the findings in human populations.

Gaps and Recommendations: Need for more detailed clinical studies to validate the efficacy and safety of *Polyporus umbellatus* in humans.

Further exploration of the potential synergistic or antagonistic effects of multi-component mixtures derived from *Polyporus umbellatus* is required to optimize its therapeutic use.

APPENDIX C

Standardization Techniques Herbal Medicine

1. A critical review on drug standardization

Author: Mishra, D. A., & Tiwari, D. K. (n.d.). A CRITICAL REVIEW ON DRUG STANDARDIZATION. World Journal of Pharmaceutical Research.

Location: India

Year: 2022

Standardization Technique: GOOD MANUFACTURING PRACTICES (GMP), PHARMACODYNAMICS, PHARMACOKINETICS, DOSAGE, STABILITY, SHELF-LIFE, TOXICITY EVALUATION, CHEMICAL PROFILING

Secondary Characterization Standardization Techniques: Good Manufacturing Practices (GMP), Pharmacodynamics, pharmacokinetics, dosage, stability, shelf-life, toxicity evaluation, chemical profiling

Aim: Chromatography (TLC, HPLC, GC), Electrophoresis, DNA Fingerprinting, Metabolomic techniques, Differential Pulse Polarography, X-ray Diffraction, Capillary Electrophoresis

Key Findings: To develop reliable, specific, and sensitive quality control methods using a combination of classical and modern instrumental methods of analysis to coordinate between the quality of raw materials, in-process materials, and final products.

Purpose of Standardization: The review highlights the necessity for standardization and quality assurance of herbal medicines,

emphasizing the importance of authenticity, purity, and assay in ensuring consistent quality.

Method Effectiveness: To ensure the quality, safety, and efficacy of herbal medicines through reproducible quality.

Method Limitation: Effective in providing a reproducible quality through the implementation of GMP and various analytical and pharmacological evaluations.

Gaps and Recommendations: Challenges include the inherent variability of natural products and the complexity of herbal formulations.

2. A review of emerging analytical techniques for standardization of herbal medicinal products

Author: Azhar, I., Basheer, E., Javaid, Z., Sohail, K., & Sheikh, M. (n.d.). A REVIEW OF EMERGING ANALYTICAL TECHNIQUES FOR STANDARDIZATION OF HERBAL MEDICINAL PRODUCTS.

Location: Pakistan

Year: 2017

Standardization Technique: CHROMATOGRAPHIC FINGERPRINTING

Secondary Characterization Standardization Techniques: Chromatographic fingerprinting

Aim: UV/DAD, MS, ELSD, LC-DAD/MS

Key Findings: To review emerging analytical techniques for the standardization of herbal medicinal products to ensure their safety, efficacy, and quality

Purpose of Standardization: Emphasizes the importance of comprehensive analytical techniques for addressing the complex chemical nature of herbal medicines and ensuring consistent quality control

Method Effectiveness: To minimize batch-to-batch variation, enhance product reliability, and ensure safety, efficacy, and quality

Method Limitation: Effective in providing comprehensive analysis and ensuring consistency and reliability

Gaps and Recommendations: Complexities in handling diverse and complex herbal matrices; need for extensive validation

3. A review on the Standardization of herbal medicines

Author: Kumari, D. R., & Kotecha, D. M. (2016). A review on the Standardization of herbal medicines. 7(02).

Location: India

Year:2016

Standardization Technique: GOOD MANUFACTURING PRACTICES (GMP), GOOD AGRICULTURAL PRACTICES (GAP)

Secondary Characterization Standardization Techniques: Good Manufacturing Practices (GMP), Good Agricultural Practices (GAP)

Aim: Chromatographic techniques (HPLC-MS, GC-MS), DNA fingerprinting, Pharmacognostic evaluation

Key Findings: To review various standardization techniques for herbal drugs and emphasize their importance in ensuring the safety, efficacy, and quality of herbal medicines.

Purpose of Standardization: The article emphasizes the critical need for stringent standardization protocols to ensure the quality,

safety, and efficacy of herbal medicines, highlighting the role of modern analytical techniques alongside traditional methods.

Method Effectiveness: To ensure consistency, safety, and efficacy across different batches of herbal products, thereby gaining public trust and integrating herbal products into mainstream healthcare.

Method Limitation: Effective in ensuring product safety and consistency, critical for integrating herbal medicines into formal health systems.

Gaps and Recommendations: Complexity in standardizing natural products due to their inherent variability and the sophisticated nature of modern analytical techniques required.

4. Advances in Fingerprint Analysis for Standardization and Quality Control of Herbal Medicines

Author: Noviana, E., Indrayanto, G., & Rohman, A. (2022). Advances in Fingerprint Analysis for Standardization and Quality Control of Herbal Medicines. *Frontiers in Pharmacology*, 13, 853023. <https://doi.org/10.3389/fphar.2022.853023>

Location: Indonesia

Year: 2022

Standardization Technique: FINGERPRINT ANALYSIS (CHEMICAL AND DNA-BASED METHODS)

Secondary Characterization Standardization Techniques: Fingerprint analysis (Chemical and DNA-based methods)

Aim: High-Performance Liquid Chromatography (HPLC), Thin-Layer Chromatography (TLC), Mass Spectrometry (MS), Capillary Electrophoresis, DNA-based methods

Key Findings: To evaluate and discuss the progress and applications of various fingerprinting techniques for the standardization and quality control of herbal medicines

Purpose of Standardization: The review highlights the critical role of fingerprinting techniques in assessing the quality of herbal drug materials and formulations, providing a standardized approach to ensure product consistency.

Method Effectiveness: To ensure reproducible quality, efficacy, and safety of herbal medicines by assessing their complete chemical profiles.

Method Limitation: Highly effective in differentiating between authentic and adulterated herbal medicines and in identifying the origins of plant species.

Gaps and Recommendations: Complexity of data interpretation, high dependency on sophisticated equipment and software for data analysis.

5. An overview of advances in the standardization of herbal drugs

Author: Choudhary, N., & Sekhon, B. S. (2011). An overview of advances in the standardization of herbal drugs. 2(2).

Location: India

Year: 2011

Standardization Technique: GOOD MANUFACTURING PRACTICES (GMP), GOOD AGRICULTURAL PRACTICES (GAP)

Secondary Characterization Standardization Techniques: Good Manufacturing Practices (GMP), Good Agricultural Practices (GAP)

Aim: Chromatographic techniques (HPLC, GC), DNA fingerprinting, Capillary electrophoresis, Polarographic techniques

Key Findings: To review the advances in the extraction and characterization of herbal medicines, including the use of herbal nanomedicines for standardization.

Purpose of Standardization: Advances in extraction methods like microwave-assisted extraction and supercritical phase extraction are significant. The use of various spectroscopic and chromatographic techniques in combination has been effective for herbal drug standardization.

Method Effectiveness: To establish a consistent biological activity, chemical profile, or quality assurance program for production and manufacturing of herbal drugs.

Method Limitation: Effective in enhancing the bioavailability and therapeutic potential of herbal medicines through advanced extraction and standardization techniques.

Gaps and Recommendations: Challenges include the complexity of herbal matrices and the need for sophisticated equipment and methodologies for effective standardization.

6. Analytical Method Development for Quality Control and Standardization of Medicinal Plants: A Critical Review

Author: Analytical Method Development for Quality Control and Standardization of Medicinal Plants: A Critical Review. (n.d.). Research & Reviews: Journal of Herbal Science.

Location: Iran

Year: 2021

Standardization Technique: CHROMATOGRAPHIC METHODS (TLC, GC, HPLC)

Secondary Characterization Standardization Techniques: Chromatographic methods (TLC, GC, HPLC)

Aim: Spectroscopic techniques (UV, MS, FT-IR), biological assays

Key Findings: To discuss and review the different analytical methods used for the standardization and quality control of herbal medicines.

Purpose of Standardization: The review highlights the integration of various analytical techniques, including both chromatographic and spectroscopic methods, to effectively standardize and ensure the quality control of herbal medicines.

Method Effectiveness: To ensure the safety, efficacy, and quality of herbal medicines through comprehensive analytical testing and quality control measures.

Method Limitation: The methods are portrayed as effective in ensuring consistent quality and safety of herbal products.

Gaps and Recommendations: Challenges include the high variability of natural products and the complexity of fully characterizing herbal matrices.

7. Anti-Inflammatory Activity and Quality Control of *Erysimum cheiri* (L.) Crantz

Author: Mosleh, G., Azadi, A., Khademian, S., Heidari, R., & Mohagheghzadeh, A. (2021). Anti-Inflammatory Activity and Quality Control of *Erysimum cheiri* (L.) Crantz. *BioMed Research International*, 2021, 1–12. <https://doi.org/10.1155/2021/5526644>

Location: Iran

Year: 2021

Standardization Technique: PHYSICOCHEMICAL ANALYSIS, INCLUDING ASH VALUES AND LOSS ON DRYING

Secondary Characterization Standardization Techniques: Physicochemical analysis, including ash values and loss on drying

Aim: High-Performance Thin Layer Chromatography (HPTLC), Fourier Transform Infrared (FT-IR) Spectroscopy

Key Findings: To evaluate the anti-inflammatory activity of *Erysimum cheiri* and perform quality control and characterization tests for different organs of the herb.

Purpose of Standardization: The study confirmed strong anti-inflammatory activity of *Erysimum cheiri* compared to diclofenac sodium. It also provided comprehensive physicochemical, HPTLC, and FT-IR profiles of various plant organs, establishing foundational data for quality control.

Method Effectiveness: To ensure consistent quality and establish pharmacopeial standards for *Erysimum cheiri* as a potential therapeutic agent, particularly given its use in traditional Persian medicine.

Method Limitation: Effective in identifying bioactive components such as flavonoids and cardenolides across different plant organs, which are linked to the herb's anti-inflammatory properties.

Gaps and Recommendations: Challenges include the complex nature of herbal matrices and the variability in bioactive component concentrations across different parts of the plant.

8. Breaking the challenge of polyherbal quality control using UV and HPLC fingerprints combined with multivariate analysis

Author: Roshan, A. A., Hathout, R. M., El-Ahmady, S. H., Singab, A. B., & Gad, H. A. (2022). Breaking the challenge of polyherbal quality control using UV and HPLC fingerprints combined with multivariate analysis. *Phytochemical Analysis*, 33(2), 320–330. <https://doi.org/10.1002/pca.3089>

Location: Egypt

Year: 2022

Standardization Technique: MULTIVARIATE ANALYSIS (MVA)

Secondary Characterization Standardization Techniques: Multivariate analysis (MVA)

Aim: Ultraviolet–visible (UV-Vis) spectroscopy, High-Performance Liquid Chromatography (HPLC) with Photo Diode Array detection (PDA)

Key Findings: To propose a simple, reliable, and efficient method for the qualitative and quantitative analysis of a polyherbal product using UV-Vis spectroscopy or HPLC-PDA data combined with multivariate analysis.

Purpose of Standardization: The study demonstrates the reliability and efficiency of using HPLC and UV spectroscopy combined with multivariate statistical analysis for ensuring the consistency of polyherbal preparations.

Method Effectiveness: To ensure the quality and consistency of polyherbal formulations by assessing their complete chemical profiles.

Method Limitation: Highly effective in distinguishing between accepted and unaccepted samples, with excellent selectivity and sensitivity.

Gaps and Recommendations: Complexity in analyzing the diverse and complex nature of polyherbal matrices; relies heavily on sophisticated analytical techniques and data interpretation skills.

9. Chromatographic finger print analysis of budmunchiamines in *Albizia amara* by HPTLC technique

Author: Roshan, A. A., Hathout, R. M., El-Ahmady, S. H., Singab, A. B., & Gad, H. A. (2022). Breaking the challenge of polyherbal quality control using UV and HPLC fingerprints combined with multivariate analysis. *Phytochemical Analysis*, 33(2), 320–330. <https://doi.org/10.1002/pca.3089>

Location: India

Year: 2010

Standardization Technique: HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC)

Secondary Characterization Standardization Techniques: High-Performance Thin-Layer Chromatography (HPTLC)

Aim: UV Spectroscopy, FTIR, ¹H NMR, MASS, CHN Analysis

Key Findings: To develop a simple, rapid, and selective HPTLC method for the separation and determination of budmunchiamines in *Albizia Amara*.

Purpose of Standardization: The HPTLC method provided effective identification and separation of macrocyclic alkaloids budmunchiamines L4 and L5, with the developed fingerprints aiding in the quality control and standardization of herbal formulations.

Method Effectiveness: To ensure the quality consistency and stability of herbal extracts or products by visible observation and comparison of standardized fingerprint patterns.

Method Limitation: The HPTLC method was found to be linear, precise, accurate, and suitable for quality control and standardization of herbal formulations.

Gaps and Recommendations: The limitations were not explicitly discussed, but challenges might include the reliance on precise conditions and the complexity of matching chromatographic profiles.

10. Chromatographic Fingerprint: A Modern Scientific Tool for Standardization of Traditional Medicines

Author: Shukla, S. S., Sharma, V., Gidwani, B., Vyas, A., Dharwal, S. J., & Kumar Pandey, R. (2021). Chromatographic Fingerprint: A Modern Scientific Tool for Standardization of Traditional Medicines. *Research Journal of Pharmacy and Technology*, 4003–4010. <https://doi.org/10.52711/0974-360X.2021.00694>

Location: India

Year: 2021

Standardization Technique: CHROMATOGRAPHIC FINGERPRINTING

Secondary Characterization Standardization Techniques: Chromatographic fingerprinting

Aim: HPLC, TLC, GC, MS

Key Findings: To discuss the utilization of chromatographic fingerprinting as a tool for the standardization and quality control of traditional medicines.

Purpose of Standardization: The article emphasizes the effectiveness of chromatographic fingerprinting in ensuring the quality, safety, and consistency of herbal medicines by providing a detailed chemical profile that represents the therapeutic properties and composition.

Method Effectiveness: To ensure the identity, authenticity, and lot-to-lot consistency of herbal formulations, facilitating better quality control and consumer trust.

Method Limitation: Highly effective in identifying and confirming the chemical constituents of herbal medicines, ensuring that products are consistent and meet quality standards.

Gaps and Recommendations: Complexity in handling diverse herbal matrices and the high dependency on sophisticated equipment for analysis.

11. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug

Author: Pandey, A., & Tripathi, S. (n.d.). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug.

Location: India

Year: 2014

Standardization Technique: PHARMACOPICIAL STANDARDS (SPECIFYING NUMERICAL VALUES FOR STRUCTURAL, ANALYTICAL, AND PHYSICAL PROPERTIES)

Secondary Characterization Standardization Techniques: Pharmacopieal standards (specifying numerical values for structural, analytical, and physical properties)

Aim: Chromatographic methods (TLC, HPLC), physical and chemical parameter assessments

Key Findings: To provide an overview of the standardization, extraction, and phytochemical screening strategies for herbal drugs, aiming to ensure the quality and purity of herbal products.

Purpose of Standardization: Emphasized the need for robust standardization processes including advanced chemical knowledge and physical constants to assure quality and purity of herbal drugs. Various extraction methods discussed offer different advantages and disadvantages depending on the desired outcome.

Method Effectiveness: To confirm the identity, quality, and purity of herbal drugs, ensuring they meet the specified standards for medicinal use.

Method Limitation: Effective in ensuring that herbal drugs meet specific, predefined quality standards through a combination of chemical, botanical, and spectroscopic methods.

Gaps and Recommendations: Variability in herbal drug composition due to natural sources and the challenge of selecting the most appropriate extraction method to maximize the yield and quality of active constituents.

12. Current Status and Major Challenges to the Safety and Efficacy Presented by Chinese Herbal Medicine

Author: Zhou, X., Li, C.-G., Chang, D., & Bensoussan, A. (2019). Current Status and Major Challenges to the Safety and Efficacy Presented by Chinese Herbal Medicine. *Medicines*, 6(1), 14. <https://doi.org/10.3390/medicines6010014>

Location: Australia

Year: 2019

Standardization Technique: IMPLEMENTATION OF STRICTER SAFETY AND QUALITY CONTROL

STANDARDS; DEVELOPMENT OF GLOBAL REGULATORY STANDARDS.

Secondary Characterization Standardization Techniques: Implementation of stricter safety and quality control standards; development of global regulatory standards.

Aim: Chromatography, DNA barcoding, toxicological studies.

Key Findings: To address the major challenges related to the safety and efficacy of Chinese Herbal Medicine (CHM) and to propose methods for improving regulatory, safety, and quality standards.

Purpose of Standardization: The review outlines significant safety risks due to poor quality control, mislabeling, and inherent toxicity of some CHM products. The efficacy of CHM often remains poorly understood or unsupported by rigorous scientific evidence.

Method Effectiveness: To enhance the safety, efficacy, and global acceptance of CHM by aligning it with international safety standards similar to those for Western pharmaceuticals.

Method Limitation: Effective in identifying gaps and proposing frameworks for improving safety and efficacy through enhanced regulations and quality controls.

Gaps and Recommendations: Challenges in integrating traditional CHM practices with modern scientific methodologies and international standards.

13. Development and standardization of novel herbal formula for the management of kidney disease

Author: Movaliya, V., & Zaveri, M. (n.d.). Development and standardization of novel herbal formula for the management of kidney disease.

Location: India

Year: 2019

Standardization Technique: DEVELOPMENT OF HERBAL FORMULATIONS USING GOOD MANUFACTURING PRACTICES (GMP)

Secondary Characterization Standardization Techniques: Development of herbal formulations using Good Manufacturing Practices (GMP)

Aim: HPLC, UV spectroscopy, MTT assay for nephroprotective activity

Key Findings: To develop and standardize a novel herbal formulation for the management of kidney disease, ensuring safety, efficacy, and quality.

Purpose of Standardization: The developed polyherbal formulation showed significant nephroprotective activity, indicating its potential effectiveness in kidney disease management.

Method Effectiveness: To ensure consistent quality and therapeutic efficacy of the herbal formula, and to provide a scientifically validated alternative to conventional treatments for kidney disease.

Method Limitation: The method was effective in creating a standardized formulation that showed promising nephroprotective results in preclinical studies.

Gaps and Recommendations: Challenges include the complexity of scaling up production while maintaining batch-to-batch consistency and ensuring the stability of active constituents.

14. Effect of Storage on the Bioactivity of Drepanoalpha® (An Anti-Sickle Cell Disease Polyherbal Formula) and Comparative Biochemical Profile of Different Batches

Author: Ngbolua, K.-N., Gbolo, B. Z., Tshidibi, J. D., Tshibangu, D. S. T., Memvanga, P. B., & Mpiana, P. T. (2018). Effect of Storage on the Bioactivity of Drepanoalpha® (An Anti-Sickle Cell Disease Polyherbal Formula) and Comparative Biochemical Profile of Different Batches. *Sickle Cell Disease*, 4(4).

Location: Democratic Republic of the Congo

Year: 2018

Standardization Technique: LYOPHILIZATION (FREEZE-DRYING) OF HERBAL INFUSIONS FOR IMPROVED STABILITY AND STANDARDIZATION.

Secondary Characterization Standardization Techniques: Lyophilization (freeze-drying) of herbal infusions for improved stability and standardization.

Aim: Quantitative analysis of phytochemical groups (total polyphenols, flavonoids, anthocyanins) and mineral content (Fe, Mn, Zn).

Key Findings: To assess the impact of storage time on the bioactivity, flavonoid and anthocyanin content, and mineral profile of Drepanoalpha® across different batches.

Purpose of Standardization: The study demonstrated that storage time did not significantly affect the anti-sickling activity, flavonoid content, or mineral profile of the Drepanoalpha® formula. It maintained its efficacy and safety over one to two years of storage at room temperature.

Method Effectiveness: To ensure the consistent therapeutic quality and stability of Drepanoalpha® over time, particularly for use in managing sickle cell disease.

Method Limitation: The freeze-drying process effectively preserved the bioactivity and chemical integrity of Drepanoalpha® across different storage periods.

Gaps and Recommendations: The reliance on correct storage conditions to maintain efficacy and the complexity involved in lyophilizing and reconstituting the herbal formulation.

15. Formulation and Standardization of Herbal Medicinal Products: A Review of the Formulation Considerations, Quality Control and Safety of Herbal Products

Author: Lutoti, S., Okwany, P., Ajayi, C. O., & Oloro, J. (n.d.). Formulation and Standardization of Herbal Medicinal Products: A Review of the Formulation Considerations, Quality Control and Safety of Herbal Products.

Location: Uganda

Year: 2020

Standardization Technique: IMPLEMENTATION OF PHARMACOPEIAL STANDARDS FOR RAW MATERIALS AND FINAL PRODUCT SPECIFICATIONS.

Secondary Characterization Standardization Techniques: Implementation of pharmacopeial standards for raw materials and final product specifications.

Aim: Chromatographic methods (TLC, HPLC), physical and chemical parameter assessments, phytochemical screening.

Key Findings: To discuss the formulation considerations, quality control measures, safety evaluations, and the stability and shelf-life determination of herbal medicinal products.

Purpose of Standardization: The review emphasizes the critical importance of comprehensive quality control measures, standardized formulation processes, and rigorous safety evaluations to ensure the efficacy, safety, and quality of herbal medicines.

Method Effectiveness: To establish and maintain the quality, safety, and efficacy of herbal medicinal products through well-defined standards and rigorous testing protocols.

Method Limitation: Effective in ensuring that herbal products meet defined quality specifications and safety standards, thereby facilitating safer and more reliable herbal treatments.

Gaps and Recommendations: Challenges include the variability in raw materials due to natural sources, difficulty in standardizing complex mixtures, and the requirement for sophisticated analytical techniques.

16. High-Performance Liquid Chromatography (HPLC) as a Tool for Standardization of Complex Herbal Drugs

Author: Govindarajan, R., Tejas, V., & Pushpangadan, P. (2019). High-Performance Liquid Chromatography (HPLC) as a Tool for Standardization of Complex Herbal Drugs. *Journal of AOAC INTERNATIONAL*, 102(4), 986–992. <https://doi.org/10.5740/jaoacint.18-0378>

Location: India

Year: 2019

Standardization Technique: USE OF HPLC FOR THE STANDARDIZATION OF COMPLEX HERBAL DRUGS,

FOCUSING ON QUANTIFICATION AND PROFILING OF ACTIVE COMPONENTS.

Secondary Characterization Standardization Techniques: Use of HPLC for the standardization of complex herbal drugs, focusing on quantification and profiling of active components.

Aim: HPLC-diode array detection (HPLC-DAD), GC-MS, capillary electrophoresis (CE)-DAD, and HPLC-MS.

Key Findings: To review the application of various HPLC techniques for the standardization of complex herbal drugs and their formulations, ensuring quality control.

Purpose of Standardization: HPLC methods provide a powerful tool for the detailed chemical profiling of herbal drugs, allowing for the identification and quantification of bioactive compounds and ensuring product consistency.

Method Effectiveness: To ensure the consistency of biological activity and chemical profiles in complex herbal drugs, thereby supporting quality assurance in production and manufacturing.

Method Limitation: HPLC is effective in creating detailed chemical profiles of complex herbal formulations, essential for quality control and standardization.

Gaps and Recommendations: High dependence on precise HPLC conditions for reproducibility and the complexity of interpreting chromatographic data.

17. Metabolomics of Medicinal Plants - A Versatile Tool for Standardization of Herbal Products and Quality Evaluation of Ayurvedic Formulations

Author: Mukherjee, P. K., Harwansh, R. K., Bahadur, S., Biswas, S., Kuchibhatla, L. N., Tetali, S. D., & Raghavendra, A. S. (2016).

Metabolomics of Medicinal Plants—A Versatile Tool for Standardization of Herbal Products and Quality Evaluation of Ayurvedic Formulations. *Current Science*, 111(10), 1624. <https://doi.org/10.18520/cs/v111/i10/1624-1630>

Location: India

Year: 2016

Standardization Technique: USE OF METABOLOMICS FOR STANDARDIZATION, FOCUSING ON COMPREHENSIVE PROFILING OF SECONDARY METABOLITES.

Secondary Characterization Standardization Techniques: Use of metabolomics for standardization, focusing on comprehensive profiling of secondary metabolites.

Aim: Advanced mass spectrometry-based platforms such as GC-MS and LC-MS for the separation, identification, and quantification of metabolites.

Key Findings: To emphasize the importance of metabolomics for the quality evaluation and scientific validation of herbal products, particularly Ayurvedic formulations.

Purpose of Standardization: Despite the potential use of metabolomics, the data on Indian medicinal plants and spices are extremely limited, and there is a lack of metabolomic studies on common Ayurvedic formulations like Triphala and Trikatu.

Method Effectiveness: To ensure the quality, safety, and efficacy of herbal products through comprehensive metabolite profiling.

Method Limitation: Metabolomics is a powerful tool that provides a detailed and comprehensive analysis of the chemical constituents of medicinal plants, aiding in the standardization and quality control of herbal products.

Gaps and Recommendations: The article highlights the scarcity of data and the need for more comprehensive metabolomic studies of Indian medicinal plants and Ayurvedic formulations.

18. Physicochemical analysis and standardization of andrographolide and gallic acid by rp-hplc in vilocym premix: a herbal veterinary formulation

Author: Kotagiri, R., Sharma, A., Lata, P., & Thakur, D. (n.d). PHYSICOCHEMICAL ANALYSIS AND STANDARDIZATION OF ANDROGRAPHOLIDE AND GALLIC ACID BY RP-HPLC IN VILOCYM PREMIX: A HERBAL VETERINARY FORMULATION. World Journal of Pharmaceutical Research.

Location: India

Year: 2019

Standardization Technique: GOOD MANUFACTURING PRACTICES (GMP)

Secondary Characterization Standardization Techniques: Good Manufacturing Practices (GMP)

Aim: HPLC, UV spectroscopy, MTT assay for nephroprotective activity

Key Findings: To develop and standardize a novel herbal formulation for the management of kidney disease, ensuring safety, efficacy, and quality.

Purpose of Standardization: The developed polyherbal formulation showed significant nephroprotective activity, indicating its potential effectiveness in kidney disease management.

Method Effectiveness: To ensure consistent quality and therapeutic efficacy of the herbal formula, and to provide a scientifically validated alternative to conventional treatments for kidney disease.

Method Limitation: The method was effective in creating a standardized formulation that showed promising nephroprotective results in preclinical studies.

Gaps and Recommendations: Challenges include the complexity of scaling up production while maintaining batch-to-batch consistency and ensuring the stability of active constituents.

19. Pitfalls of the selection of chemical markers for the quality control of medicinal herbs

Author: Chan, S. S.-K., Li, S.-L., & Lin, G. (2020). Pitfalls of the selection of chemical markers for the quality control of medicinal herbs. *Journal of Food and Drug Analysis*, 15(4). <https://doi.org/10.38212/2224-6614.2398>

Location: China

Year: 2007

Standardization Technique: ACTIVE PRINCIPLE

Secondary Characterization Standardization Techniques: Active Principle

Aim: Chemical Fingerprinting, Group Markers

Key Findings: To explore the various categories of chemical markers used in the quality control of medicinal herbs and discuss the pitfalls associated with selecting inappropriate markers.

Purpose of Standardization: The article discusses seven categories of chemical markers, highlighting the complexities and potential issues in selecting appropriate markers for the quality control of

medicinal herbs. It emphasizes the importance of selecting markers that have a direct correlation with the therapeutic effects of the herbs.

Method Effectiveness: To ensure the medicinal herb's clinical efficacy and safety by using chemical markers that accurately represent the herb's therapeutic potential.

Method Limitation: The discussion points out that while active principles are ideal for quality control, they are often not used due to availability issues. The use of other types of markers, like analytical or group markers, can lead to inadequate quality control because they may not correlate with the herb's therapeutic effects.

Gaps and Recommendations: The article emphasizes that the selection of inappropriate markers can lead to significant issues in quality control, such as the inability to predict the clinical efficacy and safety of the herb.

20. Preparation and Standardization of Vaginal Suppository from *Punica granatum* Flower Extract Known as “Golnar” in Persian Medicine

Author: Fayazmanesh, S., Toliyat, T., Eftekhari, M., Amin, G., Hajimahmoodi, M., & Khanavi, M. (2021). Preparation and Standardization of Vaginal Suppository from *Punica granatum* Flower Extract Known as “Golnar” in Persian Medicine. *Indian Journal of Pharmaceutical Education and Research*, 55(3s), S784–S789. <https://doi.org/10.5530/ijper.55.3s.185>

Location: Iran

Year: 2021

Standardization Technique: PREPARATION AND STANDARDIZATION OF A VAGINAL SUPPOSITORY USING GOOD MANUFACTURING PRACTICES (GMP).

Secondary Characterization Standardization Techniques: Preparation and standardization of a vaginal suppository using Good Manufacturing Practices (GMP).

Aim: Physicochemical tests (weight variation, melting point, dissolution test), total phenolic content and tannin assays.

Key Findings: To develop and standardize a vaginal suppository using *Punica granatum* flower extract for its anti-hemorrhagic properties traditionally used in Persian medicine to treat excessive menstrual bleeding.

Purpose of Standardization: The study formulated vaginal suppositories that maintained uniform appearance, consistent weight, and stable melting points. The suppositories effectively released 100% of the extract within 90 minutes of administration, demonstrating the formula's potential as an anti-hemorrhagic agent.

Method Effectiveness: To ensure the suppositories' consistent quality, effectiveness, and safety for treating menorrhagia, leveraging the traditional use of *Punica granatum* in Persian medicine.

Method Limitation: Effective in achieving uniform and stable preparation of vaginal suppositories, confirming the consistent release and bioavailability of active phenolic compounds.

Gaps and Recommendations: Challenges in maintaining the stability of active ingredients throughout the formulation process and limited data on the long-term stability and clinical efficacy of the suppositories.

21. Quality control and standardization of Quercetin in herbal medicines by spectroscopic and chromatographic techniques

Author: Kagawad, P., Gharge, S., Jivaje, K., Hiremath, S. I., & Suryawanshi, S. S. (2021). Quality control and standardization of

Quercetin in herbal medicines by spectroscopic and chromatographic techniques. *Future Journal of Pharmaceutical Sciences*, 7(1), 176. <https://doi.org/10.1186/s43094-021-00327-y>

Location: Iran

Year: 2021

Standardization Technique: USE OF SPECTROSCOPIC AND CHROMATOGRAPHIC TECHNIQUES SPECIFICALLY TAILORED FOR THE ANALYSIS AND QUALITY CONTROL OF QUERCETIN IN HERBAL FORMULATIONS.

Secondary Characterization Standardization Techniques: Use of spectroscopic and chromatographic techniques specifically tailored for the analysis and quality control of Quercetin in herbal formulations.

Aim: UV spectrophotometry, HPLC, HPTLC

Key Findings: To review and summarize various spectroscopic and chromatographic methods used for the qualitative and quantitative analysis of Quercetin in herbal medicines.

Purpose of Standardization: The spectroscopic and chromatographic methods are pivotal in ensuring the quality and standardization of herbal medicines containing Quercetin, helping to determine the purity and concentration of this key flavonoid.

Method Effectiveness: To ensure that herbal products containing Quercetin meet quality standards for safety, efficacy, and consistent pharmacological activity.

Method Limitation: The discussed methods are effective for the detailed and accurate analysis of Quercetin, allowing for reliable standardization and quality control.

Gaps and Recommendations: The review highlights the need for validated and robust analytical methods to handle the complexity of herbal matrices and to accurately quantify Quercetin.

22. Quality Markers of Traditional Chinese Medicine: Concept, Progress, and Perspective

Author: Li, Y., Xie, Y., He, Y., Hou, W., Liao, M., & Liu, C. (2019). Quality Markers of Traditional Chinese Medicine: Concept, Progress, and Perspective. *Engineering*, 5(5), 888–894. <https://doi.org/10.1016/j.eng.2019.01.015>

Location: China

Year: 2019

Standardization Technique: USE OF QUALITY MARKERS (Q-MARKERS) SPECIFICALLY DEVELOPED FOR TRADITIONAL CHINESE MEDICINE (TCM), BASED ON INTRINSIC CHEMICALS RELATED TO THE SAFETY AND EFFICACY OF THE HERBS.

Secondary Characterization Standardization Techniques: Use of quality markers (Q-markers) specifically developed for Traditional Chinese Medicine (TCM), based on intrinsic chemicals related to the safety and efficacy of the herbs.

Aim: Multidisciplinary approaches including conventional phytochemistry, analytical technology, computational approaches, and biosynthetic pathway analysis.

Key Findings: To enhance the understanding and implementation of TCM quality control through the development and application of quality markers, addressing the complexity and multifaceted nature of TCM.

Purpose of Standardization: Emphasizes the need for a more sophisticated quality control system in TCM, which should include the

identification of specific quality markers that correlate with the therapeutic properties and safety of the products.

Method Effectiveness: To ensure the consistency, safety, and effectiveness of TCM products by establishing quality markers that reflect the therapeutic and safety profiles of the herbs.

Method Limitation: The concept of quality markers has shown promise in refining the quality control processes for TCM, enabling a more targeted and scientifically valid assessment of herbal products.

Gaps and Recommendations: The complexity of identifying appropriate quality markers that accurately reflect the therapeutic properties of TCM due to the diverse and complex nature of herbal compositions.

23. Review article: standardization and evaluation of herbal drugs is need of the hour in present era

Author: Chopra, P., & Sindhu, R. K. (2019). REVIEW ARTICLE: STANDARDIZATION AND EVALUATION OF HERBAL DRUGS IS NEED OF THE HOUR IN PRESENT ERA. 1.

Location: India

Year: 2020

Standardization Technique: PHYSICAL ASSESSMENT (IDENTITY AND SCREENING FOR IMPURITIES)

CHEMICAL ASSESSMENT (IDENTIFICATION OF HERB, COLOR REACTION, PRINCIPLES, AND ASSAY)

BIOLOGICAL ASSESSMENT (THERAPEUTIC ACTIVITY DETERMINATION USING BIO-ORGANISMS)

Secondary Characterization Standardization Techniques: Physical Assessment (identity and screening for impurities) Chemical Assessment (identification of herb, color reaction, principles, and assay) Biological Assessment (therapeutic activity determination using bio-organisms)

Aim: Chromatography (including Thin Layer Chromatography, High-performance Liquid Chromatography, High-Performance Thin Layer Chromatography, and Liquid Chromatography-Mass Spectroscopy) DNA Fingerprinting, Radioactive Contamination evaluation, Markers (for calibration and analysis) and NMR Spectroscopy

Key Findings: To evaluate the quality and purity of crude drugs through various standardization techniques and ensure the safety, efficacy, and quality of herbal drugs.

Purpose of Standardization: Various physical, chemical, and biological assessments are vital for determining the purity and efficacy of herbal drugs.

Secondary techniques like chromatography and DNA fingerprinting help in further characterizing and confirming the consistency and quality of herbal materials.

Method Effectiveness: To ensure the safety, efficacy, and quality of herbal drugs.

To reduce adulteration and improve the reliability of herbal products in the market.

Method Limitation: Standardization techniques are effective in identifying and confirming the quality of herbal materials. Helps in minimizing adulteration and enhancing the therapeutic effectiveness of herbal drugs.

Gaps and Recommendations: High complexity and cost involved in some secondary characterization techniques. Dependence on highly specialized equipment and expertise.

24. Role of Markers in the Standardization of Herbal Drugs: A Review

Author: Kushwaha, S. K. S., Kushwaha, N., Maurya, N., & Rai, A. K. (n.d.). Role of Markers in the Standardization of Herbal Drugs: A Review.

Location: India

Year: 2010

Standardization Technique: CHEMICAL FINGERPRINT-
ING FOR QUALITY CONTROL

USE OF SPECIFIC MARKERS FOR QUANTITATIVE
STUDIES

USE OF CHEMICAL MARKERS FOR QUALITY EVAL-
UATION AND STABILITY ASSESSMENT

UTILIZATION OF HPLC-UV FOR DIFFERENTIA-
TION OF HERBAL MEDICINES AND ASSESSMENT OF
PROCESSING METHODS

IDENTIFICATION AND QUANTIFICATION OF
ADULTERANTS AND PROPRIETARY PRODUCTS US-
ING ANALYTICAL AND ACTIVE MARKERS

Secondary Characterization Standardization Techniques:
Chemical fingerprinting for quality control

Use of specific markers for quantitative studies

Use of chemical markers for quality evaluation and stability assessment

Utilization of HPLC-UV for differentiation of herbal medicines and assessment of processing methods

Identification and quantification of adulterants and proprietary products using analytical and active markers

Aim: DNA-based molecular markers for species authentication and differentiation

Polymerase Chain Reaction (PCR)-based methods for genotyping and identifying genetic variation

Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) for qualitative determination of impurities

Gas Chromatography, Column Chromatography, High-Performance Liquid Chromatography, and Spectrophotometric methods for detailed chemical profiling

Key Findings: To enhance the safety and efficacy of herbal medicines through proper quality control measures, using both chemical and DNA markers for standardization.

Purpose of Standardization: Chemical and DNA markers are essential for the standardization and quality control of herbal medicines. Markers assist in various stages of herbal medicine production from raw material selection to final product assessment.

Method Effectiveness: To ensure reproducible quality of herbal medicines which contributes significantly to their safety and efficacy.

To facilitate the integration of modern scientific techniques with traditional knowledge in herbal drug technology.

Method Limitation: Effective in authenticating and ensuring consistent quality of herbal products.

Provides a basis for reproducible therapeutic outcomes.

Gaps and Recommendations: High dependency on environmental and genetic factors which can vary the marker profiles. Challenges in the production and identification of reliable markers due to their sensitivity to conditions like temperature and light.

25. Standardization of herbal drugs

Author: Patil, P. P., Tamboli, D. S. A., Jamadar, M. J., & Chougule, V. B. (n.d.). STANDARDIZATION OF HERBAL DRUGS.

Location: India

Year: 2018

Standardization Technique: VISCOSITY MEASUREMENT
FOR LIQUID DRUGS

MELTING POINT DETERMINATION FOR PURE
PHOTOCHEMICAL CONSTITUENTS

SOLUBILITY TESTS TO DETECT ADULTERANTS

MOISTURE CONTENT AND VOLATILE MATTER
DETERMINATION TO MINIMIZE DEGRADATION

OPTICAL ROTATION TO DETERMINE THE OPTI-
CAL PROPERTIES OF COMPOUNDS

REFRACTIVE INDEX TO VERIFY PURITY AND
COMPOSITION

ASH VALUES AND EXTRACTIVES TO MEASURE
INORGANIC COMPONENTS AND CONTAMINATION
LEVELS

DETERMINATION OF EXTRACTABLE MATTER
THROUGH HOT EXTRACTION AND COLD MACERA-
TION

BITTERNESS VALUE MEASUREMENT TO ASSESS
THERAPEUTIC POTENTIAL AS APPETIZING AGENTS

HEMOLYTIC ACTIVITY TO EVALUATE THE PRES-
ENCE OF SAPONINS

SWELLING INDEX TO DETERMINE THE VOLUME
TAKEN UP BY SWELLING UNDER SPECIFIED CONDI-
TIONS

FOAMING INDEX TO MEASURE SAPONIN CON-
TENT BY THE PERSISTENCE OF FOAM

Secondary Characterization Standardization Techniques: Vis-
cosity measurement for liquid drugs

Melting Point determination for pure photochemical consti-
tuents

Solubility tests to detect adulterants

Moisture Content and Volatile Matter determination to min-
imize degradation

Optical Rotation to determine the optical properties of com-
pounds

Refractive Index to verify purity and composition

Ash Values and Extractives to measure inorganic components and contamination levels

Determination of Extractable Matter through hot extraction and cold maceration

Bitterness Value measurement to assess therapeutic potential as appetizing agents

Hemolytic Activity to evaluate the presence of saponins

Swelling Index to determine the volume taken up by swelling under specified conditions

Foaming Index to measure saponin content by the persistence of foam

Aim: Chemical Examination to detect various phytoconstituents (alkaloids, carbohydrates, glycosides, phytosterols, fixed oils, fats, saponins, phenolic compounds, tannins, proteins, amino acids, gums, mucilage, and volatile oils) Chromatography (TLC, HPTLC, HPLC, GLC) for separation, identification, impurity detection, and assay of herbal drugs in formulations or extracts

Biological and Pharmacological Standardization methods including bioassay and biological standardization for drugs that can't be assayed chemically

Key Findings: To ensure the consistent quality, safety, and therapeutic effectiveness of herbal drugs through comprehensive standardization techniques.

Purpose of Standardization: Effective standardization techniques significantly improve the quality and safety of herbal medicines.

Comprehensive techniques including physical, chemical, and biological methods are necessary for the robust standardization of herbal drugs.

Method Effectiveness: To establish a consistent biological activity or chemical profile.

To assure quality across different batches of production and during manufacturing processes.

Method Limitation: Standardization methods are effective in establishing the quality and safety of herbal drugs, ensuring that each batch of herbal medicine is consistent with the next.

Gaps and Recommendations: Complexity and the requirement for diverse analytical techniques which may not be available in all settings.

Possible variations in results depending on the specific conditions and parameters used during testing.

26. Standardization of Herbal Drugs – A Overview

Author: Umamaheswari, D., Muthuraja, R., Kumar, M., & Venkateswarlu, B. S. (2021). Standardization of Herbal Drugs – A Overview. *International Journal of Pharmaceutical Sciences Review and Research*, 68(1). <https://doi.org/10.47583/ijpsrr.2021.v68i01.033>

Location: India

Year: 2015

Standardization Technique: DETERMINATION OF TOTAL ASH VALUE TO MEASURE INORGANIC MATERIALS NATURALLY FOUND IN OR ADDED TO THE DRUG.

DETERMINATION OF EXTRACTIVE VALUES TO APPROXIMATE THE MEASURE OF CHEMICAL CONSTITUENTS EXTRACTED WITH VARIOUS SOLVENTS.

DETERMINATION OF HEAVY METALS TO ENSURE SAFETY BY QUANTIFYING TOXIC METAL CONTENT.

PESTICIDE RESIDUE ANALYSIS TO DETECT CHEMICALS FROM AGRICULTURAL PRACTICES.

Secondary Characterization Standardization Techniques: Determination of total ash value to measure inorganic materials naturally found in or added to the drug. Determination of extractive values to approximate the measure of chemical constituents extracted with various solvents.

Determination of heavy metals to ensure safety by quantifying toxic metal content.

Pesticide residue analysis to detect chemicals from agricultural practices.

Aim: Morphological or organoleptic evaluation (including the assessment of size, shape, color, odor, taste, and texture).

Microscopic evaluation to identify and authenticate herbal drugs based on histological characteristics.

Chemical evaluation including identification tests for constituents like volatile oils, balsams, resins, and gums.

Key Findings: To ensure the quality, purity, and identity of herbal drugs through comprehensive standardization processes.

Purpose of Standardization: Effective standardization is crucial for maintaining the therapeutic efficacy and safety of herbal medicines.

A combination of morphological, microscopic, and chemical evaluations is essential for thorough standardization.

Method Effectiveness: To confirm the identity and enhance the quality and purity of herbal drugs.

To provide consistent therapeutic efficacy and safety in herbal medicine use.

Method Limitation: Standardization techniques effectively ensure the reproducibility and reliability of herbal medicines by verifying their active ingredients and eliminating contaminants.

Gaps and Recommendations: Complexity and variability in herbal compositions require diverse and rigorous testing methods, which can be resource-intensive.

Some standardization methods may not cover all possible contaminants or active compounds due to the vast diversity of herbal materials.

27. Standardization of herbal medicines - A review

Author: Kunle. (2012). Standardization of herbal medicines—A review. *International Journal of Biodiversity and Conservation*, 4(3). <https://doi.org/10.5897/IJBC11.163>

Location: Nigeria, China

Year: 2012

Standardization Technique: PHYSICAL EVALUATION: INCLUDING DETAILED BOTANICAL, MACROSCOPIC, AND MICROSCOPIC DESCRIPTIONS TO ENSURE IDENTITY AND PURITY.

QUALITY CONTROL AND STANDARDIZATION OF HERBAL MEDICINES: SETTING STANDARDS FOR INHERENT CHARACTERISTICS AND PARAMETERS TO ASSURE QUALITY, EFFICACY, SAFETY, AND REPRODUCIBILITY.

DETERMINATION OF FOREIGN ORGANIC MATTER, TOTAL ASH VALUE, AND MOISTURE CONTENT TO ESTABLISH PURITY AND QUALITY.

CONTROL OF STARTING MATERIAL, FOCUSING ON AUTHENTICATION AND ENSURING REPRODUCIBILITY OF HERBAL INGREDIENTS.

Secondary Characterization Standardization Techniques: Physical evaluation: Including detailed botanical, macroscopic, and microscopic descriptions to ensure identity and purity.

Quality control and standardization of herbal medicines: Setting standards for inherent characteristics and parameters to assure quality, efficacy, safety, and reproducibility.

Determination of foreign organic matter, total ash value, and moisture content to establish purity and quality.

Control of starting material, focusing on authentication and ensuring reproducibility of herbal ingredients.

Aim: Microscopic evaluation to authenticate herbal materials and identify any adulterants or impurities. Chemical evaluation involving the identification and quantification of phytochemical constituents.

Biological evaluation through bioassays to standardize and evaluate potency and safety.

Analytical methods such as chromatography (HPTLC, HPLC, GC) and spectroscopy to ensure consistent quality and potency.

Key Findings: To establish quality parameters for the collection, handling, processing, and production of herbal medicines and employ these parameters to ensure the safety of the global herbal market.

Purpose of Standardization: The article emphasizes the need for rigorous standardization and quality control measures to ensure the safety, efficacy, and quality of herbal medicines globally.

It discusses the variability and challenges in herbal medicine quality due to genetic, cultural, and environmental factors.

Method Effectiveness: To ensure that herbal medicines are safe, effective, and of consistent quality.

To provide assurance of quality, efficacy, and safety through reproducible processes.

Method Limitation: The standardization processes described are effective in controlling the quality of herbal medicines, ensuring that they meet predefined standards for safety and efficacy.

Gaps and Recommendations: Variability in herbal medicine constituents due to natural and external factors makes standardization challenging.

The active principles of many herbal medicines are unknown, complicating the establishment of standardization parameters.

28. Standardization of Poly-herbal Formulations: A Comprehensive Review

Author: Santosh, J., & Jyotiram, S. (2016). Standardization of Poly-herbal Formulations: A Comprehensive Review. *Research Journal of Pharmacognosy and Phytochemistry*, 8(2), 85.
<https://doi.org/10.5958/0975-4385.2016.00016.9>

Location: India

Year: 2016

Standardization Technique: GOOD MANUFACTURING PRACTICES (GMPS) ADHERENCE

RATIONALIZING COMBINATIONS IN POLY-HERBAL DRUGS

QUALITY CONTROL MEASURES THROUGHOUT THE PRODUCTION PROCESS INCLUDING HARVESTING, EXTRACTION, AND PURIFICATION

IMPLEMENTATION OF STANDARD OPERATING PROCEDURES FOR MANUFACTURING PROCESSES

Secondary Characterization Standardization Techniques: Good Manufacturing Practices (GMPs) adherence

Rationalizing combinations in polyherbal drugs

Quality control measures throughout the production process including harvesting, extraction, and purification

Implementation of standard operating procedures for manufacturing processes

Aim: Pharmacognostic evaluation: Analyzing color, odor, taste, texture, size, shape, microscopical characters, and histological parameters

Physico-chemical parameters: Evaluating foreign matter, ash values, moisture content, viscosity, pH, and other physical characteristics

Chemical parameters: Chemical tests for assessing potency and identifying active principles

Chromatographic and spectroscopic analysis: Utilizing methods like TLC, HPLC, HPTLC, GC, UV, IR, FT-IR, AAS, LC-MS, and GC-MS for detailed analysis of components

Key Findings: To improve the standardization of poly-herbal formulations, ensuring safety, efficacy, and quality in line with global standards.

Purpose of Standardization: Standardization of poly-herbal formulations is complex due to the variability of plant constituents.

Advanced analytical techniques are crucial for thorough standardization.

Implementing comprehensive standardization processes is essential for validating the safety and efficacy of poly-herbal drugs.

Method Effectiveness: To guarantee the purity, safety, potency, and efficacy of poly-herbal medicines.

To establish reliable quality control profiles for herbal materials and their formulations.

Method Limitation: The described standardization methods are effective in ensuring consistent and reliable quality of poly-herbal formulations.

These methods help in minimizing variability and ensuring the reproducibility of therapeutic effects.

Gaps and Recommendations: Complexity and high variability in herbal compositions present challenges in standardization.

Difficulty in defining active components for some herbs complicates the establishment of clear standards.

29. Traditional Approaches towards Standardization of Herbal Medicines -A Review

Author: Patra, K. C., Pareta, S. K., Harwansh, R. K., & Kumar, K. J. (2010). Traditional Approaches towards Standardization of Herbal Medicines -A Review. 2.

Location: India

Year: 2010

Standardization Technique: PROTOCOL-BASED STANDARDIZATION (STANDARDIZATION OF SINGLE PLANT MATERIAL, SOP OF PREPARATION OF EXTRACTS, STANDARDIZATION OF PLANT EXTRACT, SOP OF FINISHED PRODUCT, STANDARDIZATION OF FORMULATIONS)

TESTS FOR HEAVY/TOXIC METALS (LEAD, CADMIUM, MERCURY, ARSENIC)

MICROBIAL CONTAMINATION TESTING (TOTAL VIABLE AEROBIC COUNT, TOTAL ENTEROBACTERIACEAE, AND TOTAL FUNGAL COUNT)

SPECIFIC PATHOGEN TESTING (E. COLI, SALMONELLA SPP., S. AUREUS, PSEUDOMONAS AERUGINOSA)

PESTICIDE RESIDUE ANALYSIS (DDT, HCH, ENDOSULFAN, ALDERIN, MALATHION, AND PARATHION)

TEST FOR AFLATOXINS (B1, B2, G1, G2)

CHELATING AGENT TESTING (FOR BHASMA, LEPA, ASWARISTA, ETC.)

STABILITY ASSESSMENT AND SHELF LIFE TESTING

SAFETY ASSESSMENT; DOCUMENTATION OF SAFETY BASED ON EXPERIENCE OR TOXICOLOGICAL STUDIES

EFFICACY ASSESSMENT BY ETHNOMEDICINAL INFORMATION AND BIOLOGICAL ACTIVITY EVALUATION

Secondary Characterization Standardization Techniques: Protocol-based standardization (Standardization of Single Plant Material, SOP of Preparation of Extracts, Standardization of Plant Extract, SOP of Finished Product, Standardization of Formulations)

Tests for heavy/toxic metals (Lead, Cadmium, Mercury, Arsenic) Microbial contamination testing (total viable aerobic count, total Enterobacteriaceae, and total fungal count)

Specific pathogen testing (E. coli, Salmonella spp., S. Aureus, Pseudomonas aeruginosa)

Pesticide residue analysis (DDT, HCH, Endosulfan, Alderin, Malathion, and Parathion)

Test for aflatoxins (B1, B2, G1, G2)

Chelating agent testing (for bhasma, lepa, Aswarista, etc.)

Stability assessment and shelf life testing

Safety assessment; documentation of safety based on experience or toxicological studies

Efficacy assessment by ethnomedicinal information and biological activity evaluation

Aim: Chromatographic methods (HPLC, TLC, GC, capillary electrophoresis)

Spectroscopic methods (IR, NMR, UV)

DNA fingerprinting for species authentication

Use of marker compounds for identification and setting specifications

Hyphenated techniques (HPLC-MS, GC-MS, etc.)

Key Findings: To integrate modern scientific techniques with traditional knowledge to improve the quality control and standardization of herbal medicines, ensuring their safety, efficacy, and quality.

Purpose of Standardization: The complexity and holistic nature of traditional Indian medicine require comprehensive and integrative standardization methods.

Emphasis on both qualitative and quantitative methods is necessary to address the multi-target and multi-channel characteristics of herbal medicines.

Method Effectiveness: To assure the quality, safety, and efficacy of herbal medicines.

To establish reliable and reproducible quality control profiles for herbal medicines.

Method Limitation: The described methods are effective in providing a systematic approach to the standardization of herbal medicines, incorporating both traditional and modern scientific techniques.

Gaps and Recommendations: The inherent variability in herbal medicine components makes standardization challenging.

Difficulty in fully capturing the holistic properties of complex herbal systems through conventional methods.

30. Who (world health organization) guidelines for standardization of herbal drugs

Author: Pradhan, N., Gavali, J., & Waghmare, N. (2015). WHO (WORLD HEALTH ORGANIZATION) GUIDELINES FOR STANDARDIZATION OF HERBAL DRUGS. 3(8).

Location: India

Year:2015

Standardization Technique: SENSORY EVALUATION
(VISUAL MACROSCOPY, COLOR, ODOR, TASTE, FRACTURE)

DETERMINATION OF FOREIGN MATTER (ORGANIC AND INORGANIC)

DETERMINATION OF ASH VALUES (TOTAL ASH, ACID-INSOLUBLE ASH, WATER-SOLUBLE ASH)

DETERMINATION OF EXTRACTIVE VALUES
(COLD MACERATION, HOT EXTRACTION, AND ETHANOL EXTRACTION)

DETERMINATION OF MOISTURE CONTENT AND VOLATILE MATTER

PHARMACOLOGICAL PARAMETERS SUCH AS BITTERNESS VALUE, HAEMOLYTIC ACTIVITY, AND ASTRINGENT PROPERTIES

Secondary Characterization Standardization Techniques: Sensory evaluation (visual macroscopy, color, odor, taste, fracture)

Determination of foreign matter (organic and inorganic)

Determination of ash values (total ash, acid-insoluble ash, water-soluble ash)

Determination of extractive values (cold maceration, hot extraction, and ethanol extraction)

Determination of moisture content and volatile matter

Pharmacological parameters such as bitterness value, haemolytic activity, and astringent properties

Aim: Microscopy for identification of histological characters

Chromatographic fingerprinting (HPTLC, HPLC/Densitometric chromatography, GLC, TLC) for chemical profiling

Measurement of swelling and foaming indexes

Toxicological testing for heavy metals, pesticide residues, microbial contamination, and aflatoxins

Key Findings: To standardize herbal drugs through a combination of traditional and modern scientific techniques to ensure their quality, efficacy, and safety.

Purpose of Standardization: WHO guidelines facilitate the standardization of herbal drugs by providing a structured approach to evaluating their quality through various biochemical, microbiological, and pharmacological tests.

The complexity and variability of herbal drugs necessitate comprehensive and robust standardization to ensure safety and therapeutic efficacy.

Method Effectiveness: To ensure the safety, efficacy, and consistent quality of herbal drugs.

To support the reliable use of herbal medicines in healthcare by providing standardized procedures for their evaluation.

Method Limitation: The WHO guidelines for standardization effectively address the complex nature of herbal drugs by incorporating diverse analytical techniques to assess their quality and safety.

Gaps and Recommendations: Variability in natural constituents due to geographical and environmental factors poses challenges to standardization.

Batch-to-batch variation in plant compounds can impact the consistency of herbal drugs.

31. Application of Metabolomics in the Diagnosis of Non-Alcoholic Fatty Liver Disease and the Treatment of Traditional Chinese Medicine

Author: Kim, J. H., Jung, E. J., Lee, Y. J., Kim, C. Y., & Jeon, J.-S. (2021). Application of Linear Gradient Solvent System in Centrifugal Partition Chromatography Facilitating Bioassay-Guided Fractionation of Yongdamsagan-Tang, Traditional Oriental Decoction. Evidence-Based Complementary and Alternative Medicine, 2021, 1–11. <https://doi.org/10.1155/2021/7552169>

Location: China

Year: 2022

Standardization Technique: THE ARTICLE MAINLY DISCUSSES THE USE OF METABOLOMICS IN THE DIAGNOSIS

Secondary Characterization Standardization Techniques: The article mainly discusses the use of metabolomics in the diagnosis

Aim: Ultra-performance liquid chromatography-orbitrap mass spectrometry (UPLC-Orbitrap MS)

Ultra-high-performance liquid chromatography-quadrupole time-of-flight tandem mass spectrometry (UPLC-Q/TOF-MS)

Direct infusion-tandem mass spectrometry (DI-MS/MS)

Key Findings: To review the use of metabolomics in the diagnosis and treatment of non-alcoholic fatty liver disease (NAFLD), particularly in the context of traditional Chinese medicine (TCM)

Purpose of Standardization: Metabolomics can identify biomarkers that reflect the metabolic state of the body more accurately than traditional biomarkers, allowing for early diagnosis and better understanding of the progression of NAFLD.

TCM combined with metabolomics provides new insights into therapeutic mechanisms, potentially improving treatment strategies for NAFLD.

Method Effectiveness: The article aims to improve the diagnosis and therapeutic monitoring of NAFLD using metabolomics to standardize the identification of metabolic biomarkers

Method Limitation: Metabolomics is effective in identifying potential biomarkers for NAFLD and understanding the disease's progression and response to treatment.

Gaps and Recommendations: Challenges include the variability of metabolomics results depending on the biological samples and methods used.

There is still a need for clinical validation of biomarkers identified through metabolomics

32. *Aconitum carmichaelii* Debeaux: A Systematic Review on Traditional Use, and the Chemical Structures and Pharmacological Properties of Polysaccharides and Phenolic Compounds in the Roots

Author: Fu, Y.-P., Zou, Y.-F., Lei, F.-Y., Wangenstein, H., & Inngjerdingen, K. T. (2022). *Aconitum carmichaelii* Debeaux: A systematic review on traditional use, and the chemical structures and pharmacological properties of polysaccharides and phenolic

compounds in the roots. Journal of Ethnopharmacology, 291, 115148. <https://doi.org/10.1016/j.jep.2022.115148>

Location: China, Switzerland

Year: 2022

Standardization Technique: THE REVIEW DOES NOT SPECIFY DIRECT STANDARDIZATION TECHNIQUES OUTSIDE OF CHARACTERIZATION, FOCUSING INSTEAD ON THE DESCRIPTION AND ANALYSIS OF CHEMICAL COMPOUNDS AND PHARMACOLOGICAL PROPERTIES

Secondary Characterization Standardization Techniques: The review does not specify direct standardization techniques outside of characterization, focusing instead on the description and analysis of chemical compounds and pharmacological properties

Aim: UPLC-Q/TOF-MS (Ultra-Performance Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry) for phytochemical profiling, particularly targeting polysaccharides and phenolic compounds.

Various phytochemical extraction and isolation methods discussed broadly without specification in the excerpt provided.

Key Findings: To provide a comprehensive and systematic review of the traditional use, phytochemical, and pharmacological studies of *Aconitum carmichaelii*, focusing on non-alkaloid natural products such as polysaccharides and phenolic compounds

Purpose of Standardization: Identification of different types of polysaccharides, both neutral and acidic, and 39 phenolic compounds like flavonoids, phenylpropanoids, lignans, neolignans, and benzoic acid derivatives from the roots.

Pharmacological studies on these compounds showed varied biological effects such as hypoglycemic, hypolipidemic, cardiovascular, immunomodulatory, anti-tumor, and neuropharmacological activities.

Phenolic compounds studies were limited compared to polysaccharides.

Method Effectiveness: To document and analyze the phytochemical constituents of *Aconitum carmichaelii* for better understanding and potential therapeutic applications, ensuring consistency and reliability in research and use.

Method Limitation: Effective in cataloging and summarizing a wide range of chemical compounds and their potential effects.

Provided a detailed analysis of polysaccharides and their structure-activity relationships.

Gaps and Recommendations: Limited detailed discussion on standardization processes outside of chemical profiling.

More extensive pharmacological studies are needed to substantiate many of the biological activity claims.

33. Standardization of Poly-herbal Formulations: A Comprehensive Review

Author: Santosh, J., & Jyotiram, S. (2016). Standardization of Poly-herbal Formulations: A Comprehensive Review. *Research Journal of Pharmacognosy and Phytochemistry*, 8(2), 85.
<https://doi.org/10.5958/0975-4385.2016.00016.9>

Location: India

Year: 2016

Standardization Technique: THE STANDARDIZATION PROCESS FOR POLY-HERBAL FORMULATIONS

INVOLVES ESTABLISHING QUALITY CONTROL MEASURES RIGHT FROM THE IDENTIFICATION OF PLANTS, COLLECTION SEASON AND AREA, EXTRACTION AND PURIFICATION PROCESSES, AND RATIONALIZING THE COMBINATION OF POLY-HERBAL DRUGS.

DEVELOPMENT OF STANDARD OPERATING PROCEDURES (SOPS) FOR MANUFACTURING PROCESSES TO DEVELOP PHARMACOPEIAL STANDARDS FOR AYURVEDIC PREPARATIONS

Secondary Characterization Standardization Techniques: The standardization process for poly-herbal formulations involves establishing quality control measures right from the identification of plants, collection season and area, extraction and purification processes, and rationalizing the combination of poly-herbal drugs.

Development of standard operating procedures (SOPs) for manufacturing processes to develop pharmacopeial standards for Ayurvedic preparations

Aim: Pharmacognostic evaluation includes color, odor, taste, texture, size, shape, microscopical characters, and histological parameters.

Physico-chemical parameters include foreign matter, total ash, acid-insoluble ash, swelling and foaming index, assay, successive extractive values, moisture content, viscosity, pH, disintegration time, friability, hardness, and flow capacity.

Chemical parameters cover screening, isolation, identification, and purification of chemical components through tests like color reactions, limit tests, and chemical tests.

Chromatographic and spectroscopic analysis using techniques like TLC, HPLC, HPTLC, GC, UV, IR, FT-IR, AAS, LC-MS, GC-MS, and fluorimetry.

Microbiological parameters include evaluations of pharmacological activity and bioassays indicating the strength of the drug or their preparations.

Key Findings: To enhance the quality, safety, potency, and efficacy of poly-herbal formulations through the establishment of robust standardization and quality control measures

Purpose of Standardization: Standardization of herbal drugs is challenging due to the complex nature and variability of plant-based drugs. Modern analytical techniques are crucial in overcoming these challenges.

Quality control of crude drugs and herbal formulations is essential for their acceptance in modern systems of medicine

Method Effectiveness: nan

Method Limitation: Effective application of modern analytical and traditional methods provides a comprehensive approach to the standardization of poly-herbal formulations, ensuring consistency and therapeutic efficacy.

Gaps and Recommendations: The inherent variability in plant constituents and the complex interactions in poly-herbal formulations pose significant challenges in establishing standardized quality control parameters

34. Post-Harvest Processing Methods Have Critical Roles in the Contents of Active Ingredients of *Scutellaria*

baicalensis Georgi

Author: Zhang, L., Zhang, X., & Liang, Z. (2022). Post-Harvest Processing Methods Have Critical Roles in the Contents of Active Ingredients of *Scutellaria baicalensis* Georgi. *Molecules*, 27(23), 8302. <https://doi.org/10.3390/molecules27238302>

Location: China

Year: 2022

Standardization Technique: THE ARTICLE MAINLY DISCUSSES THE EFFECTS OF POST-HARVEST PROCESSING METHODS ON THE ACTIVE INGREDIENTS OF SCUTELLARIA BAICALENSIS, WITHOUT SPECIFICALLY DETAILING A STANDARDIZATION TECHNIQUE OUTSIDE OF THESE PROCESSING METHOD

Secondary Characterization Standardization Techniques: The article mainly discusses the effects of post-harvest processing methods on the active ingredients of *Scutellaria baicalensis*, without specifically detailing a standardization technique outside of these processing method

Aim: High-performance liquid chromatography (HPLC) to evaluate the contents of active ingredients like baicalin, baicalein, wogonoside, and wogonin in different processing scenario

Key Findings: To determine the best post-harvest processing method for *Scutellaria baicalensis* by exploring the effects of fresh and traditional processing on its active ingredients and evaluating different drying techniques.

Purpose of Standardization: Fresh processing methods preserved the four active ingredients better compared to traditional processing methods.

Among the drying techniques, sun drying and shade drying methods had more similar results, while oven drying grouped differently in cluster analysis.

Fresh processing could reduce moisture content effectively and was suggested as an optimal post-harvest processing solution under sun drying conditions

Method Effectiveness: To optimize post-harvest processing in a way that maximizes the retention of active ingredients in *Scutellaria baicalensis*, enhancing the quality and efficacy of the herb.

Method Limitation: The use of HPLC allowed for precise quantification of active ingredients and effectiveness assessment of different post-harvest processing methods

Gaps and Recommendations: Dependency on specific environmental conditions for different drying methods which may not be consistently replicable across different settings or scales

35. *Pistacia atlantica* Desf.: A review of its traditional uses, phytochemicals and pharmacology

Author: Mahjoub, F., MD, Ph.D candidate, Department of Persian medicine, School of Persian and complementary medicine, Mashhad University of Medical Sciences, Mashhad, Iran, Akhavan Rezayat, K., Assistant Professor, MD, Department of Internal Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, Yousefi, M., Assistant Professor, MD, Ph.D, Department of Persian Medicine, School of Persian and Complementary Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, Mohebbi, M., Assistant Professor, MD, Endocrine research center, Mashhad University of Medical Sciences, Mashhad, Iran, Salari, R., Assistant Professor of Drug Control, Ph.D, Department of Pharmaceutical Sciences in Persian Medicine, School of Persian and complementary medicine, Mashhad University of Medical Sciences, Mashhad, Iran, & Prof. Roshanak Salari (Pharm.D, Ph.D) Address: School of complementary and Persian medicine, Azadi square, Mashhad, Iran. E-mail: salarir@mums.ac.ir Tel: +98-51-38829279, Fax: +98-51-38535980. (2018). *Pistacia atlantica* Desf. A review of its traditional uses, phytochemicals and pharmacology. *Journal of Medicine and Life*, 11(3), 180–186. <https://doi.org/10.25122/jml-2017-0055>

Location: Iran

Year: 2018

Standardization Technique: STANDARDIZATION TECHNIQUES WERE NOT EXPLICITLY DETAILED IN THE PROVIDED CONTENT, FOCUSING INSTEAD ON THE REVIEW OF TRADITIONAL USES AND PHARMACOLOGICAL PROPERTIES

Secondary Characterization Standardization Techniques: Standardization techniques were not explicitly detailed in the provided content, focusing instead on the review of traditional uses and pharmacological properties

Aim: Chemical characterization using GC (Gas Chromatography) and GC/MS (Gas Chromatography/Mass Spectrometry) for identifying essential oils and other chemical components.

Phytochemical analysis to identify various chemical entities, including terpenoids, phenolic compounds, fatty acids, and sterols.

Key Findings: To review the traditional uses, phytochemical composition, and pharmacological properties of *Pistacia atlantica*, highlighting its potential applications in modern medicine

Purpose of Standardization: *Pistacia atlantica* has a wide range of pharmacological activities, including antimicrobial, antioxidant, antihyperlipidemic, and anticancer effects.

The plant is rich in various bioactive compounds, such as terpenoids and phenolic compounds, which contribute to its diverse therapeutic properties

Method Effectiveness: The purpose of standardization highlighted in the article is to ensure the quality, safety, and efficacy of *Pistacia atlantica* derivatives used in herbal medicine

Method Limitation: GC and GC/MS were effective in identifying and quantifying the essential oils and bioactive compounds in *Pistacia atlantica*, providing a basis for its pharmacological applications.

Phytochemical analysis was crucial in elucidating the active constituents responsible for the observed pharmacological activities.

Gaps and Recommendations: The complexity of phytochemical profiles and variability in natural product composition pose challenges in standardization.

Limited data on the long-term efficacy and safety of these bioactive compounds in human clinical settings.

36. Chemical Markers for the Quality Control of Herbal Medicines: An Overview

Author: Li, S., Han, Q., Qiao, C., Song, J., Lung Cheng, C., & Xu, H. (2008). Chemical markers for the quality control of herbal medicines: An overview. *Chinese Medicine*, 3(1), 7.
<https://doi.org/10.1186/1749-8546-3-7>

Location: China

Year: 2008

Standardization Technique: ACTIVE MARKERS CONTRIBUTE TO CLINICAL EFFICACY; ANALYTICAL MARKERS HAVE NO CLINICAL OR PHARMACOLOGICAL ACTIVITIES; NEGATIVE MARKERS DEMONSTRATE ALLERGENIC OR TOXIC PROPERTIES. ALL MARKERS MAY CONTRIBUTE TO THE EVALUATION, STANDARDISATION AND SAFETY ASSESSMENT OF HERBAL MEDICINES

Secondary Characterization Standardization Techniques: active markers contribute to clinical efficacy; analytical markers have no clinical or pharmacological activities; negative markers demonstrate

allergenic or toxic properties. All markers may contribute to the evaluation, standardization and safety assessment of herbal medicines

Aim: Use of various chromatographic techniques (GC-FID, HPLC-UV, HPLC-ELSD, HPLC-MS) to identify and quantify chemical markers

Key Findings: To review the effective use of chemical markers in the quality control of herbal medicines and discuss the selection criteria considering the roles and physicochemical factors which may affect the use of these markers

Purpose of Standardization: Chemical makers are crucial for ensuring the quality of herbal medicines through the authentication of genuine species, evaluation of post-harvest handling, assessment of intermediates and finished products, and detection of harmful or toxic ingredients.

The effectiveness of quality control depends heavily on the selection of appropriate chemical markers

Method Effectiveness: nan

Method Limitation: The use of chemical markers is effective for quality control as it allows for the identification and quantification of specific bioactive, toxic, or general components within a herbal medicine

Gaps and Recommendations: Difficulty in identifying specific therapeutic components in many herbal medicines due to the complexity of their chemical profiles.

Some markers are not specific to a particular herb, which can lead to challenges in standardization and quality control

37. Polyherbal and Submerge Fermented Medicines of Ayurveda: Convergence of Tradition with Scientific Trends and Needs

Author: Sekar, S., & Vinothkanna, A. (2019). Polyherbal and submerge fermented medicines of Ayurveda: Convergence of tradition with scientific trends and needs. *South African Journal of Botany*, 121, 410–417. <https://doi.org/10.1016/j.sajb.2018.12.009>

Location: India

Year: 2018

Standardization Technique: DETAILED DESCRIPTION OF THE FERMENTATION PROCESS AND THE ROLE OF SPECIFIC FLOWERS

Secondary Characterization Standardization Techniques: Detailed description of the fermentation process and the role of specific flowers

Aim: To establish a consistent quality and efficacy of Arishta and Asava, facilitating their acceptance in global healthcare settings

Key Findings: To bridge the gap between traditional Ayurvedic medicinal practices and modern scientific methodologies.

To explore and document the scientific principles underlying the preparation and therapeutic use of Arishta and Asava.

Purpose of Standardization: Various phytochemicals extracted through the fermentation process, such as flavonoids and phenolics, which are crucial for the therapeutic properties of these formulations

Method Effectiveness: nan

Method Limitation: The review method effectively compiles and presents scattered traditional knowledge alongside modern research findings, facilitating a comprehensive understanding of the subject

Gaps and Recommendations: Reliance on existing literature which may have varying levels of scientific rigor and detail.

Limited direct experimental data on the pharmacokinetics and dynamics of the bioactive components involved.

38. Comparative Chemical Profiling of a Polyherbal Formulation with Respect to its Ingredient Plants Using Spectrophotometric and High-performance Thin-layer Chromatographic Techniques

Author: Sulaiman, C., Shafna, T., Advaya, G. R., Ramesh, P. R. V., Mahesh, K. P., Praveen, M., Anandan, E. M., & Balachandran, I. (2023). Comparative Chemical Profiling of a Polyherbal Formulation with Respect to its Ingredient Plants Using Spectrophotometric and High-performance Thin-layer Chromatographic Techniques. *Pharmacognosy Magazine*, 19(4), 917–925.
<https://doi.org/10.1177/09731296231200365>

Location: India

Year: 2023

Standardization Technique: SPECTROPHOTOMETRIC ESTIMATION OF MAJOR CLASS COMPOUNDS INCLUDING TOTAL PHENOLICS IN THE POLYHERBAL FORMULATION AND ITS INDIVIDUAL INGREDIENT PLANTS

Secondary Characterization Standardization Techniques: Spectrophotometric estimation of major class compounds including total phenolics in the polyherbal formulation and its individual ingredient plants

Aim: High-performance thin-layer chromatography (HPTLC) for comparative chemical profiling of the polyherbal formulation and its individual ingredients.

The use of spectrophotometric methods to quantify phenolic content, providing a preliminary chemical pattern of the plant extracts

Key Findings: To carry out a comparative phytochemical evaluation of an important Ayurvedic polyherbal formulation with its ingredient plants, highlighting the chemical transformations due to synergistic and antagonistic interactions during preparation

Purpose of Standardization: The phytochemical contents of the finished formulation differ from those of the ingredient plants, which might be due to the synergistic and antagonistic interactions among various chemical components extracted.

HPTLC-based comparative chromatograms illustrated differences and similarities in specific compounds across the plant ingredients and the finished formulation

Method Effectiveness: To ensure the quality of the selected finished formulation in relation to its ingredients through the identification and comparison of chemical constituents.

To validate the traditional use and pharmacological properties of the formulation through modern analytical techniques

Method Limitation: The HPTLC method provided detailed chemical profiles that enabled the visualization of differences and similarities in the chemical composition between the formulation and its ingredients.

Spectrophotometric methods effectively quantified phenolic compounds, aiding in assessing the concentration of these bioactive components

Gaps and Recommendations: Chemical variability within the herbal components can impact the consistency of results, making standardization challenging.

HPTLC and spectrophotometric methods depend heavily on the precision of the technique and conditions under which the analysis is performed, potentially affecting reproducibility.

39. "Software Assisted Multi-Tiered Mass Spectrometry Identification of Compounds in Traditional Chinese Medicine: *Dalbergia odorifera* as an Example

Author: Wang, M., Yao, C., Li, J., Wei, X., Xu, M., Huang, Y., Mei, Q., & Guo, D. (2022). Software Assisted Multi-Tiered Mass Spectrometry Identification of Compounds in Traditional Chinese Medicine: *Dalbergia odorifera* as an Example. *Molecules*, 27(7), 2333. <https://doi.org/10.3390/molecules27072333>

Location: China

Year: 2022

Standardization Technique: THE ARTICLE MAINLY DISCUSSES MULTI-TIERED MASS SPECTROMETRY (MS) TECHNIQUES FOR THE IDENTIFICATION OF COMPOUNDS IN TRADITIONAL CHINESE MEDICINE, FOCUSING ON *DALBERGIA ODORIFERA*. THE MAIN TECHNIQUE OF MS DOES NOT INCLUDE ANY SPECIFIC STANDARDIZATION PROCESSES BEYOND CHARACTERIZATION.

Secondary Characterization Standardization Techniques: The article mainly discusses multi-tiered mass spectrometry (MS) techniques for the identification of compounds in Traditional Chinese Medicine, focusing on *Dalbergia odorifera*. The main technique of MS does not include any specific standardization processes beyond characterization.

Aim: Multi-tiered mass spectrometry using different methods such as direct infusion mass spectrometry (DI-MS), liquid chromatography-mass spectrometry (LC-MS), and high-resolution mass spectrometry (HRMS) for detailed compound analysis

Key Findings: To demonstrate the application of software-assisted multi-tiered mass spectrometry in identifying complex compounds within *Dalbergia odorifera*, a commonly used material in Traditional Chinese Medicine, and to discuss the potential benefits of this approach in enhancing the understanding and quality control of herbal medicines.

Purpose of Standardization: Successful identification of multiple bioactive compounds in *Dalbergia odorifera* using advanced mass spectrometry techniques.

Demonstration of the potential of these techniques to provide deeper insights into the chemical profiles of traditional medicines, which can aid in their standardization and quality control

Method Effectiveness: To enhance the quality control measures of Traditional Chinese Medicine by accurately identifying and quantifying the chemical constituents using sophisticated mass spectrometry techniques

Method Limitation: High effectiveness in identifying and characterizing complex molecular structures within herbal medicines, proving crucial for ensuring quality and efficacy

Gaps and Recommendations: High complexity and cost associated with advanced mass spectrometry techniques.

Potential challenges in interpreting vast amounts of data generated by multi-tiered MS analysis

40. Unlocking Therapeutic Potential: Comprehensive Extraction, Profiling, and Pharmacological Evaluation of Bioactive Compounds from *Eclipta alba* (L.) Hassk. for Dermatological Applications

Author: Myo, H., Liana, D., & Phanumartwiwath, A. (2023). Unlocking Therapeutic Potential: Comprehensive Extraction, Profiling, and Pharmacological Evaluation of Bioactive Compounds from *Eclipta alba* (L.) Hassk. for Dermatological Applications. *Plants*, 13(1), 33. <https://doi.org/10.3390/plants13010033>

Location: Thailand

Year: 2004

Standardization Technique: DEVELOPING FORMULATIONS, AND ENSURING PRODUCT QUALITY ALL DEPEND ON THE IDENTIFICATION AND CHARACTERIZATION OF BIOACTIVE COMPONENTS IN THAT EXTRACT.

Secondary Characterization Standardization Techniques: developing formulations, and ensuring product quality all depend on the identification and characterization of bioactive components in that extract.

Aim: Comprehensive extraction, profiling, and pharmacological evaluation techniques using high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and other chromatographic methods to characterize the bioactive compounds in *Eclipta alba*

Key Findings: To explore the therapeutic potential of *Eclipta alba* for dermatological applications through comprehensive extraction, profiling, and evaluation of its bioactive compounds.

To evaluate these compounds' antibacterial, antifungal, antioxidant, anti-inflammatory, and cytotoxic properties in vitro and in vivo.

Purpose of Standardization: Identification of several bioactive compounds in *Eclipta alba*, including flavonoids, coumestans, and triterpenes, which have potential therapeutic effects for various skin conditions.

Demonstrated effectiveness of these compounds against skin disease-causing microbes, inflammatory skin diseases, and other dermatological conditions.

Method Effectiveness: To ensure the reproducibility and consistency of results in research and application of *Eclipta alba* extracts in dermatology.

To develop standardized extracts and formulations for therapeutic use in skin care and treatment.

Method Limitation: Effective in identifying a wide range of bioactive compounds and demonstrating their pharmacological activities.

Provided insights into the mechanisms of action and potential clinical applications of *Eclipta alba* extracts

Gaps and Recommendations: Variability in the bioactive compound profiles depending on environmental and cultivation conditions.

Limited studies on the long-term safety and efficacy of these compounds in human clinical settings