



REVIEW ARTICLE

ADVANCEMENTS IN ANALYTICAL TECHNIQUES FOR THE IDENTIFICATION OF PHYTOCONSTITUENTS IN HERBAL MEDICINES

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ABSTRACT: Actually, herbal medicines are an essential part of traditional systems of medicine and are now finding acceptance in modern medicine. However, owing to the bio pharmacological activities of their phytoconstituents, standardization, quality control, and scientific validation face several challenges. Secondary metabolites that are bioactive compounds within the herbal remedies are mostly present within matrix-encapsulated, and matrix variations are not alike which makes the identification and quantification difficult. This review aims at discussing several challenges that are being faced in the phytochemical research, and the evolution in analytical technologist that is being used to overcome these challenges. Contemporary flow-based chromatographic techniques like UPLC and hyphenated methodologies inclusive of GC-MS quickly and accurately separate and quantify the contained phytoconstituents in a highly sensitive manner. At the same time, spectroscopy methods, such as Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared Spectroscopy (FTIR), enable the analysis of complexes and their structures with little sample pre-treatment required. Hyphenated techniques like LC-MS, HPLC-DAD are amalgamation of two or more than two conventional techniques, which have optimized procedures and detailed solutions for complex herbal matrix. Besides traditional analytical methodologies including conventional laboratory tests, more recent techniques involve portable miniaturized analytical systems, and application of artificial intelligence. These encompass artifacts that enable analysis at the scene or on-site enhancements that use machine learning algorithms to improve analysis of data obtained. However, the common issues are still there, including the cost, technical knowledge, and permission requirements. This review underscores these developments while underlining the concern on safety, quality, and international recognition of these herbal products. Linking conventional practices with innovation, these methods release the potential of phytoconstituents for enhancing the prospect for the world's health.

Key Words: Phytoconstituents, Herbal medicines, Analytical techniques, Chromatography, spectroscopic methods

1. INTRODUCTION

Anticipated to be the mainstay of ethnopharmacology, traditional medicines mostly based on plant extracts are slowly being appreciated in contemporary medicine. They contain various bioactive compounds known phytoconstituents and these possessed pharmacological activities such as anti-inflammatory, antioxidant, antimicrobial and so on [1]. Due to the current need for holistic and ecofriendly approaches to using natural resources, herbal medicines are a valuable source of relief for chronic diseases, lifestyle diseases, and diseases that are not easily treated with chemical drugs [2]. However, the identification and characterization of phytoconstituents contain certain limitations barring their seemingly boundless therapeutic applications. Herbal preparations are complex in nature comprising a huge number of components of distinct chemical classes and with different concentrations and mutual linkages [3]. listeners; Other things like the conditions of

growing either in the laboratory or out in the farms, the region of origin and the method of preparation for consumption all add to the variations on the chemical makeup of Moringa. Conventional techniques of analysis fail to provide adequate sensitivity, selectivity, and robustness to closely study and quantify these complex assemblies, thus posing the need for sophisticated and accurate methods [4].

This article presents a detailed critique of the state of the art concerning novel analysis strategies which has transformed phytocomponents analysis. It goes into detail in explaining the new methods like chromatography, spectroscopy and hyphenated techniques, which are far more precise and faster than other conventional forms of techniques [5].

By outlining the applications of and future work that should be done regarding these advancements, this review seeks to broaden the gap between conventional knowledge of herbal

medicine and strictly scientific evidence to integrate the two so as to make the remedies safe, effective, and accepted around the world [6].

2. PHYTOCONSTITUENTS IN HERBAL MEDICINES

2.1 Definition and Classification

Bioactive compounds are naturally occurring constituents in plants which are responsible for curative actions. They are reactive compounds that constitute additions to the therapeutic effects of the plant derived herbs. There are various classifications of organic compounds broad category which is determined by the chemical composition and maybe the functional groups present in the compound [7]:

- **Alkaloids:** Alkaloids that possess a relevant pharmaceutical impact, for example, morphine, which is an anesthetic, and quinine, which is used to treat malaria.
- **Flavonoids:** Flavonoids, mostly of the flavonol and flavone type, and specifically quercetin and kaempferol containing anti-oxidant & anti-inflammatory precipitations.
- **Terpenoids:** A diverse group with antimicrobial and anti-cancer activities for instance artemisinin and menthol.
- **Glycosides:** Conjugates with sugar residues including cardenolides (for instance, digoxin), which are terribly vital in cardiovascular therapies.
- **Others:** Saponins, tannins, lignans and phenolic acids are also involved in number of therapeutic uses [8].

This classification highlights the diversity of phytoconstituents and their wide-ranging pharmacological potential.

2.2 Therapeutic Applications

Phytoconstituents play a major role in the medical properties of plants and lots are being used in today's congress. Examples include:

- **Curcumin (from turmeric):** Known for its anti-inflammatory and anti-cancer properties.
- **Resveratrol (from grapes):** A potent antioxidant with cardiovascular protective effects.
- **Epigallocatechin gallate (EGCG, from green tea):** Demonstrated attributes like anti-obesity, anti-diabetic attributes and has the potential to defend against Cancer cells.
- **Saponins (from ginseng):** Employed for their ability to act as natural stress and more importantly, immune system enhancers. These compounds act as lead compounds in drug development and the requisite knowledge about these compounds underlines the significance of innovative theranostics. [9].

2.3 Challenges in Extraction and Analysis

Despite their potential, the analysis and standardization of phytoconstituents pose significant challenges:

- **Stability Issues:** There are a lot of possibilities that many phytoconstituents are very sensitive to environmental conditions such as light exposure, temperature and the pH level, thereby undergo degradation during the extraction period as well as during storage.
- **Matrix Interference:** Herbal medicines come with a cocktail of phytoconstituents whose interactions make isolation and quantification very challenging.
- **Standardization Challenges:** Stability and variability of the cultivation, harvesting, and processing of plants are the producers of phytoconstituent profiles. Another factor which prevents standardization of herbal products is the above noted variability [10].

Table 1: Classification of phytoconstituents with examples and therapeutic uses [11]

Phytoconstituent Type	Examples	Therapeutic Uses
Alkaloids	Morphine, Quinine	Analgesic, Antimalarial
Flavonoids	Quercetin, Kaempferol	Antioxidant, Anti-inflammatory
Terpenoids	Artemisinin, Menthol	Antimicrobial, Anti-cancer
Glycosides	Digoxin, Stevioside	Cardiotonic, Sweetener
Saponins	Ginsenosides, Dioscin	Adaptogenic, Immunomodulatory
Tannins	Ellagic acid, Catechins	Astringent, Antioxidant
Lignans	Sesamin, Podophyllotoxin	Anti-cancer, Hepatoprotective
Phenolic Acids	Caffeic acid, Gallic acid	Anti-inflammatory, Antioxidant
Coumarins	Umbelliferone, Scopoletin	Anticoagulant, Anti-inflammatory
Polysaccharides	Beta-glucan, Pectin	Immune-boosting, Prebiotic
Anthraquinones	Aloe-emodin, Chrysophanol	Laxative, Anti-inflammatory
Sterols	Beta-sitosterol, Stigmasterol	Cholesterol-lowering, Anticancer

3. ANALYTICAL TECHNIQUES: AN OVERVIEW

3.1 Traditional Techniques

Traditionally, conventional analytical methods have remained the cornerstone in the discovery and description of phytoconstituents in herbal remedies. These include ordinary tube tests, thin layer chromatography, and spectro photometer tests. Although they as an initial attempt of giving awareness about the phytochemical composition, they possess enormous drawbacks [12].

- **Thin-Layer Chromatography (TLC):** Useful for quick screening but lacks precision in quantification and separation of complex mixtures [13].

- **Spectrophotometry:** Adequate for bulk analysis but unable to differentiate between structurally similar compounds [14].
- **Gravimetric and Volumetric Methods:** Simple but not suitable for detecting trace amounts of bioactive compounds [15].

The conventional methods being lengthy, tedious, manpower oriented and lack the selectivity and sensitivity needed to tackle multidimensional herbal matrices. These limitations have led to formulation of complex analytical techniques [16].

3.2 Advancements in Analytical Techniques

Modifications in the logic of phytoconstituent analysis came with advanced analytical techniques that provide enhanced sensitivity, accuracy and reproducibility [17].

Chromatographic Methods:

- **High-Performance Liquid Chromatography (HPLC):** Provides excellent separation and quantification capabilities for complex mixtures.
- **Gas Chromatography-Mass Spectrometry (GC-MS):** Ideal for volatile compounds with high sensitivity.
- **Ultra-Performance Liquid Chromatography (UPLC):** Enhances speed and resolution, making it a preferred choice for high-throughput analyses [18].

Spectroscopic Methods:

- **Nuclear Magnetic Resonance (NMR):** Non-destructive and highly specific for structural elucidation.
- **Fourier Transform Infrared Spectroscopy (FTIR):** Rapid identification of functional groups and chemical bonds.
- **Mass Spectrometry (MS):** Exceptional for detecting compounds in trace amounts and identifying unknown phytoconstituents [19].

Compared to conventional qualitative approaches, these sophisticated methods overcome the shortcomings allowing precise and faster evaluation of herbal products.

3.3 Integrated and Multimodal Approaches

Hybrid strategy in analytical methods are used to combine more than one method and results in more accurate and thorough results [20].

Hyphenated Techniques:

- **LC-MS (Liquid Chromatography-Mass Spectrometry):** A technique that incorporates the selectiveness of chromatography to the identification capabilities of mass spectrometry.

- **GC-MS (Gas Chromatography-Mass Spectrometry):** Gives qualitative and quantitative evaluation of various unpredictable compounds with increased accuracy.
- **HPLC-DAD (High-Performance Liquid Chromatography with Diode Array Detection):** Permits identification of several compounds at once due to the differences in their spectra [21].

Multimodal Approaches: Metabolomics and chemometrics are applied in combination to compile information from the multiple sources to consider phytoconstituent profiles [22].

These combined strategies increase the robustness of phytoconstituent characterization, specifically in the natural products with multiple components, using sample matrix Interference elimination and improved variability. Altogether these two works form the most advanced analytical tool available at present linking the age old practice of using herbal drugs to the most scientific technique available today [23].

Table 2: Comparison of traditional and advanced analytical techniques[24]

Feature	Traditional Techniques	Advanced Techniques
Sensitivity	Low	High
Specificity	Moderate	High
Accuracy	Variable	High
Reproducibility	Low	High
Throughput	Low	High
Time Efficiency	Time-consuming	Rapid
Cost	Low	High
Sample Preparation	Simple	Extensive
Ability to Analyze Complex Matrices	Limited	Excellent
Suitability for Standardization	Inconsistent	Highly suitable

4. ADVANCEMENTS IN ANALYTICAL TECHNIQUES

4.1 Chromatographic Methods

Chromatography has evolved overtime and becoming one of the powerful techniques used in the emerging, separation, identification, and quantification of phytoconstituents in herbal medicines [25].

- **High-Performance Liquid Chromatography (HPLC):** HPLC is preferably used due to its high accuracy and ability to separate mixtures which may be composed of several compounds. It gives highly accurate and reproducible results; it can be used to quantify bio-active chemical constituents in both crude herbal extracts and the finished products [26].
- **Ultra-Performance Liquid Chromatography (UPLC):** UPLC is a new technology compared to HPLC composed of smaller particle size and high-pressure capability that yields a faster analysis with higher resolutions than the HPLC. This method is useful for high throughput analysis and is associated with some advantages such as increased throughput but at the same time does not lose the sensitivity of the analysis and its accuracy [27].

- **Advances in Gas Chromatography (GC):** GC retains its position as a method of choice for volatile compounds and essential oils. Recent advancement in GC has improved the techniques sensitivity and selectivity especially when combined with detectors such as FID or GC-MS [28].

4.2 Spectroscopic Techniques

Spectroscopy provides rapid and non-destructive methods for identifying phytoconstituents based on their structural and chemical properties.

- **Nuclear Magnetic Resonance (NMR):** A critical appeal of NMR is in the identification of structure of phytoconstituents in a molecular aspect. Because of its high specificity it is useful in distinguishing compounds with similar structures making it instrumental in natural product chemistry [29].
- **Fourier Transform Infrared Spectroscopy (FTIR):** FTIR is applied for functional group analysis and for chemical bond determination in a compound. It is a fast method financially efficient appropriate for early-stage analysis of data [30].
- **Mass Spectrometry (MS):** MS gives unmatched sensitivity where even a trace presence of phytoconstituents in a matrix can be detected. This method is especially useful for unknown compounds and the fingerprinting of multi-herbal formulations [31].

4.3 Hyphenated Techniques

Hyphenated techniques combine two or more analytical methods, leveraging their strengths to overcome the limitations of standalone techniques.

Techniques like LC-MS, GC-MS, and HPLC-DAD:

- **LC-MS (Liquid Chromatography-Mass Spectrometry):** A type of system that integrates chromatography for the separation of components with mass spectrometry for the detection of substances most useful for handling compounds in solution.
- **GC-MS (Gas Chromatography-Mass Spectrometry):** Combines GC's capacity of the separation of compounds with MS for compound identification, found for essential oil analysis.
- **HPLC-DAD (High-Performance Liquid Chromatography with Diode Array Detection):** Allows for following identification of several compounds enhancing identification based on the UV-Vis spectra [32].

Advantages of Hyphenation in Complex Matrices: Combined techniques further specificity, sensitivity and accuracy aimed at a comprehensive investigation of multicomponent herbal preparations. They are especially useful when it comes to detection of the secondary or trace elements which could be important for therapeutic effects [33].

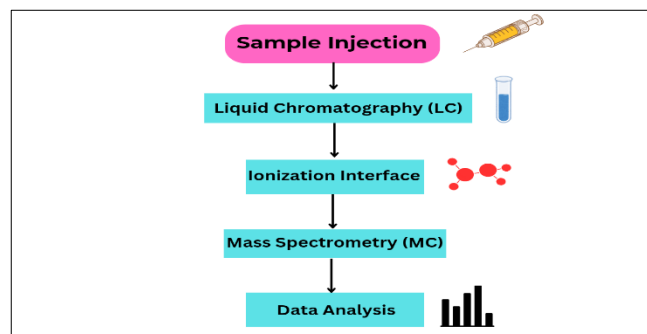


Fig. 1: Workflow of a hyphenated technique (e.g., LC-MS) [34].

Table 3: Applications of advanced techniques with examples of phytoconstituents [35]

Advanced Technique	Example Phytoconstituents	Applications
HPLC	Curcumin, Catechins	Quantification of active compounds
UPLC	Flavonoids, Phenolic acids	High-throughput compound screening
GC-MS	Essential oils, Terpenes	Analysis of volatile compounds
LC-MS	Alkaloids, Glycosides	Identification of minor components
NMR	Resveratrol, Ginsenosides	Structural elucidation
FTIR	Tannins, Saponins	Functional group analysis
HPLC-DAD	Polyphenols, Anthraquinones	Spectral-based compound profiling
Metabolomics	Metabolites in plant extracts	Metabolic fingerprinting
Chemometric Analysis	Complex phytochemical profiles	Data-driven phytoconstituent analysis
Capillary Electrophoresis	Alkaloids, Anthocyanins	Separation of charged molecules

5. APPLICATIONS IN HERBAL MEDICINE

5.1 Standardization and Quality Control

The issue of quality control of the raw herbal medicines is a very important factor in the use of these medicines as it plays a vital role in the conformity of their safety, efficacy and reproducibility. Advanced analytical techniques play a pivotal role in achieving this by:

- **Quantifying Active Constituents:** Such techniques as HPLC and UPLC aid in formulation of exact concentrations of gathered bioactive compounds while raising bar of dosage standardization between different batches [36].
- **Detecting Contaminants:** Techniques that include GC-MS and LC-MS were also significant in determining concentration of contaminants in foods and especially pesticides, heavy metals, and adulterants [37].
- **Authentication of Raw Materials:** Chemical fingerprinting analytical tools such as NMR and FTIR spectroscopy can be used in confirming the purity of plant materials to support the use of accurate formulations in the preparation of products. When implemented in the production line the following

techniques will help set quality benchmarks for herbal products among manufacturers [38].

5.2 Identification of Bioactive Compounds

To realize the pharmacological properties of herbal medicines, the bioactive compounds are essential to recognize. With the help of modern chemometric analyses, researchers were able to identify and describe the most important phytoconstituents.

- **Curcumin in Turmeric:** From HPLC and LC-MS analysis, curcumin has been determined to exhibit anti-inflammatory and antioxidant activity, and thus incorporated in numerous formulations [39].
- **Ginsenosides in Ginseng:** It was then analyzed using NMR and HPLC-DAD to reveal that Ginsenosides owns adaptogenic/ immunomodulatory properties [40].
- **Catechins in Green Tea:** The photometric derivatization technique and UPLC have been employed to quantify catechins which possess anticancer and cardio-protective effects. Such examples show how innovative approaches enhance the process of identification and differentiation of biologically active compounds to promote further legitimate utilization of plant-based remedies for diseases [41].

5.3 Enhancement of Therapeutic Potential

The therapeutic potential of herbal medicines can be enhanced through the application of advanced analytical methods in formulation development and dosage optimization.

- **Formulation Optimization:** This view has attributed the technique such as chemometric analysis and metabolomics to determine the synergistic propping up of phytoconstituents that may facilitate the formulation of highly effective remedies [42].
- **Dosage Standardization:** Analytical methods on active compounds enable establishment of dosage levels thus reducing variability and enhance therapeutic performance [43].

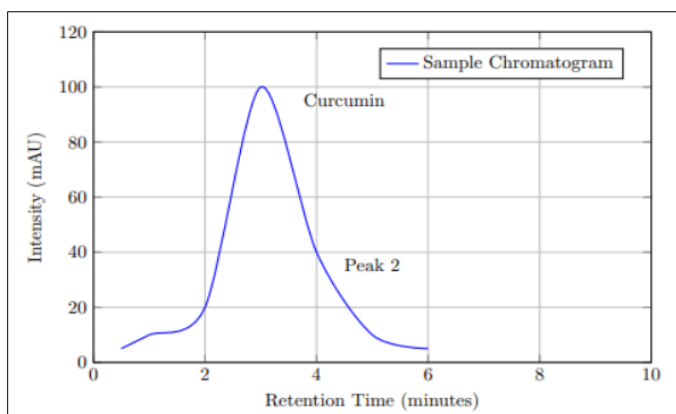


Fig. 2: Example chromatograms or spectral data for a known phytoconstituent [45]

Targeted Delivery Systems: In the development of targeted delivery systems, mass spectrometry and NMR are important in determining the compound stability and its interaction with carriers. Such innovations work to ensure that herbal medicine products offer the highest clinical value with the least side effects placing herbal medicine as a vital cornerstone in contemporary health care systems [44].

6. CHALLENGES AND LIMITATIONS

6.1 Cost and Accessibility

One of the primary challenges associated with advanced analytical techniques is their high cost and limited accessibility.

- **Equipment Costs:** Instruments such LC-MS, NMR as well as UPLC are expensive, thus cannot be afforded by small laboratories or manufacturers who operate in less-endowed environments.
- **Operational Expertise:** These techniques require personnel for operation and analysis of data, which is a challenge in areas where the workforce is still a challenge.
- **Maintenance and Upkeep:** By the current design, there are numerous operational costs such as maintenance and calibration, which add up to the cost and prove to be prohibitive in making them convenient for widespread use. To overcome these barriers, new approaches to cost reduction and capacity development need to be undertaken in order to increase the access and utilization of deep analytical capabilities [46].

6.2 Complex Herbal Matrices

Herbal medicines are composed of highly complex mixtures, which pose significant challenges during analysis.

- **Reproducibility Issues:** Differences in type and stage of plant cultivation, geographical source, and time of harvesting lead to variations of phytoconstituent levels, and thus making replication of such studies hard [47].
- **Data Complexity:** Metabolite identification is always extensive and generates much data; other secondary analytes also produce voluminous data. Indeed, the interpretation of these data is possible in the context of the usage of specific software and with the help of specialists [48].
- **Matrix Interference:** Since there are many compounds in the herbal matrices, superimposition or suppression of signals including peaks is a major challenge in identification and quantification of analytes. There will need to be additional, more durable penetration of sound methodologies and the incorporation of modern computational aids such as chemometrics for analyses [49].

6.3 Regulatory and Standardization Issues

The lack of globally harmonized regulations for herbal medicines remains a significant limitation in their acceptance and integration into modern healthcare.

- **Inconsistent Standards:** Inconstancy of quality standards from one country to another results in differences in product assessment and hence the quality of products cannot be guaranteed.
- **Documentation Gaps:** Traditional knowledge does not have adequate documentation, and lack of scientific research information causes difficulties that lead to delayed regulatory approval.
- **Regulatory Barriers:** Still, advanced techniques, although applied efficiently in certain situations, are not as firmly prescribed and applied in different regulatory frameworks as basic ones, which contributes to inconsistency further. These concerns require the elaboration of international cooperation and the development of standard requirements and further regulatory schemes for qualifying herbal products [50].

7. FUTURE DIRECTIONS

Therefore, the future of phytoconstituent analysis of herbal medicines depends on the advancement of new technology, especially in collaboration between countries. The recently developed miniaturized analytical devices are expected to revolutionize the field by providing portable and field analytical methods for real time analysis [51]. Some of them include handheld spectrometers, where they allow on the spot analysis of raw materials and the final products, and portable chromatographs. Their cost and simplicity also make them ideal for use especially in environments with limited resources thereby enabling the wider use of complex analyses strategies [52].

It is a known fact that future of analyzing data is in the hands of artificial intelligence (AI) and machine learning (ML). These technologies are particularly well suited to dealing with the very large and diverse information loads that result from the various forms of analytical methodologies [53]. The possible application of Artificial Intelligence related tools can help to pattern compounds' interactions, they can also predict optimized analytical procedures, serve to enhance the Phytoconstituent analysis function and noticeably increase its overall accuracy. In the same way, formulation design and/or therapeutic outcomes can also be modeled and predicted using ML models thus expanding knowledge and facts from empirical formulation to scientific formulation [54].

Combination on an international realm is important in addressing the issues of variation and variance of regulations in herbal remedies. It is suggested that global databases of phytoconstituents should be set up, with study and analytical techniques to be unified across it so as to improve on the research methodology in the field [55]. They should undergo

cross-country collaborations to share knowledge and funds that enhance formulation of universal protocols on how to analyze and standardize the herbal products. These collective efforts will help in promoting the use of analytical model-based approaches in their health-care systems, and for making herbal products safer, effective and affordable [56].

CONCLUSION

Analyses of such advancement highlight the fact that modern analytical procedures have solved some of the biggest problems in the identification, quantification and characterization of phytoconstituents in herbal medicine research. In the recent past, advanced chromatographic techniques like HPLC and UPLC along with powerful spectroscopic TechNet like NMR and Fining have improved the sensitivity, specificity, and speed of photo determination. In addition, this presentation has revealed the advantage of co-utilizing more specific hyphenated systems such as LC-MS and GC-MS for comprehensive characterization of intricate herbal matrices that have enhanced the standardization and quality assessment of medicinal herbs. Such advancements have brought out herbals from folklore practices to acknowledged therapeutic systems easing acceptance of herbals in the prevailing health care systems around the globe.

Nevertheless, challenges like high costs, technical skilled manpower, and incomorphic nature of regulations still linger thus steering expansion of these techniques. Future research should include introducing cheap and mobile analytical instrumentation for a broad range of users. The use of artificial intelligence and machine learning in enhancing disc analysis, model prediction, as well as the enhancement in therapeutic formulations creates boundless possibilities. Also, international cooperation is important while coordinating the elaboration of guidelines for phytoconstituent identification as well as while creating large scale phytoconstituent databases for increasing the confidence in research done with CAMs and HM.

This future direction should be embraced to effectively open future avenues in the direction of herbal medicine that will help integrate phytoconstituents in a safe, effective and scientific manner. Such endeavors will help not only to progress the studied field and provide evidence for the acceptance of herbal remedies in the global population of patients.

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REFERENCES:

1. Shin SA, Joo BJ, Lee JS, Ryu G, Han M, Kim WY, Park HH, Lee JH, Lee CS. Phytochemicals as anti-inflammatory agents in animal models of prevalent inflammatory diseases. *Molecules*. 2020 Dec 15;25(24):5932.
2. Pathak A, Gupta AP, Pandey P. Herbal Medicine and Sustainable Development Challenges and Opportunities. *Herbal Medicine Phytochemistry: Applications and Trends*. 2024 Jan 27:1-26.

3. Efferth T, Koch E. Complex interactions between phytochemicals. The multi-target therapeutic concept of phytotherapy. *Current drug targets*. 2011 Jan 1;12(1):122-32.
4. Pferschy-Wenzig EM, Bauer R. The relevance of pharmacognosy in pharmacological research on herbal medicinal products. *Epilepsy & behavior*. 2015 Nov 1;52:344-62.
5. Tarighat MA, Barnwal A, Tariq M, Mishra P, Choudhary S, Jain M, Abdi G. Utilizing HPLC for Efficient Metabolite Purification. In *Advances in Metabolomics 2024* Dec 17 (pp. 139-177). Singapore: Springer Nature Singapore.
6. Amabie T, Izah SC, Ogwu MC, Hait M. Harmonizing Tradition and Technology: The Synergy of Artificial Intelligence in Traditional Medicine. In *Herbal Medicine Phytochemistry: Applications and Trends 2024* Jul 10 (pp. 2103-2125). Cham: Springer International Publishing.
7. Hussein RA, El-Anssary AA. Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. *Herbal medicine*. 2019 Jan 30;1(3):11-30.
8. Mutha RE, Tatiya AU, Surana SJ. Flavonoids as natural phenolic compounds and their role in therapeutics: An overview. *Future journal of pharmaceutical sciences*. 2021 Dec;7:1-3.
9. Riaz M, Rahman NU, Zia-Ul-Haq M, Jaffar HZ, Manea R. Ginseng: A dietary supplement as immune-modulator in various diseases. *Trends in Food Science & Technology*. 2019 Jan 1;83:12-30.
10. Srivastava S, Misra A. Quality control of herbal drugs: Advancements and challenges. *New Age Herbs: Resource, Quality and Pharmacognosy*. 2018:189-209.
11. Alamgir AN, Alamgir AN. Phytoconstituents—active and inert constituents, metabolic pathways, chemistry and application of phytoconstituents, primary metabolic products, and bioactive compounds of primary metabolic origin. *Therapeutic Use of Medicinal Plants and their Extracts: Volume 2: Phytochemistry and Bioactive Compounds*. 2018:25-164.
12. Kowalska T, Sajewicz M. Thin-layer chromatography (TLC) in the screening of botanicals—its versatile potential and selected applications. *Molecules*. 2022 Oct 5;27(19):6607.
13. Hess AV. Digitally enhanced thin-layer chromatography: an inexpensive, new technique for qualitative and quantitative analysis. *Journal of chemical education*. 2007 May;84(5):842.
14. Cuyckens F, Claeys M. Mass spectrometry in the structural analysis of flavonoids. *Journal of Mass spectrometry*. 2004 Jan;39(1):1-5.
15. Ares AM, Valverde S, Bernal JL, Nozal MJ, Bernal J. Extraction and determination of bioactive compounds from bee pollen. *Journal of pharmaceutical and biomedical analysis*. 2018 Jan 5;147:110-24.
16. Ding R, Yu L, Wang C, Zhong S, Gu R. Quality assessment of traditional Chinese medicine based on data fusion combined with machine learning: A review. *Critical reviews in analytical chemistry*. 2024 Oct 2;54(7):2618-35.
17. Jain SK, Khabiya R, Dwivedi A, Soni P, Soni V. Approaches and Challenges in Developing Quality Control Parameters for Herbal Drugs. *New Avenues in Drug Discovery and Bioactive Natural Products*. 2023 Aug 1:54-82.
18. Naushad M, Khan RM. *Ultra Performance Liquid Chromatography Mass Spectrometry*. Abingdon: Taylor and Francis Group. 2014;288.
19. Nascimento PT, Santos AF, Yamamoto CI, Tose LV, Barros EV, Goncalves GR, Freitas JC, Vaz BG, Romao W, Scheer AP. Fractionation of asphaltene by adsorption onto silica and chemical characterization by atmospheric pressure photoionization fourier transform ion cyclotron resonance mass spectrometry, fourier transform infrared spectroscopy coupled to attenuated total reflectance, and proton nuclear magnetic resonance. *Energy & Fuels*. 2016 Jul 21;30(7):5439-48.
20. Curran PJ, Hussong AM. Integrative data analysis: the simultaneous analysis of multiple data sets. *Psychological methods*. 2009 Jun;14(2):81.
21. Pragst F, Herzler M, Erxleben BT. Systematic toxicological analysis by high-performance liquid chromatography with diode array detection (HPLC-DAD). *Clinical Chemistry and Laboratory Medicine (CCLM)*. 2004 Nov 1;42(11):1325-40.
22. Bhattacharya K, Chanu NR, Kalita R, Chakraborty A, Deka S, Bordoloi R. Role of Analytical Methods in Herbal Drug Discovery. In *New Avenues in Drug Discovery and Bioactive Natural Products 2023* Aug 1 (pp. 1-34). Bentham Science Publishers.
23. Yau WP, Goh CH, Koh HL. Quality control and quality assurance of phytomedicines: Key considerations, methods, and analytical challenges. *Phytotherapies: efficacy, safety, and regulation*. 2015 Apr 24:18-48.
24. Tu Y, Li L, Wang Z, Yang L. Advances in analytical techniques and quality control of traditional Chinese medicine injections. *Journal of Pharmaceutical and Biomedical Analysis*. 2021 Nov 30;206:114353.
25. Wu H, Guo J, Chen S, Liu X, Zhou Y, Zhang X, Xu X. Recent developments in qualitative and quantitative analysis of phytochemical constituents and their metabolites using liquid chromatography–mass spectrometry. *Journal of pharmaceutical and biomedical analysis*. 2013 Jan 18;72:267-91.
26. Siddique I. Unveiling the Power of High-Performance Liquid Chromatography: Techniques, Applications, and Innovations. *European Journal of Advances in Engineering and Technology*. 2021 Sep 21;8(9):79-84.
27. Nguyen DT, Guillaume D, Rudaz S, Veuthey JL. Fast analysis in liquid chromatography using small particle size and high pressure. *Journal of separation science*. 2006 Aug;29(12):1836-48.
28. Marriott PJ, Shellie R, Cornwell C. Gas chromatographic technologies for the analysis of essential oils. *Journal of chromatography A*. 2001 Nov 30;936(1-2):1-22.
29. Arora C, Bharti D, Tiwari BK, Kumar A, Verma DK, Singh B. Characterization techniques used for the analysis of phytochemical constituents. *Phytochemicals in medicinal plants: biodiversity, bioactivity and drug discovery*. Walter de Gruyter GmbH & Co KG, Germany p. 2023 Jun 6:131.
30. Siddique IM. *Exploring Functional Groups and Molecular Structures: A Comprehensive Analysis using FTIR Spectroscopy*. development. 2024 Feb 28;1:2.
31. Bierla K, Godin S, Lobinski R, Szpunar J. Advances in electrospray mass spectrometry for the selenium speciation: Focus on Se-rich yeast. *TrAC Trends in Analytical Chemistry*. 2018 Jul 1;104:87-94.
32. Saint-Marcoux F, Lachâtre G, Marquet P. Evaluation of an improved general unknown screening procedure using liquid-chromatography-electrospray-mass spectrometry by comparison with gas chromatography and high-performance liquid-chromatography—diode array detection. *Journal of the american society for mass spectrometry*. 2003 Jan;14(1):14-22.
33. Mukherjee PK, Kar A, Biswas S, Chaudhary SK, Banerjee S. Hyphenated analytical techniques for validation of herbal

- medicine. In Evidence-based validation of herbal medicine 2022 Jan 1 (pp. 811-827). Elsevier.
34. Oedit A, Vulto P, Ramautar R, Lindenburg PW, Hankemeier T. Lab-on-a-Chip hyphenation with mass spectrometry: strategies for bioanalytical applications. Current opinion in biotechnology. 2015 Feb 1;31:79-85.
 35. Fierascu RC, Fierascu I, Ortan A, Georgiev MI, Sieniawska E. Innovative approaches for recovery of phytoconstituents from medicinal/aromatic plants and biotechnological production. Molecules. 2020 Jan 12;25(2):309.
 36. Song W, Qiao X, Chen K, Wang Y, Ji S, Feng J, Li K, Lin Y, Ye M. Biosynthesis-based quantitative analysis of 151 secondary metabolites of licorice to differentiate medicinal Glycyrrhiza species and their hybrids. Analytical chemistry. 2017 Mar 7;89(5):3146-53.
 37. Ma F, Wu R, Li P, Yu L. Analytical approaches for measuring pesticides, mycotoxins and heavy metals in vegetable oils: A review. European journal of lipid science and technology. 2016 Mar;118(3):339-52.
 38. Noviana E, Indrayanto G, Rohman A. Advances in fingerprint analysis for standardization and quality control of herbal medicines. Frontiers in Pharmacology. 2022 Jun 2;13:853023.
 39. Urošević M, Nikolić L, Gajić I, Nikolić V, Dinić A, Miljković V. Curcumin: Biological activities and modern pharmaceutical forms. Antibiotics. 2022 Jan 20;11(2):135.
 40. Wenli S, Shahrajabian MH, Qi C. Therapeutic roles of goji berry and ginseng in traditional Chinese. Journal of Nutrition and Food Security. 2019 Nov 10;4(4):293-305.
 41. Okafor JN, Meyer M, Le Roes-Hill M, Jideani VA. Flavonoid and phenolic acid profiles of dehulled and whole Vigna subterranea (L.) Verde seeds commonly consumed in South Africa. Molecules. 2022 Aug 18;27(16):5265.
 42. Shaari K, Jahangir M, Shami AA, Akhtar MT. Analytical platforms and methodologies in herbal metabolomics. In Evidence-Based Validation of Herbal Medicine 2022 Jan 1 (pp. 587-602). Elsevier.
 43. Tyson RJ, Park CC, Powell JR, Patterson JH, Weiner D, Watkins PB, Gonzalez D. Precision dosing priority criteria: drug, disease, and patient population variables. Frontiers in pharmacology. 2020 Apr 22;11:420.
 44. Domingo C, Saurina J. An overview of the analytical characterization of nanostructured drug delivery systems: towards green and sustainable pharmaceuticals: a review. Analytica Chimica Acta. 2012 Sep 26;744:8-22.
 45. Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC. Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. Journal of Pharmacognosy and Phytochemistry. 2017;6(1):32-6.
 46. Aderinto N, Olatunji D, Abdulbasit M, Edun M. The essential role of neuroimaging in diagnosing and managing cerebrovascular disease in Africa: a review. Annals of Medicine. 2023 Dec 12;55(2):2251490.
 47. Naik GG, Sahu AN, Kaushik V, Kaushik A, Sarkar BK. Phytopharmaceuticals and herbal drugs: prospects and safety issues in the delivery of natural products. In Phytopharmaceuticals and Herbal Drugs 2023 Jan 1 (pp. 215-248). Academic Press.
 48. Cambiaghi A, Ferrario M, Masseroli M. Analysis of metabolomic data: tools, current strategies and future challenges for omics data integration. Briefings in bioinformatics. 2017 May 1;18(3):498-510.
 49. Zhou JL, Qi LW, Li P. Herbal medicine analysis by liquid chromatography/time-of-flight mass spectrometry. Journal of chromatography A. 2009 Oct 30;1216(44):7582-94.
 50. Maskus KE, Wilson JS, Otsuki T. Quantifying the impact of technical barriers to trade. World Bank Policy Research Working Paper. 2000;2512:1-51.
 51. Duan C, Li J, Zhang Y, Ding K, Geng X, Guan Y. Portable instruments for on-site analysis of environmental samples. TrAC Trends in Analytical Chemistry. 2022 Sep 1;154:116653.
 52. Okai-Ugbaje S, Ardzewewska K, Imran A. A mobile learning framework for higher education in resource constrained environments. Education and Information Technologies. 2022 Sep;27(8):11947-69.
 53. Saggi MK, Jain S. A survey towards an integration of big data analytics to big insights for value-creation. Information Processing & Management. 2018 Sep 1;54(5):758-90.
 54. Bannigan P, Aldeghi M, Bao Z, Häse F, Aspuru-Guzik A, Allen C. Machine learning directed drug formulation development. Advanced Drug Delivery Reviews. 2021 Aug 1;175:113806.
 55. Upton R, David B, Gafner S, Glasl S. Botanical ingredient identification and quality assessment: strengths and limitations of analytical techniques. Phytochemistry Reviews. 2020 Oct;19(5):1157-77.
 56. Baker JT, Borris RP, Carté B, Cordell GA, Soejarto DD, Cragg GM, Gupta MP, Iwu MM, Madulid DR, Tyler VE. Natural product drug discovery and development: new perspectives on international collaboration. Journal of natural products. 1995 Sep;58(9):1325-57.

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