Global Scholar Publications

Global Journal of Research in
Chemistry and Pharmacy

Journal homepage: https://gsjournals.com/gjrcp/

ISSN: 2980-4213 (Online)

(Review Article)

Check for updates

INNOVATIONS IN EXTRACTION, ISOLATION, AND IDENTIFICATION TECHNOLOGIES FOR PLANT SECONDARY METABOLITES

Avinash Gadare *

B. Pharmacy Student, Modern College of Pharmacy, Pune, India.
* Corresponding Author

Global Journal of Research in Chemistry and Pharmacy, 2023, 02(01), 001–006

Publication history: Received on 03 January 2023; revised on 26 January 2023; accepted on 28 January 2023

Article DOI: https://doi.org/10.58175/gjrcp.2023.2.1.0007

ABSTRACT

The extraction, isolation, and identification of plant secondary metabolites represent fundamental steps in natural product research and drug discovery. Traditional extraction methods such as maceration and Soxhlet extraction, while widely employed, are associated with prolonged processing times, high solvent consumption, thermal degradation of thermolabile compounds, and significant environmental burden. This review critically examines recent innovations in green extraction technologies including Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE), and Pressurized Liquid Extraction (PLE), which offer enhanced efficiency, reduced solvent usage, and improved selectivity. Furthermore, this review explores advanced hyphenated chromatographic-mass spectrometric techniques including LC-MS/MS, GC-MS, and HPLC-DAD-MS that enable comprehensive profiling of complex alkaloids, terpenoids, and phenolics. The integration of extraction and analytical platforms represents a paradigm shift toward streamlined, high-throughput natural product analysis. Despite significant progress, challenges including matrix interference, scalability limitations, and high operational costs persist. This review provides a comprehensive overview of these technologies, critically evaluates their applications and limitations, and identifies emerging trends including green solvent development, microextraction techniques, and process optimization strategies.

Keywords: Green extraction; Ultrasound-Assisted Extraction; Microwave-Assisted Extraction; Hyphenated techniques; Secondary metabolites; Natural product analysis

1 INTRODUCTION

Plant secondary metabolites encompass a structurally diverse array of compounds including alkaloids, terpenoids, phenolics, flavonoids, and saponins, many of which exhibit significant pharmacological activities. The efficient extraction, isolation, and identification of these compounds are essential for drug discovery, quality control of herbal medicines, and understanding plant biochemistry. Conventional extraction techniques such as maceration, percolation, and Soxhlet extraction, though established, suffer from several inherent limitations including lengthy extraction times, high solvent consumption, potential degradation of thermolabile compounds, and environmental concerns associated with large-scale solvent usage (da Silva et al., 2022).

The principles of green analytical chemistry have catalyzed the development of alternative extraction methodologies that minimize environmental impact while maintaining or improving extraction efficiency (da Silva et al., 2022). Green extraction procedures are defined as those employing alternative solvents, reducing energy consumption, in addition to ensuring the safety and high quality of the extract (da Silva et al., 2022). The twelve principles of green chemistry, particularly those concerning the use of solvents and safer reaction conditions, and waste prevention, have guided the development of these innovative approaches (da Silva et al., 2022). Two of the twelve principles of green chemistry concern the use of solvents and safer reaction conditions, and waste prevention, as the toxic and volatile nature of many organic solvents, particularly chlorinated hydrocarbons, commonly used in large quantities for organic reactions, created an alert to human health and the environment (da Silva et al., 2022).

Simultaneously, advances in analytical instrumentation have transformed the identification and characterization of plant secondary metabolites. Hyphenated techniques combining separation methods with spectroscopic detection have enabled the comprehensive profiling of complex natural product mixtures, facilitating dereplication and accelerating the discovery of novel bioactive compounds (Mukherjee et al., 2022).

This review aims to provide a critical evaluation of recent innovations in extraction, isolation, and identification technologies for plant secondary metabolites, with particular emphasis on green extraction techniques and advanced hyphenated analytical platforms.

2 REEN EXTRACTION TECHNOLOGIES

2.1 Ultrasound-Assisted Extraction (UAE)

Ultrasound-Assisted Extraction utilizes acoustic cavitation to disrupt plant cell walls, enhancing solvent penetration and mass transfer (Yusoff et al., 2022). The mechanism involves the formation, growth, and collapse of microbubbles in the extraction solvent, generating localized high temperatures and pressures that facilitate the release of intracellular compounds. UAE offers several advantages including reduced extraction time, lower solvent consumption, and operation at ambient temperatures, making it particularly suitable for thermolabile compounds (Yusoff et al., 2022).

The ultrasonic-assisted extraction is accepted as a green extraction technique due to its high performance with less solvent and time consumption, as well as suitability for thermo-sensitive compounds (Yusoff et al., 2022). The mechanism and advantages of ultrasound-assisted extraction have been discussed, particularly focusing on the extraction of bioactive compounds such as phenolics, flavonoids, thymols, saponins and proteins (Yusoff et al., 2022). Studies have systematically reviewed the optimization of extraction variables to reveal their significant contribution to the yield and quality change of bioactive compounds (Yusoff et al., 2022).

For alkaloid extraction, UAE has demonstrated superior efficiency compared to conventional methods, with studies reporting enhanced yields and reduced extraction times. However, limitations include potential degradation of sensitive compounds under intense cavitation conditions and challenges in scalability from laboratory to industrial applications.

2.2 Microwave-Assisted Extraction (MAE)

Microwave-Assisted Extraction employs microwave energy to heat the extraction solvent and plant matrix, accelerating the extraction process through selective heating of polar compounds. MAE offers rapid extraction times, typically achieving complete extraction in minutes compared to hours for conventional methods. The current use of microwaves implements green technology and satisfies the demand for getting the maximum yield of the target compound (Microwave: An Ecologically Innovative, 2022). MAE promises to be a novel extraction technique for the extraction of phytoconstituents from natural sources than other conventional extraction techniques (Microwave: An Ecologically Innovative, 2022).

Microwave-assisted extraction is proved to be advantageous as this method permits specificity, higher yield, selectivity, and extraction efficiency over traditional methods such as Soxhlet extraction, maceration and others along with reduced time and solvent consumption, less environmental pollution, and reduced degradation of thermolabile constituents (Microwave: An Ecologically Innovative, 2022).

MAE has been extensively applied to the extraction of phenolic compounds, terpenoids, and other secondary metabolites. The technique's efficiency is attributed to the rapid heating and increased solvent penetration into the plant matrix. However, limitations include the requirement for solvents with high dielectric constants and potential degradation of thermosensitive compounds.

2.3 Supercritical Fluid Extraction (SFE)

Supercritical Fluid Extraction, most commonly employing supercritical CO_2, offers a solvent-free extraction alternative with tunable selectivity through pressure and temperature modulation (Srivastava et al., 2022). The utilization of highly toxic, expensive, and eco-unfriendly organic solvents for the extraction process has been replaced by carbon dioxide (CO_2) in its supercritical state (Srivastava et al., 2022). The most significant advantage of supercritical CO_2 extraction is after the extraction process there was no trace amount of solvent present in extracted compounds since the residual CO_2 can be easily depressurized and vented (Srivastava et al., 2022).

Supercritical CO_2 attracts interest because of its high compressibility, non-toxic, non-flammable, and physiological compatibility (Srivastava et al., 2022). The significant parameters that affect the extraction process are temperature, pressure, solvent flow rate, and extraction time, which are optimized predominantly to improve the yield of the extraction (Srivastava et al., 2022). The addition of co-solvents enhances the extraction of polar compounds (Srivastava et al., 2022).

SFE is particularly advantageous for extracting non-polar to moderately polar compounds and is compatible with food and pharmaceutical applications due to the non-toxic nature of CO_2. However, the technique requires specialized equipment and operational expertise, limiting its widespread adoption.

2.4 Pressurized Liquid Extraction (PLE)

Pressurized Liquid Extraction, also known as accelerated solvent extraction, employs elevated temperatures and pressures to enhance extraction efficiency. The technique reduces extraction time and solvent consumption while maintaining high extraction yields. PLE has been successfully applied to the extraction of various secondary metabolite classes, including phenolics, terpenoids, and alkaloids. High-pressure technologies including pressurized liquid extraction have been reviewed for the recovery of bioactive molecules from various sources (Li et al., 2022).

2.5 Green Solvents

The development of green solvents has emerged as a complementary strategy to green extraction techniques. Natural Deep Eutectic Solvents (NADES) and Ionic Liquids (ILs) have gained attention as environmentally benign alternatives to conventional organic solvents (Cecchi et al., 2022; da Silva et al., 2022). Consumption of large volumes of toxic organic solvents in chemical processes can trigger both health and environmental problems (Cecchi et al., 2022). A diverse range of more sustainable solvents including deep eutectic solvents, bio-based solvents, supercritical carbon dioxide, and gas-expanded liquids has been introduced and their applications explored in both research and industry (Cecchi et al., 2022).

In recent years, a new class of green solvents called natural deep eutectic solvents (NADES) have been considered a valid alternative to conventional solvents (Cecchi et al., 2022). Compared with conventional organic solvents, NADES have attracted considerable attention since they are sustainable, biodegradable, and non-toxic but also are easy to prepare, and have low production costs (Cecchi et al., 2022). These solvents offer tunable physicochemical properties and biodegradability, addressing the environmental concerns associated with chlorinated hydrocarbons and volatile organic solvents (Cecchi et al., 2022).

Table 1: Comparison of Green Extraction Technologies for Plant Secondary Metabolites

Technique	**Principle**	**Advantages**	**Limitations**	**Suitable Compound Classes**
UAE *(Ultrasound-Assisted)*	Acoustic cavitation	Rapid extraction, low temperature, reduced solvent usage	Scalability challenges, risk of degradation	Alkaloids, phenolics, terpenoids
MAE *(Microwave-Assisted)*	Microwave heating	Very rapid, high yield, low solvent volume	Requires polar solvents, risk of thermal degradation	Phenolics, flavonoids, terpenoids
SFE *(Supercritical Fluid)*	Supercritical fluid solvation	Solvent-free, tunable selectivity	High equipment cost, specialized expertise required	Non-polar to moderately polar compounds

PLE *(Pressurized Liquid)*	Elevated pressure and temperature	Rapid extraction, reduced solvent usage	High equipment cost, risk of thermal degradation	Broad range of compound classes
NADES / ILs *(Deep Eutectic Solvents / Ionic Liquids)*	Unique solvating properties	Biodegradable, highly tunable selectivity	High viscosity, limited commercial availability	Polar compounds, phenolics

3 ADVANCED ANALYTICAL TECHNIQUES FOR IDENTIFICATION

3.1 Hyphenated Chromatographic-Mass Spectrometric Techniques

The characterization of plant secondary metabolites has been revolutionized by hyphenated techniques coupling separation methods with mass spectrometric detection. Hyphenated techniques make it possible to get broad chemical profiles of herbal extracts/medicine/formulations (Mukherjee et al., 2022). Liquid Chromatography-Mass Spectrometry (LC-MS) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) have become the methods of choice for the analysis of plant secondary metabolites, offering high sensitivity, selectivity, and the capability for structural elucidation (Mukherjee et al., 2022).

The hyphenated technique is an advanced form which is the link of a separation and spectroscopic technique to achieve the benefits of both (Mukherjee et al., 2022). The outstanding progresses in hyphenated systems have considerably extended their uses in natural products analysis (Mukherjee et al., 2022). The influence of linking separation and spectroscopic systems has been proved for both quantitative and qualitative analysis of unidentified phytoconstituents in the extracts/fractions (Mukherjee et al., 2022).

High-Resolution Mass Spectrometry (HRMS) has further enhanced analytical capabilities, enabling the accurate mass determination necessary for confident compound identification. The combination of HRMS with separation techniques provides comprehensive profiling of complex plant extracts, facilitating metabolomic studies and dereplication strategies.

Gas Chromatography-Mass Spectrometry (GC-MS) remains valuable for the analysis of volatile secondary metabolites, particularly terpenoids and essential oil components. The technique offers high chromatographic resolution and reproducible mass spectral libraries for compound identification.

3.2 Integrated Analytical Platforms

The integration of extraction and analytical techniques has emerged as a powerful strategy for streamlined natural product analysis. These integrated platforms reduce sample handling, minimize compound degradation, and enhance overall analytical efficiency. Case studies have demonstrated successful profiling of various alkaloids and other secondary metabolites using integrated approaches.

Table 2: Hyphenated Techniques for Secondary Metabolite Identification

Technique	Separation	Detection	Application	Sensitivity	Selectivity
LC-MS	Liquid chromatography	Mass spectrometry	Non-volatile compounds	High	High
LC-MS/MS	Liquid chromatography	Tandem MS	Structural elucidation	Very high	Very high
GC-MS	Gas chromatography	Mass spectrometry	Volatile compounds	High	High
HPLC-DAD-MS	High-performance LC	Diode array + MS	Comprehensive profiling	High	High
UHPLC-HRMS	Ultra-high performance LC	High-resolution MS	Metabolomics	Very high	Very high

4 INTEGRATION STRATEGIES AND EMERGING TRENDS

The integration of extraction and analytical techniques represents a significant advancement in natural product research. These integrated approaches offer enhanced efficiency, reduced compound degradation, and improved throughput. Emerging trends include the development of microextraction techniques for minimal sample consumption, ambient mass spectrometry for rapid analysis, and AI-driven process optimization.

The combination of green extraction techniques with advanced hyphenated analytical platforms enables comprehensive characterization of complex secondary metabolite mixtures. The application of statistical methods in the evaluation of chemical processes is considered as one of the main principles of green chemistry that can be useful for improvements in the efficiency of chemical procedures (Cecchi et al., 2022). Ultrasonic technology has also been combined with other processing technologies to improve quality, stability, safety and security (Yusoff et al., 2022).

Despite these advances, challenges including matrix interference, high equipment costs, and the need for skilled operators persist (da Silva et al., 2022). The ongoing development of user-friendly platforms and comprehensive spectral databases promises to address these limitations.

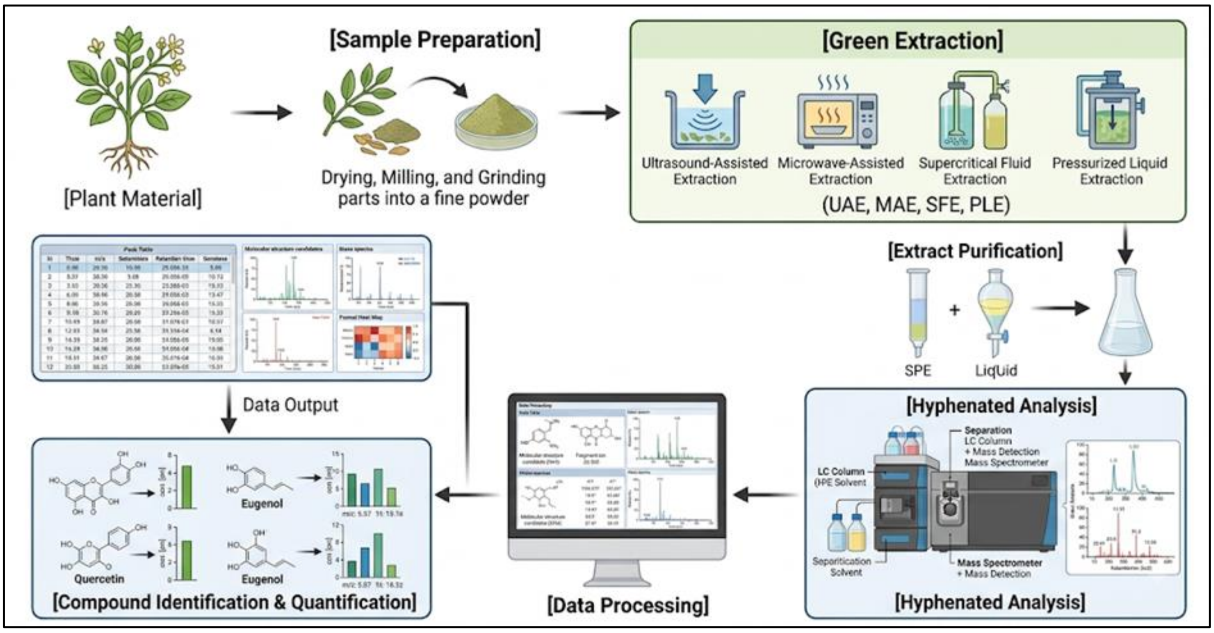

Figure 1: Schematic Representation of Integrated Extraction and Analytical Workflow for Plant Secondary Metabolite Analysis

Figure 1 illustrates the comprehensive workflow for plant secondary metabolite analysis, integrating green extraction technologies with advanced hyphenated analytical platforms for streamlined natural product characterization.

5 FUTURE PERSPECTIVES

The future of secondary metabolite extraction, isolation, and identification lies in the continued integration of green technologies with advanced analytical platforms. The application of artificial intelligence and machine learning approaches are expected to play increasing roles in process optimization, compound identification, and data interpretation. The development of comprehensive spectral databases and the application of molecular networking strategies will accelerate dereplication and compound discovery.

Emerging trends include the application of microextraction techniques for minimal sample consumption, ambient mass spectrometry for rapid analysis, and the development of portable analytical devices for field applications. The continued evolution of green solvents, including the development of designer solvents with tailored properties, promises to further enhance extraction efficiency and selectivity. The integration of chemometric approaches for the optimization of processes related to the extraction of bioactive compounds is also expected to advance (Cecchi et al., 2022).

6 CONCLUSION

Innovations in extraction, isolation, and identification technologies have transformed the field of natural product research. Green extraction techniques including UAE, MAE, SFE, and PLE offer significant advantages over conventional methods, including reduced environmental impact, enhanced efficiency, and improved compound integrity (da Silva et al., 2022; Microwave: An Ecologically Innovative, 2022; Srivastava et al., 2022; Li et al., 2022). Advanced hyphenated analytical platforms enable comprehensive profiling of complex secondary metabolite mixtures, facilitating drug discovery and quality control (Mukherjee et al., 2022). The development of green solvents including NADES and bio-based solvents offers sustainable alternatives to conventional organic solvents (Cecchi et al., 2022). The continued integration of these technologies, coupled with emerging computational approaches, promises to accelerate natural product research and development.

REFERENCES

[1] Cecchi, A., Cecchi, L., and Cecchi, M. (2022). Natural Deep Eutectic Solvents (NADESs) Combined with Sustainable Extraction Techniques: A Review of the Green Chemistry Approach in Food Analysis. Foods, 12(1), 56.

[2] da Silva, R.F., Carneiro, C.N., do C. de Sousa, C.B., et al. (2022). Sustainable extraction bioactive compounds procedures in medicinal plants based on the principles of green analytical chemistry: A review. Microchemical Journal, 175, 107184.

[3] Li, J., Pettinato, M., Campardelli, R., De Marco, I., and Perego, P. (2022). High-Pressure Technologies for the Recovery of Bioactive Molecules from Agro-Industrial Waste. Applied Sciences, 12(7), 3642.

[4] Microwave: An Ecologically Innovative, Green Extraction Technology. (2022). Current Analytical Chemistry, 18(8).

[5] Mukherjee, P.K., Kar, A., Biswas, S., Chaudhary, S.K., and Banerjee, S. (2022). Hyphenated analytical techniques for validation of herbal medicine. In Elsevier eBooks (pp. 811-827). Elsevier.

[6] Srivastava, R., Kumar, A., and Singh, P. (2022). Intensification of supercritical fluid in the extraction of flavonoids: A comprehensive review. Process Biochemistry, 117, 45-58.

[7] Yusoff, I.M., Mat Taher, Z., Rahmat, Z., and Chua, L.S. (2022). A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. Food Research International, 157, 111268.

[8] Wong, Y.S., Yusoff, R., and Ngoh, G.C. (2022). Phenolic compounds extraction by assistive technologies and natural deep eutectic solvents. Reviews in Chemical Engineering.

[9] Alvi, T., Asif, Z., and Iqbal Khan, M.K. (2022). Clean label extraction of bioactive compounds from food waste through microwave-assisted extraction technique-A review. Food Bioscience, 101580.

[10] Joshi, R.R., Gupta, K.R., and Patil, S.S. (2022). Hyphenated technique- a boon to analytical world. International Journal of Pharma Sciences, 3(11), 4184-4191.