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ADVANCES IN GREEN AND CONVENTIONAL EXTRACTION METHODS FOR PHYTOCHEMICALS IN HALOPHYTIC PLANTS

Nicoleta-Olimpia CIOARĂ (ANDREI)[1, 2], Amalia Carmen MITELUȚ[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăști Blvd, District 1, Bucharest, Romania
[2]National Institute of Research and Development for Biological Sciences,
296 Splaiul Independentei, 060031, District 6, Bucharest, Romania

Corresponding author email: nicol_andrei@yahoo.com

Abstract

Halophytes are plants adapted to grow in high-salinity environments such as salt marshes and coastal areas. Halophytic plants exhibit biochemically distinct metabolite profiles that depend on various species (e.g., Salicornia sp., Suaeda sp.) and the extraction methodology; therefore, method selection must be tailored to the target compound class and the particular taxon studied. The modern green techniques, as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE) and natural deep eutectic solvent (NADES)/deep eutectic solvent (DES) based extractions, systematically increase mass transfer, reduce solvent consumption, and often improve recovery and the preservation of labile phenolics and lipophilic constituents relative to conventional maceration or Soxhlet extraction. In conclusion, rational extraction-process design enables the selection of efficient and sustainable extraction workflows that maximize the recovery of bioactive compounds while minimizing environmental degradation and solvent footprint.

Key words: *green extractions, halophytes, conventional extraction, phytochemicals, eco-friendly solvents.*

INTRODUCTION

Halophytes represent a specialized group of salt-tolerant flora characterized by their ability to proliferate in substrates featuring high osmotic pressure and ionic toxicity (Lopes et al., 2021; Cioara (Andrei) & Mitelut, 2025). These organisms are distributed across diverse bioclimatic zones, where they have evolved complex adaptive mechanisms to navigate hypersaline conditions. This specialized physiological adaptation positions halophytes as viable candidates for alternative agriculture within saline environments. Furthermore, these taxa represent significant repositories of essential nutrients and secondary metabolites, offering substantial potential for integration into the food and pharmaceutical sectors, and not only (Oulahal & Degraeve, 2022; Palos-Hernández et al., 2022; 2025; Ribas-Taberner et al., 2025). While numerous halophytic species biosynthesize an extensive repertoire of bioactive compounds with pronounced economic and therapeutic utility, their utility is often complicated by the concomitant accumulation of supra-optimal concentrations of sodium and chloride ions. Traditional extraction techniques, such as maceration or Soxhlet extraction, continue to be widely applied; however, modern green extraction methods, including supercritical fluid extraction or ultrasound-assisted extraction, provide improved efficiency and greater environmental sustainability (Balkrishna et al., 2024).

The extraction of bioactive constituents from halophytes has garnered considerable interest in recent years, driven by the escalating demand for natural and functionally active compounds across the pharmaceutical, food, and cosmetic sectors. Bioactive constituents present in these extracts - including polyphenols, flavonoids, alkaloids, terpenoids, and glycosides - exhibit a wide spectrum of pharmacological activities, notably antioxidant, anti-inflammatory, and antimicrobial effects (Giordano et al., 2021; Aware et al., 2022; Hulkko et al., 2022). Owing to their diverse biological properties, these metabolite compounds have attracted considerable interest for their incorporation into pharmaceutical products, nutraceuticals, functional foods, and cosmetic formulations (Balkrishna et al., 2024). Nevertheless, the

effective translation of their therapeutic potential into practical applications is highly dependent on the implementation of high-performance extraction technologies capable of maximizing extraction while preserving the chemical stability and biological functionality of these compounds.

Emerging extraction paradigms - encompassing green extraction methodologies and nanotechnology-enhanced approaches - are explored with particular focus on their potential to further augment extraction performance and sustainability (Chongo, 2025). The integration of environmentally benign solvents, process intensification techniques, and nanoscale extraction platforms represents a promising frontier in bioactive compound recovery.

Bibliometric data from research and experimental results from recent studies are incorporated to elucidate the application of different extraction techniques for target classes of bioactive compounds, including phenolics, flavonoids, alkaloids, and essential oils (Chaves et al., 2020; Chávez-González et al., 2020). These examples highlight comparative extraction results, retained bioactivity, and potential industrial utility of the resultant extracts (Usman I. et al., 2022; Usman M. et al., 2023).

Recent developments in extraction methodologies have arisen in response to expanding scientific and industrial interest in the bioactive compounds. These innovative methodologies are designed to isolate these compounds from diverse biomass and from natural matrices with greater specificity and reduced environmental burden compared to traditional extraction techniques (Sanoja-López et al., 2024). Phytochemicals, bioactive constituents of plant origin, have attracted considerable scholarly and commercial attention due to their wide-ranging therapeutic properties and utility across pharmaceutical, nutraceutical, and cosmetic industries (de Lima Cherubim et al., 2020). Efficient recovery of these compounds constitutes a crucial step in exploiting their functional potential.

Emerging extraction technologies, including supercritical fluid extraction (SFE). microwave-assisted extraction (MAE). ultrasound-assisted extraction (UAE), instant controlled pressure drops, pressurized liquid extraction (PLE), and negative pressure cavitation, have demonstrated enhanced yields, diminished solvent consumption, and improved environmental sustainability relative to conventional methods (Sanoja-López et al., 2024).

For example, SFE using supercritical carbon dioxide affords selective isolation of phenolics, essential oils, and antioxidants while mitigating the need for hazardous organic solvents and preserving thermally labile constituents (Wang et al., 2021; Wang et al., 2024). Similarly, ultrasonic and microwave-assisted techniques facilitate disruption of plant cell walls and improve mass transfer, thereby accelerating extraction kinetics and reducing energy input. Synergistic combinations of extraction approaches (e.g., ultrasonic–microwave-assisted extraction) further underscores the potential for optimizing recovery efficiency.

Recent evidence underscores the potential of integrating disparate extraction modalities to optimize both the quantitative recovery of metabolites and the preservation of their biofunctional attributes (Jha & Sit, 2022). The fundamental rationale for such "hybrid" or "coupled" extraction strategies is to capitalize on the discrete strengths of various techniques, thereby inducing a synergistic effect that transcends the inherent limitations and extractive capacities of any singular methodology (Capaldi et al., 2024).

By strategically sequencing or concurrently applying diverse energy sources - such as acoustic cavitation, electromagnetic radiation, or enzymatic catalysis - researchers can achieve higher process intensification, leading to superior mass transfer rates and the stabilization of sensitive molecular structures that might otherwise be compromised in traditional monolithic systems.

Integration of these advanced extraction strategies within biorefinery frameworks underscores their alignment with circular economy principles and valorisation of plant biomass toward zero-waste paradigms (Alara et al., 2021; Alvi et al., 2022).

As scientific literature has mentioned, several factors such as the types of plant materials, extraction techniques/time, solvents, pH, temperature, pressure, and material-to-solvent ratio can influence the extraction efficiency of bioactive compounds from plant matrices.

Therefore, further investigations to optimize these factors could be advantageous for recovering bioactive compounds from halophyte extracts.

Sophisticated analytical methodologies are pivotal in the characterization and identification of secondary metabolites derived from halophytic matrices. Phytochemical profiling serves as a fundamental cornerstone in elucidating the bioactive constituents within these salt-tolerant taxa, providing the empirical basis for their pharmacological evaluation (Pai et al., 2022). For initial separation and chromatographic fingerprinting, thin-layer chromatography (TLC) and its high-performance evolution (HPTLC) function as economically viable diagnostic tools for high-throughput preliminary screening.

Conversely, high-performance liquid chromatography (HPLC) and its advanced iterations - ultra-high-performance (UHPLC) and ultra-performance liquid chromatography (UPLC) - remain the established benchmark for the sensitive quantification of polyphenolic compounds, flavonoids, and glycosides (Chuo et al., 2022). For the analysis of lipophilic and volatile fractions, gas chromatography-mass spectrometry (GC-MS) is uniquely optimized for profiling volatile oils and terpenoid assemblages (Hubschmann, 2025).

The synergy of these integrated analytical approaches fortifies quality control protocols, ensures rigorous standardization, and accelerates the trajectory of natural product-based drug discovery. Consequently, systematic phytochemical analysis continues to serve as a critical nexus, bridging ethnobotanical traditional knowledge with the rigorous requirements of contemporary pharmaceutical applications (Kumar et al., 2023).

A paramount challenge in the field of halophyte extraction involves the rigorous standardization of extracts, as the phytochemical profile of an extract is subject to significant fluctuations dictated by taxonomic specificity, geoclimatic provenance, environmental stressors, and the phenological stage at the time of harvest.

This inherent biochemical heterogeneity complicates the maintenance of inter-batch consistency, posing a substantial impediment to the development of pharmaceutical and nutraceutical formulations, where the preservation of specific bioactivity levels and toxicological safety profiles is mandatory. To mitigate these challenges, sophisticated analytical platforms, including high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) (Hubschmann, 2025), and nuclear magnetic resonance (NMR) spectroscopy, have become indispensable for the comprehensive metabolomic profiling and qualitative standardization of halophyte extracts.

The integration of these advanced instrumental techniques not only facilitates enhanced reproducibility across production cycles but also provides a quantitative framework for identifying optimized extraction methodologies that ensure the maximum retention of bioactive constituents.

The present review synthesizes recent progress in phytochemical extraction, critically examining the influence of novel techniques on human health outcomes, ecological sustainability, and industrial applications (Custodio et al., 2022). It also delineates current challenges and prospects for improving extraction efficiency and eco-sustainability, including the adoption of innovative solvents, process intensification strategies, and hybrid technologies.

Extraction methods of phytochemical constituents

The extraction of bioactive compounds represents the fundamental and most critical prerequisite for the subsequent separation and characterization of phytochemical constituents (Mandal et al., 2015; Shikov et al., 2022; Bommakanti et al., 2023; Bouizgma et al., 2025).

Phytochemical extraction separates bioactive constituents from plant matrices using methods chosen based on compound polarity, stability, and desired purity, applying conventional techniques like maceration, decoction, or Soxhlet extraction (solvent-based, often high-temperature), or modern, efficient methods such as Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE), High Voltage Electrical Discharge (HVED-assisted extraction) (Abbaspour et al., 2024) or Supercritical Fluid Extraction (SFE) (Verma &

Himshweta, 2023; Veršić Bratinčević et al., 2023).

This process necessitates a series of preparatory stages - including pre-washing, dehydration (via solar desiccation or lyophilization) and pulverization in order to ensure sample homogeneity and to maximize the surface-area-to-volume ratio, thereby enhancing solvent penetration and mass transfer. Effective extraction requires careful solvent selection: polar (water/ethanol) for phenolics or non-polar (hexane) for terpenoids, to ensure maximum yield and stability of compounds (Mgoma et al., 2025).

1. Conventional Extraction Methods

These are widely used, simple, and cost-effective methods, though they often require longer times and higher solvent volumes (Manousi et al., 2019; Martins et al., 2023).

✓ Maceration: Plant material is soaked in a solvent (e.g., ethanol, water) at room temperature for several days, requiring occasional stirring.

✓ Percolation: Solvent is passed slowly through a packed column of plant powder, ensuring continuous exposure to fresh solvent, making it more efficient than maceration.

✓ Soxhlet Extraction: Used for continuous extraction, where the sample is placed in a thimble and continuously washed with hot, vaporized solvent. It is efficient for lipids and less-soluble compounds but is not suitable for heat-sensitive substances.

✓ Hydrodistillation & Steam Distillation: Used primarily to extract volatile essential oils. Plant material is boiled in water, with compounds separating based on volatility and immiscibility.

✓ Decoction & Infusion: Specifically for water-soluble, heat-stable compounds (decoction) or delicate herbs (infusion) in water. Best for water-soluble and thermostable compounds.

2. Advanced/Modern Extraction Methods

These methods are faster, more efficient, and use less solvent (Green Extraction) (Zhang et al., 2020; Yeasmen & Orsat, 2023; Pogorzelska-Nowicka et al., 2024; Zaky et al., 2024).

➢ Ultrasound-Assisted Extraction (UAE): Uses high-frequency sound waves to induce cavitation, breaking cell walls to release compounds and improving mass transfer (Yusoff et al., 2022; Shen et al., 2023; Tymczewska et al., (2023)

➢ Microwave-Assisted Extraction (MAE): Uses electromagnetic energy to heat the sample directly, accelerating extraction and breaking down cell walls via internal pressure. Highly efficient for polar compounds.

➢ Supercritical Fluid Extraction (SFE): Employs supercritical fluids (usually) to extract lipophilic compounds under specific temperature (40–80°C) and pressure (35–70 MPa) making it ideal for thermolabile compounds and non-polar lipophilic substances (terpenoids, carotenoids).

➢ Pressurized Fluid Extraction (PFE)/Accelerated Solvent Extraction (ASE): Uses elevated pressure (50-300 psi) and temperature (50-200°C) to keep solvents in a liquid state, above their boiling point, which significantly increases extraction speed and efficiency.

➢ Enzyme-Assisted Extraction (EAE): Uses enzymes (e.g., cellulase, pectinase) to break down plant cell walls, releasing compounds (Costa et al., 2020).

➢ Pulsed Electric Field (PEF) Extraction: A non-thermal technique that increases cell membrane permeability (electroporation).

➢ Natural Deep Eutectic Solvent (NADES) Extraction: A green method using biocompatible, biodegradable solvents (Costa et al., 2022; Aktaş & Kurek, 2024; Suthar, 2025).

Table 1 Extraction of biochemical compounds from halophytes

No.	Biochemical compounds		Best Methods	Solvents	Conditions	Resources
1	Polyphenols (Flavonoids, Tannins, Phenolic Acids)		MAE, UAE and SFE are favored for high efficiency. EAE is used to break down cell walls to release bound polyphenols.	Polar solvents (methanol, ethanol, water, acetone) or their mixtures (70% ethanol-water).	Moderate temperatures (40°C–60°C) are preferred to avoid thermal degradation.	Chávez-González et al., 2020 Alara et al., 2021 Alvi et.al., 2022 Bener et al., 2022 Anaya-Esparza et al., 2023 Chociej et al., 2024 Bouizgma et al., 202
2	Terpenoids (Essential Oils, Monoterpenes, Sesquiterpenes)		HD is the traditional standard, while DS is used for volatile components. For higher quality and yield is used particularly for non-polar terpenoids.	Non-polar solvents like hexane, petroleum ether, or chloroform.	Room temperature for maceration (to protect delicate oils) or high-temperature distillation for stable terpenoids.	Usman I. et al., 2022 Usman Mm et al., 2023
3	Alkaloids		Percolation or maceration	Ethanol, ether, or chloroform.	Acidified alcohol is often used to form salts, increasing solubility.	Mandal et al., 2015 Soares et al., 2023
			Modern techniques like MAE or UAE are used to enhance yield.			Bagade & Patil, 2021 Pogorzelska-Nowicka, 2024
4	Pigments	Carotenoids/Chlorophylls (Lipophilic)	Soxhlet, cold press methods or green extractions (NADES, SFE)	Non-polar solvents (hexane, petroleum ether, chloroform, acetone)	Low temperature and protection from light are critical due to instability.	Bashir et al., 2023 Hulkko et al., 2022
		Anthocyanins (Hydrophilic)		Acidified polar solvents (methanol or ethanol with HCl).		Wu et al., 2020
5	Polysaccharides and Dietary Fiber		Hot water extraction or decoction.	Water.	Water extraction followed by ethanol precipitation (water extraction and ethanol precipitation - WEEP).	Espro et al., 2021 Martins et al., 2023 Soares et al., 2023
6	Proteins and Amino acids		Enzyme-Assisted Extraction (EAE)	Aqueous buffers (acetic acid/sodium acetate)	Low temperatures	Mandal et al., 2015 Pogorzelska-Nowicka, 2024
7	Glycosides (e.g., Saponins) and Alkaloids		Maceration, percolation or reflux extraction.	Aqueous ethanol or methanol.	Can break down the cell wall matrix to improve yield	Mandal et al., 2015
			Enzyme-assisted extraction or chromatography-based methods	Cellulases/pectinases (enzymes derived from *Aspergillus sp., Bacillus sp.*)		Martins et al., 2023 Mgoma et al., 2025

MATERIALS AND METHODS

A comprehensive and systematic literature search was performed across the Scopus, PubMed, and Web of Science databases. The comprehensive search encompassed included studies, articles, review papers, and books published from 2020 onward, as publications prior to this period did not address the central focus of the present study (green extraction methodologies) (Yeasmen et al., 2021; Kraemer et al., 2025). The search was limited to English-language articles published within selected dates in the databases.

The search strategy combined terms related to halophytes, types of extraction, bioactive compounds, and kinds of solvents using the following Boolean expression: (“extract” OR “extraction” OR “extracting”) AND (“solvent” OR “soluble” OR “solution”) AND (“bioactive compounds” OR “phytochemicals” OR “phytonutrients”) AND halophyte.

To further refine the results, a secondary search component will be incorporated using the Boolean operator “AND NOT” with the following terms: “chemical composition” OR “nutraceutical” OR “bioresources”. This approach is implemented to exclude studies not pertaining to halophytes and those extracts.

Additionally, the reference lists of all included articles were manually screened to identify further relevant studies and ensure comprehensive coverage of the literature. The reference lists predominantly include studies from recent years, as halophytes have only relatively recently become the subject of extensive scientific investigation and analytical research.

VOSviewer is a software tool for constructing and visualizing bibliometric networks. These networks may include journals, researchers, or individual publications, and they can be constructed based on citation, bibliographic coupling, co-citation, or co-authorship relations (https://guides.lib.purdue.edu/c.php?g=706710&p=10841603).

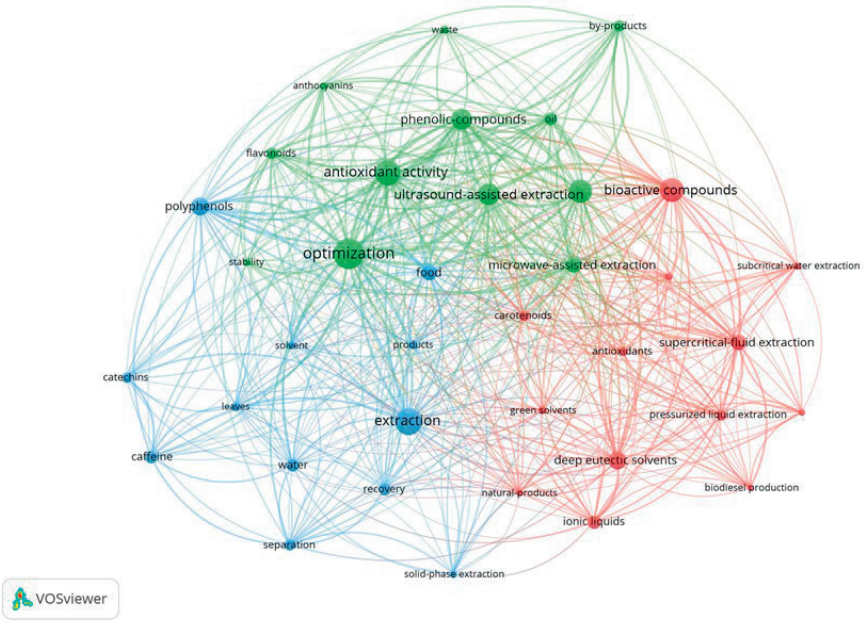

Fig. 1 Map based on bibliographic data

Cluster 1, highlighted in red, includes 13 keywords closely linked to the green extraction of natural products. Central to this cluster is "bioactive compounds", which represents bioactive compounds that combat free radicals, enhancing health benefits. The term "natural products" further emphasizes their effectiveness in applications like health, nutraceuticals, etc. Together, these keywords underscore the interconnectedness of sustainable practices and the extraction of beneficial compounds in green extraction research.

Cluster 2, highlighted in green, encompasses 12 keywords that are vital for understanding green extraction. The term "optimisation" serves as a core concept, referring to the process of isolating valuable compounds from natural sources. The keyword "antioxidant activity" indicates the focus on bioactive compounds that provide health benefits by mitigating oxidative stress. Together, these keywords illustrate the essential components and innovative techniques that characterize green extraction, emphasizing the importance of sustainability and the extraction of beneficial compounds.

Cluster 3, highlighted in blue, comprises five key terms that are essential for understanding the green extraction of natural products. The term "extraction" refers to natural substances that have significant effects on living organisms, playing a crucial role in health and nutrition. "Polyphenols" encompass the various effects these bioactive compounds have, including antioxidant, anti-inflammatory, and antimicrobial properties, highlighting their potential applications.

RESULTS AND DISCUSSIONS

Conventional extraction methods and their limitations

Decoction, infusion, and maceration represent three fundamental solid-liquid extraction methodologies extensively utilized for the isolation of bioactive secondary metabolites from diverse botanical matrices (Hidayat & Wulandari, 2021). Each technique is governed by distinct thermodynamic and kinetic parameters, which dictate its specific procedural application and resultant extractive efficiency. A critical appraisal of the operational nuances inherent to these processes is imperative for the selection of an optimized extraction protocol, as the efficacy of the methodology is strictly contingent upon the morphological characteristics of the plant material, such as

lignification and tissue density, and the physicochemical stability of the target phytoconstituents.

Decoction represents a rigorous thermal extraction methodology involving the sustained boiling of vegetal material within an aqueous medium to facilitate the liberation of bioactive secondary metabolites. This technique is specifically efficacious for the processing of recalcitrant and highly lignified tissues, such as rhizomes, barks, and seeds, where elevated temperatures are requisite to disrupt the robust cellular architecture and enhance the mass transfer of intracellular solutes.

Notwithstanding its utility, decoction is subject to several inherent limitations, including prolonged processing durations and the potential for the thermal degradation of heat-sensitive nutritional constituents. Furthermore, continuous exposure to high temperatures frequently induces the volatilization of essential oils, leading to a substantial loss of volatile phytochemicals and a potential alteration in the qualitative pharmacological profile of the resulting extract.

Infusion represents a moderate thermal extraction methodology characterized by the submersion of botanical matrices in either ambient or sub-boiling aqueous solvents, deliberately avoiding the rigorous kinetic conditions of decoction. This technique is primarily indicated for the processing of non-lignified tissues (foliar, floral, and herbaceous structures) where the preservation of labile volatile constituents is prioritized over exhaustive leaching.

While advantageous for maintaining the chemical integrity of sensitive compounds, infusion typically demonstrates a reduced total extractive yield relative to more aggressive thermal methods, as it lacks the thermal energy required to penetrate recalcitrant cellular barriers effectively. Furthermore, the methodology often proves inadequate for the comprehensive recovery of bioactive metabolites from specialized taxa, such as halophytes, where complex physiological adaptations and specialized integuments may necessitate more robust extraction parameters to facilitate complete solute liberation.

Maceration represents a classical solid-liquid extraction methodology traditionally employed in the preparation of phytopharmaceuticals, remaining a cornerstone of natural product isolation due to its inherent cost-effectiveness and technical simplicity. During this process, crushed botanical matrices are submerged in an appropriate solvent inside a hermetically sealed vessel. The system is maintained in a static state for a prolonged incubation period, ranging from several hours to several days, augmented by intermittent agitation to minimize boundary layer resistance and facilitate solute transport. This extended contact time allows for passive intra-particle diffusion of the solvent across the semi-permeable plant cell walls. Consequently, the solvent facilitates the solvation and subsequent leaching of intracellular bioactive secondary metabolites into the bulk liquid phase until a thermodynamic equilibrium is approached. Unfortunately, the process has several major disadvantages: very slow and time-consuming, consuming, and the solvent required is considerable.

The percolation technique utilizes a conical, narrow-necked apparatus known as a percolator, characterized by apertures at both the proximal and distal ends. In this technique, the solid matrix is pre-moistened with a solvent prior to its introduction into the vessel to facilitate the efficient extraction of bioactive compounds (Luksta & Spalvins, 2023). Percolation offers several distinct advantages over traditional maceration, including enhanced temporal efficiency, the ability to isolate thermolabile constituents without thermal degradation, and high suitability for the extraction of potent or high-value pharmacological agents. Conversely, the technique is subject to certain limitations, most notably substantial solvent consumption and extended processing durations when compared to Soxhlet extraction. Furthermore, the process requires work experience, as the particle size of the solid material is a critical parameter to ensure optimal extraction kinetics and prevent the formation of preferential flow paths.

The **Soxhlet extraction** protocol constitutes a preeminent methodology for the continuous, iterative solid-liquid extraction of botanical or synthetic matrices via a heated organic solvent within a dedicated glass refluxing assembly (López-Bascón & De Castro, 2020). As a highly standardized and well-established technique, it

frequently exhibits superior performance and higher recovery yields relative to conventional batch extraction methods. The defining feature of the Soxhlet mechanism is the cyclic exposure of the comminuted matrix to fresh, distilled solvent, which maintains a maximal concentration gradient and ensures the exhaustive leaching of solutes into the bulk liquid phase. While the method is widely utilized in the isolation of nutraceuticals and offers a cost-effective operational framework, it is subject to several inherent limitations. Notably, although the user mentions its use for thermolabile compounds, the continuous boiling of the extract in the collection flask frequently poses a significant risk of thermal degradation for heat-sensitive constituents. Furthermore, the process is characterized by prolonged extraction durations, high solvent-to-feed ratios, and the absence of mechanical agitation within the extraction thimble, which may limit the kinetics of mass transfer for certain recalcitrant analytes.
Hydrodistillation (HD) and **Steam Distillation (SD)** are conventional methods used to extract essential oils by vaporizing volatile compounds from plant material using heat and steam, which are then condensed and separated. HD involves immersing plant material in boiling water, while SD passes steam through the halophytic material. SD is often better for reducing distillation time, whereas HD can cause decay due to prolonged contact with boiling water.
Although HD can often result in higher yields for some halophytes, SD can produce higher-quality oils in specific applications.

Advanced extraction techniques for enhanced bioactivity

Microwave-assisted extraction (MAE) operates through the dual mechanisms of dipole rotation and ionic conduction, driven by the displacement of charged ions within both the solvent and the botanical matrix (Camel, 2000; Mketo & Nomngongo, 2023). While MAE is highly effective for the isolation of specific polyphenolic subclasses, such as flavonoids, its application is often constrained by the intense thermal kinetics involved (Aguiar et al., 2022). Consequently, more sensitive phytochemicals, including tannins and anthocyanins, may undergo significant thermal degradation or structural transformation, necessitating precise calibration of irradiation power and duration to preserve the biochemical integrity of the extract (Bhadange et al., 2024).
Ultrasound-assisted extraction (UAE) utilizes acoustic energy at frequencies exceeding 20 kHz to facilitate the mechanical disruption of botanical cell walls, thereby augmenting the effective surface area available for solvent penetration and the subsequent liberation of intracellular secondary metabolites (Dzah et al., 2020; Demesa et al., 2024). UAE accelerates extraction kinetics and significantly reduces both processing durations and solvent consumption while maximizing the total extractive yield. However, the intense energy levels associated with UAE may initiate sonochemical reactions, necessitating careful optimization of sonication power and pulse cycles to preserve the biochemical integrity of the extract (Anaya-Esparza et al., 2023, Bagade & Patil, 2021).
Supercritical fluid extraction (SFE) represents a sophisticated analytical and industrial-scale separation methodology that employs fluids maintained beyond their thermodynamic critical temperature and pressure as the primary solvent. Supercritical carbon dioxide remains the preeminent medium for SFE due to its chemically inert nature, inherent non-toxicity, and favourable critical parameters (Tc=31.1 C, Pc =73.8 bar), which facilitate the extraction of labile bioactive compounds under relatively mild thermal conditions (Dias et al., 2021; Herzyk et al., 2024). While is the established benchmark for most phytopharmaceutical and nutraceutical applications, alternative fluids such as ethane, propane, or even supercritical water may be strategically employed depending on the specific polarity, molecular weight, and solubility requirements of the target analytes.
Accelerated Solvent Extraction (ASE), also widely recognized as **Pressurized Fluid Extraction (PFE)** is characterized by minimized solvent consumption, high analytical throughput, and significantly expedited extraction kinetics compared to conventional methodologies.
To achieve optimal recovery yields of specific secondary metabolites, several critical process parameters must be meticulously calibrated; these include, but are not limited to, the

extraction temperature (T), hydrostatic pressure (P), and the duration of static and dynamic extraction cycles. The precise control of these variables allows for the exhaustive leaching of phytochemicals while maintaining a high degree of reproducibility across complex biological samples.

Enzyme-assisted extraction (EAE) is predicated on the biocatalytic cleavage of covalent linkages within the plant cell wall matrix, typically requiring an aqueous environment to facilitate enzymatic activity. By targeting specific structural polysaccharides such as cellulose, hemicellulose, and pectin, these enzymes induce the systematic disintegration of the cellular architecture, thereby significantly augmenting the permeability of the botanical substrate (Vo et al., 2023)

Notably, enzymatic reactions proceed with high efficiency under relatively mild conditions - typically characterized by moderate pH levels and low thermal input - over a condensed temporal framework ranging from several minutes to a few hours. In the majority of experimental cases, the integration of an enzymatic pretreatment phase demonstrates a substantial positive correlation with overall extraction efficacy and the qualitative enrichment of the final biochemical profile.

Deep eutectic solvents (DES) are defined as eutectic systems comprising stoichiometric mixtures of hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs), characterized by a significant depression in melting point relative to their individual components and highly correlated properties. (Serna-Vázquez et al., 2021; Aduloju et al., 2023). A specialized subclass, **natural deep eutectic solvents (NADES)**, is uniquely formulated from primary metabolites - including amino acids, saccharides, and organic acids - that are endogenously present within biological systems (Alam et al., 2021; Ferreira & Sarraguça, 2024). This intrinsic composition ensures a high degree of biocompatibility and environmental sustainability (Stanisz et al., 2024).).

Their favourable ecological profile is further underscored by their inherent biodegradability and negligible toxicity, particularly when composed of plant-derived constituents (Socas-Rodríguez et al., 2021; Bashir et al., 2023). Furthermore, NADES-mediated extractions are effectively conducted under mild operational conditions, which are critical for preserving the molecular integrity of thermolabile constituents while simultaneously reducing total energy consumption (Pinho et al., 2024). Collectively, these attributes establish NADES as a transformative medium in green chemistry, offering a synergistic balance of operational efficiency and environmental stewardship for modernized extraction processes (Ivanovic et al., 2020; Lu & Liu, 2020).

Pulsed Electric Field (PEF) technology represents a non-thermal, ecologically sustainable processing modality based on the application of high-intensity, short-duration electrical pulses. The fundamental mechanism of PEF involves the induction of electroporation, a phenomenon whereby the application of an external electric field exceeding the membrane's critical dielectric strength results in the formation of transient or permanent aqueous pores. This methodology is particularly effective for the selective isolation of proteins, bioactive secondary metabolites, and natural pigments from diverse vegetal substrates.

Factors influencing extraction efficiency and bioactivity

Extraction efficiency and the bioactivity of compounds are primarily influenced by solvent polarity, temperature, extraction time, pH, etc. Optimal conditions (e.g., proper solvent, moderate heat) maximize yield, while extreme conditions (high heat/long time) can degrade compounds, reducing bioactivity (Khan & Dashti, 2023). Advanced methods like UAE/MAE offer higher efficiency.

The cumulative yield of phytochemical extraction is intrinsically contingent upon the unique physicochemical attributes and structural stabilities of the target analytes. Empirical evidence underscores that a specific set of operational parameters - such as temperature, solvent polarity, or pH - may exert divergent or even antagonistic effects on the recovery of distinct phytochemical classes (Wu et al., 2020). For instance, while elevated temperatures may enhance the solubility and mass transfer of certain thermostable polyphenols, the same thermal energy can simultaneously induce the

oxidative degradation of more sensitive constituents, such as anthocyanins, thereby resulting in an inverse correlation between extraction efficiency and yield across disparate molecular species within the same botanical matrix (Veršić Bratinčević et al., 2023).
This phenomenon necessitates a multi-objective optimization approach when attempting to isolate a broad spectrum of secondary metabolites, as the "optimal" conditions for one chemical species may be detrimental to the recovery of another (Qasim et al., 2016).

A one-way analysis of variance (ANOVA) test can be applied to detect differences among the different extraction techniques (used at a given time) in terms of the extraction yield amount affected by solvent type and extraction time. Each test is conducted at least three times, and the data are presented as the mean ± standard deviation (SD). The data results are calculated using Microsoft Excel and analyzed with Prism software until the differences are significant at $p < 0.05$.

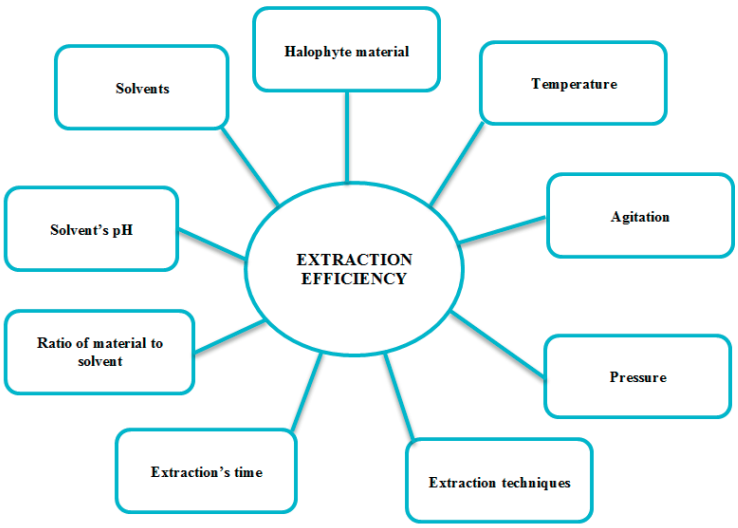

Fig. 2 Factors that can influence the extraction efficiency of halophyte's bioactive compounds

• **Solvent Selection and Polarity:** The most critical factor is solvent polarity, must match the target compounds (e.g., water/ethanol for polar phenolics and hexane for non-polar). Natural Deep Eutectic Solvents (NADES) are emerging as a sustainable, non-toxic, and highly efficient alternative to conventional organic solvents, particularly for extracting bioactive compounds from plants and food waste (Prabhune & Dey, 2023; Foroutani et al., 2024). Composed of natural metabolites such as choline chloride, sugars, or organic acids, they are characterized by strong hydrogen bonding, low volatility, and high customizability. Conventional solvents, traditionally used for extraction and industrial processes, are primarily characterized by their volatility, high solvency power, and petroleum-derived origins, but they are increasingly scrutinized due to environmental and health hazards (Claux et al., 2021).

• **Temperature:** higher temperatures generally increase solubility and mass transfer, but excessive heat can degrade bioactive compounds (Antony & Farid, 2022)

• **Extraction time** and kinetics**:** yield increases with time until reaching equilibrium, often within 30–60 minutes, after which prolonged extraction can lead to compound degradation. And heat, can also cause degradation if prolonged. Advanced techniques can significantly decrease processing time from hours to minutes.

• **Particle size and pretreatment:** reducing particle size (grinding/milling) increases the surface area, enhancing solvent penetration and yield.

- **pH levels:** pH affects the solubility and stability of specific compounds; for instance, acidic conditions (pH < 5) favor the extraction of anthocyanins.
- **Extraction method:** green technologies are more efficient than conventional methods, providing higher yields in less time while preserving bioactivity.
- **Solid-to-solvent ratio:** a higher proportion of solvent to solid can increase yield, but an excessive amount may complicate downstream processing.

The choice of parameters directly affects the structural integrity of the compounds and their final antioxidant, antimicrobial, or therapeutic potential (Rodríguez et al., 2020).

Analytical techniques for phytochemical profiling

Several methods can be used in the identification of compounds from halophyte plant extracts. Phytochemical studies play a crucial role in the identification of bioactive compounds present in halophytes (Machado et al., 2025; Szydłowska-Czerniak et al., 2025). These compounds mainly include alkaloids, flavonoids, tannins, terpenoids, and phenolic substances, which are widely recognized for their potential (Tzanova et al., 2020; Soares et al., 2023; Valisakkagari et al., 2024; Wehbe et al., 2024; Sun et al., 2025).

Physicochemical properties include total polyphenol content, total flavonoid content, antioxidant activity, and polyphenol identification (Câmara et al., 2020; Cañadas et al., 2021; Fredsgaard et al., 2023; Buscaglia et al., 2024)

Biological properties include antimicrobial properties, biodegradation, and long-term stability (et al., 2024).

For the detection of functional group, presence of multiple bonds and rings, hydrogen and carbon arrangement, as well as full structural elucidation the techniques used include Mass Spectroscopy (MS), Ultraviolet Spectroscopy (UV), Infrared Spectroscopy (IR), Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS) remain reliable and widely used. More recent advancements, including Nuclear Magnetic Resonance (NMR), Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS), Fourier Transform Infrared Spectroscopy (FTIR), metabolomics approaches, and machine learning–assisted spectral analysis, have further improved the accuracy and sensitivity of detecting and profiling complex plant metabolites.

The integration of these analytical and extraction methods contributes significantly to quality control, supports the standardization of plant-derived products, and facilitates the discovery and development of natural product-based pharmaceuticals, nutraceuticals, and more (Bener et al., 2022; Choudhary et al., 2021).

Challenges and limitations

Green extraction has increasingly gained prominence as an advanced and rapidly developing area within analytical chemistry, reflecting the broader global movement toward more sustainable and environmentally responsible industrial and scientific practices (Lefebvre et al., 2021; Majid et al., 2023). This approach seeks to reconcile the growing demand for the efficient isolation of high-purity secondary metabolites with the necessity of reducing the environmental impact associated with conventional extraction processes. Consequently, ongoing developments in this field are directed toward improving both the efficiency and selectivity of extracting biologically active compounds, while simultaneously minimizing the consumption of hazardous solvents, energy, and other resources throughout the analytical procedure. The adoption of such strategies contributes to the development of more sustainable analytical methodologies and supports the broader objective of environmentally conscious research and production.

Solvents employed in the extraction of halophyte constituents are typically selected according to their polarity, which strongly influences the efficiency and selectivity of phytochemical recovery. These solvents can be broadly classified into polar solvents, such as water and various alcohols; solvents with intermediate polarity, including acetone and dichloromethane; and nonpolar solvents, for example *n*-hexane, ether, and chloroform. The choice of solvent is therefore a critical factor in determining the range of bioactive compounds that can be isolated from plant matrices. A

variety of extraction techniques are used to obtain these compounds, ranging from conventional methods to more advanced approaches. Commonly applied procedures include maceration, decoction, infusion, percolation, and Soxhlet extraction, as well as superficial extraction. In addition, modern extraction strategies such as ultrasound-assisted extraction and microwave-assisted extraction have been increasingly adopted due to their potential to improve extraction efficiency and reduce processing time (Geow et al., 2021).

RESULTS AND DISCUSSIONS

The selection of an appropriate extraction technique represents a crucial factor in effectively harnessing the therapeutic potential of natural products. This choice influences not only the extraction yield and the resulting phytochemical profile but also, and perhaps more importantly, the preservation of the intrinsic bioactivity of the compounds present in the extract (Ruiz-Medina et al., 2025).

Conventional extraction methods are characterized by several significant limitations, most notably the protracted temporal requirements and the necessity for substantial solvent volumes, often involving labor-intensive, multi-stage iterative procedures. A critical constraint within these frameworks is the inherent susceptibility of thermolabile phytochemicals to thermal degradation or oxidative transformation during heating phases, which can significantly compromise the biochemical integrity of the resulting extract.

Despite these technical impediments, these conventional techniques maintain widespread utility in the isolation of volatile essential oils and aromatic fractions. This persistent application is primarily attributed to their operational simplicity and the minimal instrumentation overhead required, making them accessible and cost-effective alternatives to more contemporary, capital-intensive extraction technologies.

Modern extraction techniques, specifically ultrasound-assisted (UAE), microwave-assisted (MAE), enzyme-assisted (EAE), and supercritical fluid extraction (SFE) (Kumar et al., 2021; Paucar et al., 2023), represent a paradigm shift toward process intensification in phytopharmaceutical research (Islam et al., 2023). Novel methods have been demonstrated to improve selectivity, reduce solvent consumption, and enhance extraction kinetics relative to conventional approaches (Sánchez-Camargo et al., 2020).

These methodologies align with the principles of Green Chemistry, ensuring that higher recovery yields are achieved with enhanced selectivity and reduced environmental impact, positioning these "green" technologies as superior alternatives to traditional, labor-intensive benchmarks.

The review also critically evaluates key process parameters that influence extraction efficiency, such as solvent type and polarity, extraction duration, temperature regimes, and ultrasonication amplitude, and discusses strategies for their optimization in various extraction contexts (Espro et al., 2021).

CONCLUSIONS

Further research is needed into the optimization of alternative combinations of green solvents; may provide valuable insights into improving the extraction performance of halophytes (Hessel et al., 2022). In particular, the investigation of modified extraction parameters, including variations in extraction time, solvent-to-sample ratio, and other operational conditions, may further clarify the efficiency and selectivity of the process. The encouraging outcomes reported for these environmentally friendly extraction approaches also highlight their potential for broader practical utilization. Additionally, phytochemical profiling what utilizes advanced analytical techniques to separate, identify, and quantify bioactive compounds like flavonoids, terpenoids, and alkaloids is a necessity (Mondal et al., 2025). Extracts obtained through such sustainable methodologies are often enriched in high-value bioactive compounds, thereby presenting promising opportunities for different and practical applications (Lemoine et al., 2024; Lemoine et al., 2025). The integration of these extracts into healthcare, pharmacy, and agriculture applications could support the exploitation of currently underutilized halophytes and contribute to the advancement of

circular economy principles (Socas-Rodríguez et al., 2021).

This review emphasizes that the future valorisation of halophytes and their extracts will likely depend on the strategic integration of hybrid extraction approaches designed to enhance compound recovery while simultaneously addressing sustainability and economic feasibility. The implementation of a carefully selected and rigorously optimized extraction methodology therefore represents a fundamental initial stage in the effective exploitation of the extensive chemical diversity inherent to halophytes. Such an approach facilitates the transformation of these into safe and efficacious bioactive ingredients with potential applications in both pharmaceutical and nutraceutical fields.

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