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Plant-based bioactive compounds: A comprehensive review of conventional and novel extraction techniques

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Abstract
The growing demand for functional foods and nutraceuticals, known for their health benefits, has led to increased interest in natural bioactive compounds. Extracting these compounds is essential from both industrial and technological perspectives. Conventional methods like maceration, hydrodistillation and solvent extraction are commonly used, but modern eco-innovative techniques such as ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, enzyme assisted extraction, pulsed electric field and pressurized liquid extraction have gained popularity for their efficiency. The incorporation of innovative and combined novel techniques enhances extractability, leading to improved yields with higher extraction rates. Moreover, this process reduces impurities in the final extract and safeguards delicate thermo- sensitive compounds. It involves the utilization of diverse inorganic solvents and boasts low energy consumption. This review aims to assess the efficacy of various conventional, novel, and hybrid technologies utilized in extracting bioactive compounds from plant materials.

Keywords: Bioactive compounds, extraction, UAE, MAE, SFE, EAE, PEF, PLE

Introduction
In today's world, people seek to consume both essential foods for bodily functions and beneficial bioactive compounds—non-nutritional ingredients that support health. Bioactive compounds are secondary metabolites produced by organisms to regulate physiological activities and increase survival resilience (Koçak & Pazir, 2018) [1]. Bioactive compounds can be found in a variety of plant items and are classified into various classes including terpenoids, alkaloids, nitrogen-containing compounds, organosulfur compounds, and phenolics (Jha & Sit, 2022) [2].

The process of extraction plays a vital role in acquiring elements from either a solid mixture or solution, and is an integral part of preparing samples, conducting experiments and qualitatively analysing bioactive compounds (H. W. Huang *et al*., 2013) [3]. The success of qualitative and quantitative investigations of these compounds depends on extraction, which is the first stage in isolating and characterising bioactive compounds from plant materials. The solubility of the target compounds in the selected solvents, process circumstances, and precautions to prevent the co- extraction of undesirable compounds should all be taken into account during the process selection phase (Mphahlele *et al*., 2016) [4]. The two most popular methods are solvent extraction and solid- phase extraction (Kultys & Kurek, 2022) [5]. The effectiveness and efficiency of the chosen extraction methods play a significant role in the extraction of bioactive compounds from a variety of food and agricultural sources (Wen *et al*., 2020) [6].

Traditional methods such as soxhlet, hydrodistillation, and maceration with alcohol can all be used to extract bioactive chemicals. Depending on the nature of the desired compound, the proper technique should be used in order to get the best yield and purity (Kadam & Tiwari, 2013) [7]. Solvent selection, temperature, stirring, and solubility are among the variables on which traditional extraction techniques depend. However, as phytochemicals are extremely sensitive to heat and light, they are vulnerable to thermal and photodegradation, which cannot be eliminated using conventional methods due to their high molecular weight (Singh *et al*., 2015) [8]. Due to their drawbacks, which include high usage of organic solvents, and detrimental effects on the environment and costly expenses, led to development of alternative techniques like microwave, ultrasound, supercritical fluids (which predominantly employ carbon dioxide, known as SC-CO_2), enzyme, pressurized liquid, and pulsed electric field- -assisted extractions (Moreira *et al*., 2019) [9].

Maceration

The most ancient method used to extract primary and secondary metabolites involves immersing the plant material in solvents such as water, oil, or alcohol, is maceration or solid-liquid extraction (Lefebvre *et al*., 2021) [10], involves leaving the plant material with the solvent for a few hours up to several days. Afterward, the extract is filtered to eliminate any solid suspensions. Despite the relatively high solvent usage, maceration is a favorable technique for thermally labile components (Ligor *et al*., 2018) [11]. To extract plant metabolites methanol, ethanol, acetone, hexane, dichloromethane, chloroform, ethyl acetate, water and other solvents are commonly used in maceration. To aid in the maceration process, continuous agitation or induced heat is sometimes utilized (Azahar *et al*., 2020) [12]. Intermittent shaking during maceration has two functions: to remove concentrated solutions from the surface of the sample, to increase the rate of diffusion and enabling a fresh solvent to extract further yield (Azmir *et al*., 2013) [13].

Soxhlet Extraction

Franz Ritter Von Soxhlet, a German scientist, first created the extraction technique known as the Soxhlet extractor in 1879, particularly for lipid extraction. Nevertheless, it is being used to extract advantageous bioactive substances from a variety of natural sources. It also acts as a common reference for assessing novel extraction options (Azmir *et al*., 2013) [13]. This technique is more effective than other conventional techniques, except for extracting thermolabile compounds (Singh *et al*., 2015) [8]. It involves using a porous carrier, called a "thimble," made of filter paper or cellulose to hold the plant material. The extraction solvent is poured into the thimble, which is then set on a thimble holder. The sample-containing thimble is heated in the bottom flask, whereupon the solvent evaporates, condenses, and drops back into it (Manousi *et al*., 2019) [14]. When the soxhlet chamber is nearly full, the syphon arm automatically empties it, allowing the solvent to flow back down to the boiling flask. Cycles of this procedure are carried out until a concentrated extract is achieved (Azahar *et al*., 2020) [12]. González-Barrio *et al*., (2018) [15] used a Soxhlet extractor to extract a known weight of powdered sample with petroleum ether to assess the crude fat content of *Viola wittrockiana* and *Antirrhinum majus*. Similarly, Moliner *et al*., (2022) [16] extracted fresh borage (*Borago officinalis* L.) using the soxhlet method with ethanol as the solvent which gave a yield of 5.45% (mass of extract/mass of fresh flowers).

Hydrodistillation

Hydrodistillation is a widely utilized method of acquiring essential oils and bioactive substances from plant matter, like flowers or wood, that are typically not soluble in water and have high boiling temperatures. This method involves immersing the plant material in water and boiling it (Tongnuanchan & Benjakul, 2014) [17]. Hydrodistillation is a solvent-free technique that can be utilized on both damp and dry samples. It can be categorized into four types, namely water distillation, water and steam distillation, direct steam distillation, and cohobation distillation (Manousi *et al*., 2019) [14]. Hydrodistillation allows for the simultaneous extraction and separation of volatile and nonvolatile organic compounds. The azeotropic distillation is utilised to seperate volatile organic compounds from the matrix and is collected in a Florentine flask. While to extract soluble organic compounds that are not volatile, boiling water is utilized in the alambic with direct contact with the matrix. Despite its efficacy, this technique is laborious and requires substantial energy, and the high temperature used for extraction may result in the loss of some volatile components (Petigny *et al*., 2014) [18]. There are three key physicochemical procedures involved in the process: hydrolysis, hydrodiffusion and heat-induced decomposition. Nevertheless, the usage of hydrodistillation for thermolabile compound extraction is restricted because at high extraction temperatures, some volatile components may be forfeited. (Azmir *et al*., 2013) [13].

Ultrasound Assisted Extraction (UAE)

UAE is a productive technique that sits at the intersection of traditional and contemporary methods and is employed for various bioactive substances (Ligor *et al*., 2018) [11]. There are two main types of ultrasound: diagnostic ultrasound and power ultrasound. Diagnostic ultrasound uses high frequency waves (100 kHz to 1 MHz) of low intensity for non-destructive analysis to ensure quality and process management. On the other hand, power ultrasound uses low frequency waves (16 to 100 kHz) of high intensity and is commonly used in extraction and processing techniques (Rutkowska *et al*., 2017) [19]. Several molecules and biomaterials, including polysaccharides, peptides, proteins, dyes, pigments, bioactive compounds and essential oils have been extracted using ultrasound (Soquetta *et al*., 2018) [20]. Acoustic cavitation is the primary influence of ultrasound in a liquid medium (Saini & Keum, 2018) [21]. The transmission of ultrasound waves occurs in a medium, whether it is a solid, liquid, or gas. This happens by producing compression and expansion, which leads to the formation, growth, and collapse of bubbles. This process is known as "cavitation" (Moreira *et al*., 2019) [9]. The changes induced by ultrasound bring about a forceful impact of molecules, leading to the creation of shock waves and zones of high pressure (up to 50 MPa) and temperature (5500 °C) for a brief period (9-10 s). This causes harm to the cell wall, facilitating improved penetration of solvents and the extraction of intracellular compounds (Kultys & Kurek, 2022) [5]. Several factors can alter ultrasonic waves, with frequency (Hz) and amplitude (MPa) being the most significant ones. Power (W) is determined by amplitude changes over time, and intensity (W/m) is obtained by dividing power by surface area (Lefebvre *et al*., 2021) [10]. The choice of solvent has a considerable influence on the efficacy of cavitation in UAE and the transfer of acoustic energy to reactants. The most commonly used solvents include water, ionic liquids, ethylene glycol and its oligomers, glycerol, and other solvents derived from biomass (Giacometti *et al*., 2018) [22]. There are two primary devices for sonication: the ultrasonic probe and ultrasonic bath system, the former is which is a direct method of sonication and the latter is indirect applications (Manousi *et al*., 2019) [14].

Microwave Assisted Extraction (MAE)

Microwaves fall within the frequency range of 300 MHz to 300 GHz and are characterised by two oscillating fields at right angles to each other - the electric field and magnetic field (Angiolillo *et al*., 2015) [23]. Microwaves serve as a non-contact heat source, which not only enhances heating efficiency and selectivity, but also accelerates energy transfer,

response to heating control, and startup, while reducing equipment size, thermal gradient, and operation units (Li *et al*., 2013) [24]. MAE is an economical and innovative approach that combines conventional solvent techniques with microwaves. In this method, microwaves are utilized to heat solvents for extracting biologically active substances within a short span of time while also reducing the volume of solvents used (Singh *et al*., 2015) [8]. Microwave heating is much faster compared to traditional heating methods such as conduction or convection. Microwave heating works on the principle of creating heat through friction by moving dipole particles. To heat the sample and transfer the heat to the extractant, a non-polar solvent that does not absorb microwave radiation is used (Kultys & Kurek, 2022) [5]. MAE involves both ionic conduction and dipole rotation, which occur simultaneously and directly affect the molecules. Unlike traditional heating methods, microwave heating causes molecules to absorb energy without dissipating heat to the surrounding environment. As a result, polar molecules within the sample absorb energy, causing cell disruption. Faster diffusion and mass transfer from solids can be achieved by destroying cells, where heat and mass transfer work together and in unison (Ciko *et al*., 2018) [25]. Three successive processes make up the MAE extraction method. Under pressured and high-temperature conditions, the solutes are first desorption from the different active sites within the sample matrix. Secondly, the extraction fluid may diffuse into the matrix. Last but not least, depending on the sample matrix, solutes may separate into the extraction fluid (Alupului *et al*., 2012) [26]. The antioxidant potential of flowers including, *Calendula officinalis* L., *Rosa damascena* Mill., *Viola tricolor* L., *Hibiscus rosa- sinensis* L., *Cucurbita pepo* L., *Allium ursinum* L. and *Sambucus nigra* L. was evaluated using MAE (Petkova *et al*., 2021) [27].

Enzyme Assisted Extraction (EAE)

Enzymatic extraction, which involves the use of enzymes or combinations of enzymes, is frequently employed to catalyse the breakdown of compounds that are difficult to transfer due to their association with cell walls or binding to target compounds, such as pectin, within the matrix. Enzymes are utilized because of their inherent properties such as specificity and regioselectivity (Wen *et al*., 2020) [6]. Many species, such as animal organs, bacteria, fungus, or fruits and plant extracts, may serve as a source of enzymes. Oxidation-reduction enzymes, hydrolyzing enzymes, carboxylation enzymes, isomerizing enzymes, group transfer enzymes, ligases and desmolases are some of the categories under which these enzymes fall (Cheng *et al*., 2015) [28]. A variety of enzymes are available for chemical compound extraction. Cellulases, hemicellulases and pectinases are often used because of their catalytic capabilities to extract bioactive chemicals from medicinal and aromatic plants, while lipase, protease/lipase, phospholipase and lactase are frequently mentioned ones (Manousi *et al*., 2019) [14]. Enzymes engage with the plant cell wall via their active site, which induces the enzyme to alter its configuration to accommodate the substrate, thereby fostering extensive interaction between them. The conformational transformation of the enzyme instigates the rupture of bonds present within the cell wall, culminating in the liberation of the intended constituents (Nadar *et al*., 2018) [29]. Various vital factors can influence enzyme- facilitated extraction, such as time, temperature, pH, the type of solvent employed for extraction, the ratio of solid to liquid, composition and concentration of the enzyme and the proportion of enzyme to substrate (Azmir *et al*., 2013) [13]. EAE offers several benefits, especially regarding its eco-friendliness and non-toxicity, as it obviates the requirement for solvents during the process, it generates a higher yield of bioactive compounds without requiring heat. This technique is highly proficient in producing bioactive compounds by removing extraneous elements from cell walls and freeing the desired bioactives. Additionally, it helps to surmount the impediments posed by water solubility and insolubility of bioactive compounds (Kadam & Tiwari, 2013) [7]. Nevertheless, there are commercial and technical challenges associated with enzyme-assisted extraction Firstly, the expense of enzymes is relatively high, making it unfeasible for processing large quantities of raw materials. Secondly, enzymes may not be able to break down plant cell walls completely. Finally, it may not be viable for industrial application due to the restricted behaviour of enzymes under diverse environmental conditions (Puri *et al*., 2012) [30].

Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction is gaining more recognition as a method for extracting bioactive substances from natural sources because of its numerous benefits. These benefits include shorter extraction times, lower usage of organic solvents, the ability to extract thermo-sensitive substances, the production of purer extracts, and its environmentally friendly nature (Huang *et al*., 2012) [31]. A supercritical fluid is a substance that is in a state above its critical point, where it cannot exist as separate gas and liquid phases due to temperature and pressure (Kultys & Kurek, 2022) [5]. At this stage, these fluids lose their distinct gas and liquid characteristics and cannot be turned into a liquid state by manipulating temperature and pressure. (Azmir *et al*., 2013) [13]. The distinctive attributes of supercritical fluids result in them having comparable gas-like traits in viscosity, diffusion and surface tension, as well as liquid- like traits in density and solvation power. These characteristics render them appropriate for extracting bioactive substances from natural sources (Manousi *et al*., 2019) [14]. These fluids can effortlessly permeate through solid matrices, akin to gases, and can dissolve materials similarly to conventional liquids. SFE is usually carried out close to the critical point of the fluid, where slight variations in temperature or pressure can cause significant changes in the fluid's density (Ahangari *et al*., 2021) [32]. Various solvents, including methanol, ethanol, butane, pentane, hexane, nitrous oxide, sulfur hexafluoride, and fluorinated hydrocarbons, possess critical properties that enable their use in supercritical fluid extraction (Molino *et al*., 2020) [33]. However, carbon dioxide is the supercritical fluid of choice for this extraction technique due to several beneficial qualities, such as being non-toxic, ecofriendly, non-flammable, widely accessible, chemically inert, high diffusivity, low surface tension and low viscosity. Moreover, it is relatively inexpensive and can be effortlessly extracted from the extract after decompression, resulting in no solvent remnants in the end product (López-Hortas *et al*., 2022) [34]. SFE involves two primary stages: the initial dissolution of chemical substances found in the solid matrix, and the subsequent separation of dissolved compounds from the supercritical solvent (da Silva *et al*., 2016) [35]. SFE is primarily employed for extracting non-polar bioactive

compounds, including lipids and carotenoids, because of the solvents used in this approach (Soquetta *et al*., 2018) [20]. However, a polar modifier or co-solvent like water, methanol, ethanol, acetone, acetonitrile, dichloromethane or ethyl ether must be added to the supercritical fluid to boost solubility in order to extract polar chemicals like flavonoids (Ligor *et al*., 2018) [11]. Water, being a safe and easily accessible solvent, is a good complement to SC-CO_2 extraction as a polar co-solvent, particularly because it is safe for human consumption (Wrona *et al*., 2017) [36]. Several factors, including temperature, pressure, particle size, moisture content of the feed material, CO_2 flow rate, and solvent-to-feed ratio, significantly impact the efficiency of SFE (De Aguiar *et al*., 2022) [37]. An advantage of SFE is that CO_2 completely evaporates during the extraction process, leaving no solvent residues behind (Gañan *et al*., 2020) [38].

Pressurized Liquid Extraction (PLE)

Pressurized liquid extraction involves increasing extraction kinetics maintaining solvents in their liquid state by utilizing high pressure (3.5-20MPa) and elevated temperatures (313-473K), without surpassing the critical point (Xynos *et al*., 2012) [39]. The chemicals' solubilization is accelerated by the increased temperature, which lowers the solvent's viscosity and surface tension resulting in faster and more effective extraction (Sousa *et al*., 2016) [40]. Additionally, high pressure during PLE expedites filling of the extraction cells and enables the liquid solvent to penetrate the solid matrix (Joana Gil-Chávez *et al*., 2013) [41]. The PLE method has two modes: dynamic and static. In the majority of documented studies, the static mode was utilized, followed by a dynamic flush with an organic solvent (Yahya *et al*., 2018) [42]. The analyte's solubility in the solvent determines the extraction efficiency in this method along with temperature and processing time (Kultys & Kurek, 2022) [5]. The subcritical water extraction technique, also known as pressurized hot water extraction, utilizes water as a solvent. At certain pressure and temperature, water's polarity may be changed to mimic that of certain alcohols, such as methanol and ethanol. It is hence capable of dissolving a variety of medium to low polarity analytes (Plaza & Turner, 2015) [43]. Optimum selection of solvents or solvent mixtures and fine-tuning process parameters like solid-to-liquid ratios, pressure, temperature, and number and duration of extraction cycles can lead to improved efficiency (Giacometti *et al*., 2018) [22]. PLE is recognized as an environmentally friendly and effective technique, attributed to its reduced solvent consumption, absence of exposure to light and oxygen, and shorter processing time (Shang *et al*., 2017) [44]. This technique is suitable for extracting compounds that are sensitive to degradation by heat (Osorio-Tobón & Meireles, 2013) [45]. However, PLE can be costly in terms of capital and running expenses, and it has yet to be scaled up to extract the volumes required for industrial use. Furthermore, packing of cells can pose a significant issue with this method, which can negatively impact the extraction process (Tierney *et al*., 2013) [46].

Pulsed Electric Field Extraction (PEF)

Pulsed electric field extraction technique has become popular in the food and pharmaceutical industries due to its cost-effectiveness (Boulaaba *et al*., 2014) [47]. The method involves applying short pulses of high voltage to the food product that is placed between two electrodes. This process increases the membrane permeability and leads to improved extraction yields (Nowacka *et al*., 2019) [48]. A switch, power supply, energy storage element, pulse generator, activating circuit, treatment chamber, and monitoring and controlling system are some of the components included in a standard PEF system (Zhang *et al*., 2022) [49]. Depending on how the treatment chamber is set up, the PEF system may run in batch or continuous modes (Usman *et al*., 2022) [50]. To extract materials, the PEF approach applies a moderate to high intensity electric field in either batch or continuous mode, ranging from 100-300 V/cm and 20- 80 kV/cm, respectively. Two potential mechanisms for PEF's efficacy have been suggested. The first hypothesis proposes that PEF accelerates chemical reactions to increase solvent solubility, whereas the second suggests that electroporation of biological cell membranes (Xi *et al*., 2021) [51]. Electroporation is the act of increasing the permeability of cell membranes in order to transport ions and macromolecules. This reduction in resistance to diffusion through the cell membrane facilitates the extraction of bioactive compounds from cells, resulting in higher extraction yields (Salehi, 2020) [52]. It can be either reversible or irreversible, process variables including treatment temperature, energy input, pulse count, field strength, and the properties of the treated materials all have an impact on how effective PEF treatment is (Azmir *et al*., 2013) [13]. PEF provides an alternative to conventional cell disruption techniques in extraction procedures due to its non-thermal and chemical-free characteristics, as well as its continuous operation capability (Goettel *et al*., 2013) [53]. This technique has demonstrated promise in the targeted retrieval and restoration of a variety of beneficial ingredients, including but not limited to sugar, inulin, starch, proteins, polysaccharides, polyphenols, pigments, flavor compounds, and phytochemicals (Vorobiev & Lebovka, 2017) [54].

Conclusion

Traditional as well as novel extraction technologies are limelighted in this current article. The extraction of bioactive compounds from plant sources is a critical area of research driven by the rising demand for functional foods and nutraceuticals with health-promoting properties. Conventional methods have long been employed for this purpose, but with advancements in technology, novel and eco-innovative techniques have emerged as viable alternatives. Novel extraction technologies can result in larger extraction yields in less time, better product quality, and fewer environmental issues. However, these unique extraction procedures still need to be adequately developed whilst optimized conditions are needed to make a scaling process. So, there is a dire need for mechanistic studies to thoroughly understand the mechanisms leading to the extraction of bioactive compounds from different extraction methods. On the other hand, the increasing economic significance of bioactive compounds and commodities rich in these bioactive compounds may lead to find out more sophisticated extraction methods in future.

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