

## EXTRACTION METHODS OF BIOACTIVE COMPOUNDS FROM THE PLANTS

### Abstract

The escalating demand for bioactive compounds from plants has grown substantially, primarily attributed to their potential therapeutic applications across diverse industries such as pharmaceuticals, nutraceuticals, and cosmetics. This comprehensive review examines the extraction methods employed for isolating bioactive compounds from plant sources, focusing on techniques that maximise yield, maintain compound integrity, and ensure sustainability. Conventional methods, including solvent extraction, are extensively discussed, encompassing well-established techniques such as maceration, Soxhlet extraction, and hydrodistillation. These methods have gained widespread usage due to their simplicity and cost-effectiveness. In addition to traditional approaches, this review explores novel methods such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE). These emerging techniques are recognised for their capacity to enhance extraction efficiency and reduce processing times significantly. Factors influencing the extraction process and subsequent bioactive compound yield, such as solvent selection, particle size, and temperature, are thoroughly investigated. The review also scrutinises the ecological impact and economic considerations associated with each method, aligning with the growing emphasis on sustainable practices in plant-based compound extraction. Advancements in extraction technologies, including ionic liquids and enzyme-assisted extraction, are highlighted, emphasising their potential to improve specificity and selectivity in isolating bioactive compounds. The paramount importance of selecting the

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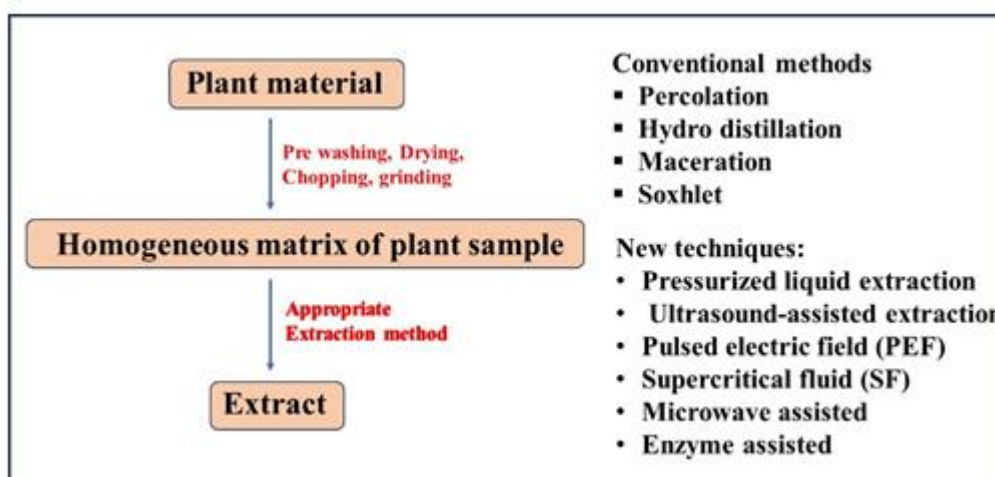
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most suitable extraction method based on the chemical nature of the target compounds and the characteristics of the plant matrix is underscored throughout the discussion. In conclusion, this review provides a comprehensive synthesis of the current state of plant extraction methods for bioactive compounds. It meticulously delineates the advantages and limitations of conventional and emerging techniques. It offers valuable insights for researchers, practitioners, and industries aiming to optimise the extraction process for enhanced bioactive compound yields while prioritising environmental and economic sustainability.

**Keywords:** Conventional extraction technique, Green extraction method, Bioactive compounds.

## I. INTRODUCTION

Uses of medicinal plants in traditional medicine have drawn immense attention from time immemorial. It is essential to look into the matter from a modern perspective. Hence, the mode and method of experimental procedures should be well-established and documented. Extraction is the first step to isolate and purify bioactive compounds from plant material. In the analysis or study of herbal or medicinal plants, extraction plays the most crucial role because it is the extraction procedure that can ensure the presence of desired chemical components in the fraction of plant extraction for subsequent chemical analysis, like isolation of bioactive markers and their characterisation. Before going to extraction, there are some basic steps that one needs to follow, which include pre-washing of the plant material followed by drying of the plant part, chopping, and grinding to yield a homogenous matrix of samples which improves the extraction kinetics by increasing the solvent contact with the sample surface [1,2]. Next comes the solvent selection part. Indeed, extracting solvent is not selected arbitrarily; the specific nature of the targeted bioactive compound(s) to be extracted guides the solvent selection. Plenty of various different solvents or mixtures/combinations of solvent systems are available to extract the desired bio-active entities from medicinal plants. Polar solvents such as acetone ( $\text{CH}_3\text{COCH}_3$ ), ethyl acetate (EtOAc), methanol (MeOH), ethanol (EtOH) or water can be used to extract hydrophilic (water-loving) compounds; on the other hand, chloroform ( $\text{CHCl}_3$ ), dichloromethane (DCM) or a mixture of MeOH/DCM in the ratio of 1:1 is used for the extraction of lipophilic (fat loving) compounds. The insoluble nature of phenolic acids and flavonoids makes their extraction troublesome. Traditional extraction techniques include reflux, Soxhlet, percolation, maceration etc., which are well-known procedures for the extraction of bioactive entities and the equipment entangled in these techniques are dissimilar from one another. Now one question may arise. What is an appropriate extraction technique? A suitable extraction method is one that balances the quality of the product, efficiency of the process, costs of production, and environmentally benign methods that should be used to extract bioactive markers from herbal plants. Besides these, various new methods, including greener approaches for sustainable and nontoxic techniques of extraction, have also been adopted in recent times. In the green method of extraction use of hazardous chemicals is avoided. Technologies like ultrasound (US), high hydrostatic pressure (HHP), supercritical fluid (SF), pulsed electric field (PEF) etc., are rapidly replacing the traditional techniques. The use of unconventional new techniques increases the extent of extraction, thereby increasing yields along with increased extraction rates. This extraction accounts for fewer impurities in the resultant extract, safeguards thermo-labile compounds, uses various inorganic solvents, and consumes less energy. In this review, our main purpose will be to discuss different conventional and novel new technologies entangled in the extraction process of bioactive motifs from medicinal plants [3,4].



**Figure 1:** A schematic illustration of the procedural steps in an extraction protocol

## II. BIOACTIVE ENTITIES PRESENT IN PLANTS

A plant's biological system encompasses both primary and secondary metabolites. Primary metabolites, like carbohydrates, amino acids, and proteins, are essential for the development and maturation of plant tissues [3]. In contrast, secondary metabolites are synthesized by the plant throughout its life to enhance its ability to survive and adapt to diverse environmental challenges. Bioactive compounds can be found in various plant-derived products and fall into different categories, including terpenoids, flavonoids, alkaloids, nitrogen-containing compounds, organosulfur compounds, and phenolic compounds. [1]. The most well-known class of terpenoids are tocotrienols, which include: Atropine, Colchicine, Morphine, Strychnine, Ephedrine, Acridine, Quinine, Nicotine, Carotenoids, Pyrrolidine, Isoquinoline, Quinoline, Lycopodium, Quinolizid, Phytosterols etc. [5]. The bioactive compounds mentioned above have various health benefits, such as anti-inflammatory properties, anti-cancer properties, anti-diabetes properties, blood circulation properties, digestion properties, etc. [6].

## III. SELECTION OF APPROPRIATE EXTRACTION PROTOCOL

Bioactive motifs can not only be derived from plant sources, it is present in every living organism, be it a microorganism or animal/marine organism [7]. But in this review, we will restrict our discussion to plants only. The quantity of bioactive entities present in plant parts is low enough and all plant parts, such as barks, leaves, stems, roots, tuber roots, gums/oleoresin, exudates, fruits, stolon, flowers, rhizomes etc. produces bioactive chemicals in fairly low quantities and at different concentrations. And for this selection of proper extraction technique is very much essential to augment the extract from plants [8]. The extractability is dependent on various elements like extraction technique, plant component, and matrix composition of plant materials, extracting solvent, pressure, temperature and time [9]. In the last couple of decades, researchers have focused more towards novel extraction techniques that are environment-friendly [10]. They have also made efforts to minimize the use of organic solvents, simplify the operational procedures, and attain a superior quality extract. Because of these advantages, novel extraction techniques are gaining much attention

over conventional techniques. Conventional extraction procedures have certain drawbacks which can easily be circumvented by these novel extraction strategies [11].

The extractability of any traditional technique mainly depends on the selection of solvents [12]. The solvent is chosen in such a way that it matches the polarity of the compound of interest. In selecting a solvent for extracting bioactive compounds, it's crucial to consider several factors. These include the compatibility of the solvent and the substance being extracted, the impact of co-solvents, how mass transfer occurs, environmental considerations, human safety, and economic viability. The following table offers examples of how bioactive components are conventionally obtained using suitable solvents.

| <b>Solvents</b>                   | <b>Class of compounds extracted using suitable solvent</b>         |
|-----------------------------------|--|
| Water (H <sub>2</sub> O)          | Tannins, Anthocyanins, Carbohydrates, Terpenoids, Saponins         |
| EtOH                              | Terpenoids, Tannins, Polyphenols, Alkaloids, Flavonol              |
| MeOH                              | Anthocyanin, Tannins, Terpenoids, Polyphenols, Saponins, Flavones, |
| CHCl <sub>3</sub>                 | Flavonoids, Terpenoids   |
| DCM                               | Terpenoids   |
| Et-O-Et                           | Terpenoids, Alkaloids  |
| CH <sub>3</sub> COCH <sub>3</sub> | Flavonoids   |

#### IV. PROPERTIES OF SOME COMMON EXTRACTING SOLVENTS

##### 1. Water

This solvent, with a polarity index of 1.000, is the most polar option one can find, and it is frequently utilized for extracting a wide range of polar substances. Water's key benefit is that it dissolves a wide variety of compounds and is also affordable, nontoxic, non-flammable, and highly polar. Although it has certain drawbacks, such as the fact that it encourages bacterial and mould growth and might cause ester bond hydrolysis. [13,14]

##### 2. Alcohol

Alcohol has a polar character (Polarity index of methanol is 0.762 and ethanol is 0.654). Since it is fully miscible with water is utilized for the extraction of polar secondary metabolites. To act as a self-preserved, the alcohol concentration should be greater than 20%. Alcohols in low concentrations are harmless, and because of their low boiling point, extracts can be concentrated with a low flame easily. Its disadvantage is that it is volatile and combustible, and it does not dissolve wax, gums, or fats. [13,14]

##### 3. Chloroform

It works as a nonpolar solvent (Polarity index is 0.259) for extracting substances like terpenoids, flavonoids, oils, and lipids. Advantages, it is colourless having pleasant smell and miscible with alcohols. Main disadvantage is that both sedative and carcinogenic. [14, 15]

##### 4. Ether

It works as a nonpolar solvent (polarity index is 0.117) that can be used to extract substances including fatty acids, terpenoids, coumarins, and alkaloids. Ether is a solvent

known for its low boiling point, capacity to mix with water, and lack of any discernible taste. Additionally, ether is stable enough not to react with metals, acids, or bases. Its disadvantage is that it has a significant degree of volatility and flammability. [14,15]

## 5. N-hexane

With a polarity index of 0.009, n-hexane is the most non-polar solvent and is used to remove wax, gums etc., present in the plant material. Hexane finds its wide use as an oil extraction material due to its ease of oil recovery, low b.p. (63-69 °C), and high solubility. [16]

## 6. Ionic liquid (green solvent)

This particular extraction solvent is very polar and incredibly heat-stable. Even at 3000°C, it can maintain a liquid condition and is suitable in high-temperature applications. It is exceedingly miscible with water and other solvents and works well for polar chemical extraction. The main advantage is that it is convenient for microwave-mediated extraction because it contains superior solvent that draws and transmits microwaves. It is extremely polar, incombustible, and appropriate for liquid-liquid extraction. [17]

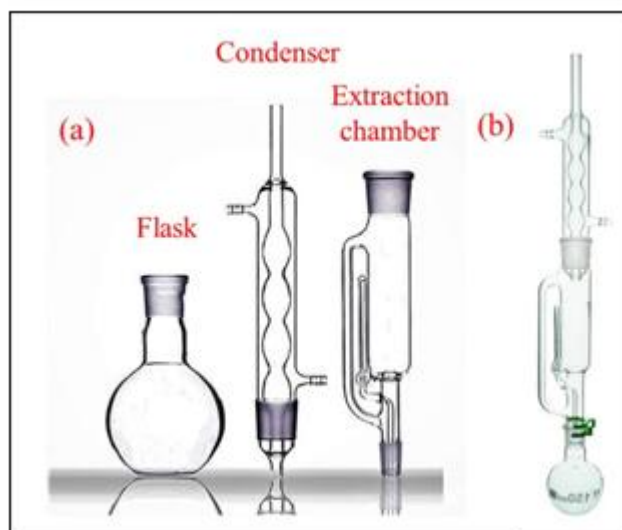
Apart from these solvents, acetone (polarity index: 0.355), ethyl acetate (polarity index: 0.228) and DCM (Polarity index: 0.309) are the other solvents which researchers frequently use for the extraction purpose.

## V. CONVENTIONAL EXTRACTION METHODS

Conventional approaches for obtaining bioactive compounds from plants typically utilize a combination of various solvents, temperature, and agitation to enhance the effectiveness of extraction. Four widely recognized techniques used for this purpose include i) Soxhlet extraction, ii) maceration, iii) hydro distillation, and iv) percolation. These methods are frequently utilized for the isolation of bioactive constituents from plant materials.

### 1. Soxhlet Extraction

The Soxhlet extractor was invented by Franz Ritter von Soxhlet, a German scientist, in 1879 [18]. It was mainly aimed at Lipid extraction; although, in recent times, it has widespread application. Soxhlet extraction is commonly employed for the extraction of valuable bioactive compounds from diverse natural sources and serves as a benchmark for evaluating innovative extraction techniques. A thimble is usually filled with an appropriate amount of dry material. The thimble is then put in the siphon-containing apparatus with a distillation flask containing the solvent of interest below. A siphon is used to aspirate the thimble-holder solution when it has reached an overflow level. The siphon mechanism circulates the solution, sending the extracted substances back into the main liquid. This process ensures that the solutes stay in the distillation flask while the solvent is returned to the solid bed of the plant in the thimble, allowing the extraction cycle to repeat. The procedure is repeated until the extraction is completed.



**Figure 2:** (a) Different parts, (b) Full set up of Soxhlet apparatus

## 2. Maceration

Maceration has traditionally been utilised in the mass production of tonic. In a very short period of time, this process became a well-liked and cost-effective method for isolating essential oils and bioactive components. The small-scale maceration process typically involves several stages. First, plant materials are finely ground to enhance their surface area, facilitating optimal mixing with the chosen solvent, referred to as the menstruum. In the maceration technique, this mixture is placed in a sealed container. Ultimately, the liquid is filtered, and the solid remnants from the extraction process, known as marc, are subjected to pressing to extract any remaining trapped solutions. Strained liquid is filtered to remove contaminants and to squeeze out liquids from them. Occasional shaking in the maceration process increases its extraction efficacy in two ways: (i) by increasing diffusion and (ii) by removing concentrated solution from the sample surface, facilitating the additional solvent to enter the menstruum and boost extraction yield.

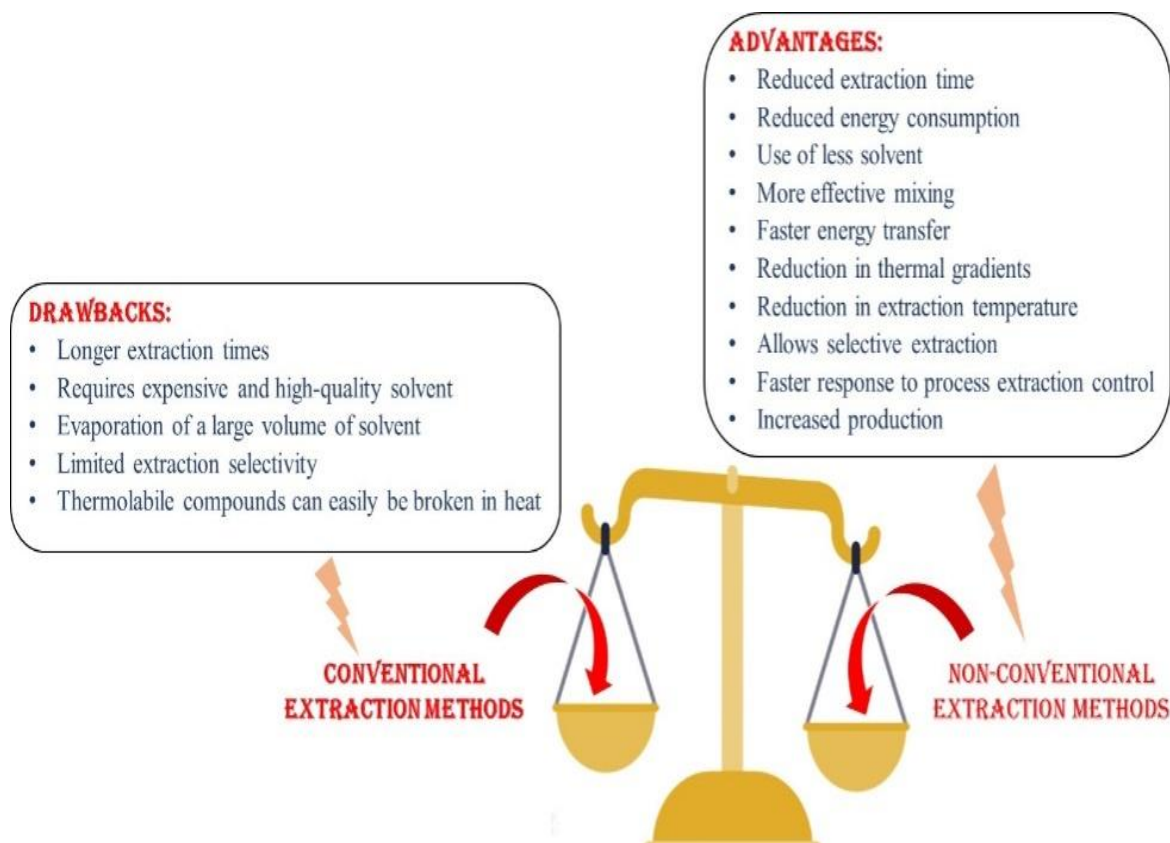
## 3. Hydro Distillation

The extraction of bioactive components and essential oils from plants by hydro distillation is a conventional process. Organic solvents are not required for this process and can be carried out without dehydrating the plant materials. Hydro distillation process can be carried out in three ways i.e., i) Water distillation, ii) water and steam distillation, and iii) direct steam distillation [19]. At first, the plant materials are packed in a separate chamber; secondly, the required amount of water is added and heated to a boil. The introduction of direct steam into the plant sample is also an alternative way of extraction. Steam and hot water are the key factors which accelerate the release of bioactive compounds from plant parts. Water condenses the vapour combination of oil and water during indirect cooling. The mixture so condensed passes from the condenser to the separator, and bioactive chemicals and oils separate automatically from the water [20]. Hydro-diffusion, hydrolysis, and heat degradation are the three major physicochemical processes of Hydro distillation. Some

volatile compounds may be lost at high temperatures during the extraction process, which limits their use in chemical extraction of thermolabile components.

#### 4. Percolation

Percolation is a process in which a liquid is slowly filtered through a filter, similar to the way coffee is typically made. The term "percolation" originates from the Latin phrase "percolare", which translates to "strain through". Unlike maceration, percolation is an ongoing process in which a fresh solvent continually replaces a saturated solvent. In a study conducted by Zhang and co-workers, refluxing was compared with percolation and other extraction techniques for the extraction of *Undaria pinnatifid*. The percolation method yielded a higher content of the main component (fuxanthin) than the other extraction method (refluxing), while extract yield was more or less similar in either method. The study was conducted in the context of a compound Chinese medicinal product, Goupil Patch, which is a combination of 29 Chinese medicinal products [21]. Fu and co-workers employed the total alkaloid content as the index, with the EtOH percolating technique being optimized by soaking Goupil Patch for 24 hours with 55% alcohol, then percolating 12 times the same amount with alcohol (55 %) [22]. Gao further optimized the percolation method for sinomenine hydrochloride and ephedrine hydrochloride by using the extracting rate as the index. This method involved soaking the medicine for an additional 24 hours with 70 % EtOH, followed by percolating 20 times the amount with 70 % EtOH; the transfer rates for these two substances were nearly similar (78.23 % for sinomenine hydrochloride and 76.92 % ephedrine hydrochloride) [23].



**Figure 3:** A schematic comparison between two extraction protocols



## VI. NON-CONVENTIONAL EXTRACTION METHODS

Conventional extraction procedure has the following major drawbacks [24]:

- Extended duration for extraction
- Requires expensive and high-quality solvent
- Evaporation of a large volume of solvent
- Limited extraction selectivity
- Thermolabile compound can easily be broken in heat

Innovative and potentially effective extraction techniques have been devised to overcome the drawbacks of traditional extraction methods. Under the umbrella of these new techniques, namely non-conventional extraction methods, following are some of the most promising approaches:

- Ultrasound aided extraction (UAE)
- Pulsed electric field-assisted extraction
- Enzyme-assisted extraction
- Microwave-assisted extraction (MAE)
- Pressurized liquid extraction (PLE)
- Supercritical fluid extraction

Some of these approaches are termed "green processes," since they meet requirements established by the EPA (Environmental Protection Agency) in the US. Achieving a reduction in chemical synthesis hazards necessitates careful preparation and the adoption of specific methodologies, which encompass [25]:

- Designing safer chemicals
- The utilisation of benign solvent additives
- Creating with a focus on optimising energy usage
- Use of less derivatives
- Use of renewable feedstocks
- Catalysis
- Atom economy
- Proper designing to prevent degradation
- Analysis of the time required for pollution prevention
- Safer chemistry designed to prevent accidents inherently.

### 1. Ultrasound Assisted Extraction (UAE)

Ultrasound refers to sound waves with frequencies typically falling between 20,000 Hertz (Hz) and 100 million Hertz (MHz), which are beyond the audible range for humans. It is a transverse wave, travels through a material by compressing and expanding and cannot pass through vacuum. This phenomenon involves cavitation, which is the formation, expansion, and subsequent collapse of bubbles. It results in the conversion of kinetic energy (motion energy) into heat within these bubbles, generating a substantial amount of energy. According to Suslick and Doktycz, these bubbles reach temperatures of approximately 5000

Kelvin, experience pressures of about 1000 atmospheres, and undergo rapid heating and cooling at rates exceeding 1010 Kelvin per second. [26] Ultrasonic-assisted extraction (UAE) was developed based on these principles. Cavitation primarily occurs in liquid media, including those with solid particles. The primary benefit of UAE is particularly evident when working with solid plant samples, as ultrasonic energy significantly enhances the release of both organic and inorganic compounds from plant tissues. [27] The probable mechanism involves the transfer of substances facilitated by ultrasound and faster penetration of solvents into plant tissues. The ultrasonic extraction technique incorporates two major physical phenomena: i) diffusion over the cell wall and ii) washing the contents of the cell after shattering the walls [28]. Sample moisture content, extent of grinding, solvent, and particle size are all critical aspects in achieving efficient and successful extraction. Besides these, the controlling parameters for ultrasonic action include pressure, temperature, frequency, and sonication time. UAE has also been used in conjunction with conventional processes to leverage the efficiency of a traditional system. To enhance the efficiency of the extraction process, an ultrasonic device is strategically placed within a solvent extraction system. [29]. The following are the benefits of UAE [30]:

- Reduced extraction time
- Reduced energy consumption
- Use of less solvent
- Enhanced blending efficiency
- Accelerated energy transmission
- Minimized thermal differences
- Lowering the extraction temperature
- Enabling specific and targeted extraction
- Reducing the size of equipment required
- Swift response to control the extraction process
- Rapid initiation of operations
- Enhanced manufacturing output
- Elimination of procedural step

UAE is proved to be an efficient extraction process for extracting bioactive compounds from medicinal plants. Rostagno *et al.* demonstrated the extraction efficacy of four (04) isoflavone derivatives from soybean using a mix-stirring technique with varying extraction periods and solvents, namely genistin, daidzin, glycitin, and malonyl genistin [31]. The authors discovered that depending on the solvent used, ultrasound can boost extraction yield. Herrera *et al.* developed a semiautomatic technique based on ultrasounds to extract phenolic chemicals such as naringin, rutin, naringenin, ellagic acid, quercetin, and kaempferol from straw berries at 0.8 s duty cycle for 30 seconds [32]. Li and colleagues found that using optimal conditions, including a solvent mixture of 70% methanol, a 20:1 ratio of solvent to sample, and a 30-minute duration, resulted in a higher recovery of chlorogenic acid from fresh leaves, bark, and dried bark of *Eucommia ulmoides* Oliv. compared to traditional extraction procedures [33]. Yang and co-workers extracted bioactive chemicals termed rutin and quercetin from *Euonymus alatus* (Thund.) Sieb using optimum sonication conditions and determined that the ultrasonic approach was more efficient than conventional methods [34]. Ionic liquid-based UAE has been shown to be particularly successful in extracting three alkaloids from *Catharanthus roseus* (vinblastine, vindoline, and catharanthine) [35]. Anthocyanins and phenolic compounds were obtained from grape peel

using ultrasonic-assisted extraction (UAE), with adjustments made to the extraction conditions, such as temperature, solvent, and duration [36,37]. For the extraction of phenolcarboxylic acids, carnolic acid, and rosmarinic acid from *Rosmarinus officinalis*, an ionic liquid-based UAE method proved to be superior in terms of efficiency and speed compared to traditional methods [38].

## 2. Pulses Electric Field Extraction

Over the last decade, pulsed electric field (PEF) treatment has emerged as a valuable method for improving the processing, drying, extraction, and diffusion of plant materials. The fundamental principle of PEF treatment is to disrupt the cell wall and membrane structure to enhance extraction. When living cells are exposed to an electric field, the electric potential traverses the cell membrane, which is composed of molecules with a dipolar nature. [39-43] As the electric potential acts on these molecules, they separate based on their charge. When the transmembrane potential reaches a critical value, typically around 1 volt, the repulsion of these charged molecules leads to the formation of pores in weaker areas of the membrane, greatly increasing its permeability. [44] PEF treatment for plant materials typically involves the use of a simple circuit that generates exponential decay pulses. The treatment chamber comprises two electrodes, and the plant material is placed inside it. Depending on the chamber's design, the PEF process can be continuous or batch-based. [45] The effectiveness of PEF treatment depends on various process parameters, including field strength, specific power input, pulse number, treatment temperature, and the properties of the material being treated. [46] PEF treatment is particularly effective in enhancing mass transfer during extraction by disrupting the membrane structure of plant materials, thereby expediting extraction and reducing extraction time. It is also employed to facilitate the release of intracellular compounds from plant tissues by increasing cell membrane permeability. When PEF treatment is applied at moderate electric field strengths (typically 500-1000 V/cm) with short exposure times, it damages the cell membrane of plant materials while minimising temperature increases. As a result, it helps preserve heat-sensitive compounds. Additionally, PEF is used as a pretreatment step before conventional extraction to streamline the extraction process. [47] In specific examples, PEF treatment has been shown to be highly effective. For instance, in the solid-liquid extraction of beetroots to extract betanin, PEF treatment at 1 kV/cm with low energy consumption (7 kJ/kg) outperformed freezing and mechanical pressing. In other studies, PEF treatment increased the recovery of phytoosterols from maize by 32.4% and isoflavonoids from soybeans by 20-21% when used as a pretreatment process. [48] Furthermore, PEF was found to enhance the extraction of bioactive compounds such as anthocyanin mono glucosides from grape by-products. [49] When applied to grape skins before maceration, PEF reduced maceration time and improved the stability of bioactive compounds like anthocyanins and polyphenols during the winemaking process. [50] Additionally, PEF treatment improved the permeability of Merlot grape skins. [51] In summary, Pulsed Electric Field (PEF) treatment has gained recognition in the past decade for its ability to enhance the processing and extraction of plant materials by disrupting cell membranes and improving extraction efficiency, particularly for heat-sensitive compounds, making it a valuable tool in various industries.

## 3. Enzyme Assisted Extraction

Enzyme-assisted extraction is a process of extracting organic compounds from plant matrices through seed cell wall hydrolysis. This method is known as Enzymatic Pre-treatment and has been found to be a novel and efficacious way to release bound compounds, thus increasing overall yield [52]. EAAE (Enzyme-Assisted Aqueous Extraction) and Enzymatic Cold Pressing (EACP) are two approaches to this process and are typically used to extract oils from a variety of seeds. In EAAE, enzymes are used to break down the cell wall, which is not possible in EACP due to the lack of Polysaccharide-Protein Colloid in this system. Adding certain enzymes such as Cellulase, Pectinase, and  $\alpha$ -amylase during the extraction process further enhances recovery by hydrolysing the Structural Polysaccharides and Lipid Bodies [53]. The key determinants of enzymic hydrolysis are the composition and concentration of the enzymic material, the particle size of the plant material, the solubility ratio of the material to water, and the time required for the hydrolysis process [54]. According to the research conducted by Dominguez and colleagues, the moisture content of the plant material is also a significant factor in the hydrolysis of the material. In the study of EACP, it was found that the oil extracted from oilseed oil by enzymic-assisted methods had a higher percentage of FFA and Phosphorous content than the oil extracted from traditional hexane-extracted oil [55]. The EAE (Enzyme-Assisted Extraction) method is acknowledged as an eco-friendly technology for extracting organic compounds and oils because it employs water as a solvent instead of using organic chemicals [56]. Meyer *et al.* demonstrated the efficacy of enzyme-based exfoliation (EAE) of phenolic anti-oxidants in wine production. This study showed a relationship between the yield of the total phenols obtained and the degree of the breakdown of the plant cell wall by the enzyme used [57]. Landbo *et al.* demonstrated an improvement in the exfoliating power of various enzymes when using EAAE to extract phenolic compounds (pectinyl acid, non-antichyanoid flavonoids, and phenocyanins) [58]. Li and colleagues conducted an experiment to determine the total phenolic content in five different citrus peels, including Yen Ben Lemon, Meyer Lemon, Grapefruit, Mandarin, and Orange. They employed various EAAE enzymes for the extraction process, and the most effective enzyme for recovering phenolic compounds was found to be Celluzyme MX, which is a cellulolytic enzyme [59]. Another important finding from this study was that the extraction of the phenolic antioxidants was significantly improved with higher enzyme concentrations [60]. Finally, Laroze *et al.* demonstrated an increase in the extraction of Phenolic Antioxidant from raspberry Solid Waste by utilization of Enzyme in Hydro-alcoholic Extraction, compared to non-enzymatic control. Enzymes may be used as an alternate source to bioactive compounds to extract phenolic compounds from agri-industrial byproducts [61]. In 2012, Gómez-García *et al.* demonstrated that phenolic compounds can be extracted from grape waste using a variety of enzymes, including celluclast and pectinex, as well as novoferm, in EAE. Novoferm was found to have the most significant effect on releasing phenolic compounds from grape waste [62].

#### 4. Microwave assisted extraction (MAE)

Microwave-assisted extraction (MAE) is an innovative method that harnesses microwave energy to extract soluble substances from various materials [63]. It is based on the principle that electromagnetic fields, which are oscillating fields of two perpendicular lengths, such as electric and magnetic fields, can be induced by the direct impact of microwaves on polar molecules [64]. Heat is produced by converting electromagnetic energy into thermal energy, a process achieved through both ionic and dipole rotational mechanisms [65]. In the ionic mechanism, heat is generated as ions encounter resistance while moving

through the medium. On the other hand, ions consistently align with changing field polarities, leading to frequent collisions between molecules and the subsequent production of heat in microwave-assisted extraction (MAE). The extraction process in MAE is believed to follow a three-step sequence: firstly, the separation of solutes from the active sites within the sample matrix at elevated temperatures and pressures; secondly, the diffusion of solvents throughout the sample matrix; and finally, the release of solutes from the sample matrix into the solvent [66]. Several benefits of MAE extraction have been identified, such as the ability to heat bioactive substances more quickly, the reduction of thermal gradients, the reduction of equipment size, and improved extract yield [67]. Additionally, MAE is a green technology as it reduces the consumption of organic solvents. For the extraction of polyphenols, caffeine, and other organic compounds, MAE yielded a higher extraction yield in 4 minutes than any other extraction method over 20 hours at room temperature. Ginsenosides can be extracted from ginseng roots in 15 minutes using a focused MAE technique, which is better than regular solvent extraction over 10 hours. Dhobi *et al.* showed that MAE is more efficient than conventional extraction methods like Soxhlet and maceration when it comes to extracting flavolignins, silybinins, and other bioactive compounds [68]. Hui *et al.* for example, used MAE to extract flavonoids, phenolics, and cinnamaldehyde from various plants under optimal conditions, and showed that it's faster and easier than other extraction methods [69]. Chiremba *et al.* used MAE for releasing bound phenolic acids in sorghum and corn fractions of different hardness [70]. MAE process is also used to extract some bioactive compounds, including cinnamaldehyde, tannin, and flavonoids, from Chinese quince. They also used designed experiments to maximize the recoveries of the extracts, as well as enhance their electron donating ability [69].

## 5. Pressurized liquid extraction (PLE)

In 1996 Richter and colleagues coined the term "Pressurized Liquid Extraction" (PLE) [71]. This method has since been referred to by various names, including Pressurized Fluid Extraction (PFE), Accelerated Liquid Extraction (ASE), Enhanced Solvent Extraction (ESE), and High-Pressure Solvent Extracting (HSPE) [72]. The core concept behind Pressurized Liquid Extraction (PLE) involves subjecting a liquid solvent to elevated pressure, surpassing its standard boiling point, in order to expedite the extraction process. Automation methods have played a pivotal role in advancing PLE techniques by diminishing both extraction time and the amount of solvent needed. PLE requires only minimal solvent quantities due to the synergy of high pressure and temperatures, resulting in a swifter extraction. Increasing the extraction temperature can enhance the solubility of analytes by raising both their solubility level and the speed at which they transfer into the solvent. Simultaneously, it reduces the viscosity of the solvent and its surface tension. As a result, this leads to an overall improvement in the extraction rates [73].

Compared to the conventional Soxhlet method, PLE is cost effective in terms of time and solvent consumption [71]. Nowadays, PLE is used to extract polar compounds, and is also seen as an alternative to supercritical fluid extractions [74]. PLE is also used to extract organic pollutants from environmental matrixes that are stable at high temperature [75]. PLE has been used to extract bioactive compounds from marine sponge. Plant-Based Extractions (PLES) Plant-based extraction (PLES) is a widely used technique for extracting natural products. Plant Based Extraction (PLES) has been widely reorganised as a Green Extraction Technique (PLES) due to the use of small quantity of organic solvents [73].

The use of PLE has been demonstrated to be effective in the extraction of bioactive motifs from a variety of plant materials. Utilizing optimized conditions, isoflavones have been extracted from soybeans that have been frozen-dried without being degraded by PLE [76]. Shen *et al.* compared ASE for the extraction of Terpenoids and Sterols from tobacco with the use of Soxhlet extraction, as well as Ultrasonically Assisted Extraction (SAE) [77]. PLE is suggested as a viable alternative to traditional methods because it offers quicker processing and lowers solvent usage. For example, flavonoids derived from spinach using a PLE-based mixture of ethanol and a 70:30 solvent at a temperature range of 50–150 °C was more effective than a 50–130 °C water solvent [78]. The results of Luthria's (2008) study demonstrated that the temperature of the solution, the pressure, the size of the particles, the flush volume, the duration of the reaction, and the solubility ratio of the solution all have an effect on the ability of PLE to extract phenolic compounds. The optimized method of PLE extraction was particularly effective in extracting lycorine, galanthamine, and alkaloids from *Narcis sujonquilla*. Additionally, the optimized method was more efficient than hot-sourced extraction, Methylene ether (MAE), and United States of America (U.S.A.); individual phenolic compounds (GCT, Epicatechin, Catechin, Gallate, Chlorogenic Acid, Caffeic Acid, and Myricetin), as well as total phenolic content, were recovered from different parts of the genus *Anastasia propolis* at optimal conditions (40 °C, 1500 psi, 15min) [79].

## 6. Supercritical Fluid Extraction

The application of supercritical fluids in extraction processes can be traced back to the initial observation made by Hogarth and Hannay-Hogarth in 1879. Nevertheless, it was Zosel who, in 1964, obtained the first patent for a method involving supercritical fluid extraction to decaffeinate coffee. Since that time, the technique has generated widespread scientific interest and has been utilised in environmental, pharmaceutical, polymer, and food analysis applications [80]. Several industrial sectors have been utilizing this method for a long time, particularly in the decaffeinated coffee production industries [81].

The three basic states of all earthly substances are Solid, Liquid and Gas. A supercritical phase, or state, is a unique condition that can be attained when a substance is subjected to temperatures and pressures exceeding its critical point. The critical point marks a specific temperature ( $T_c$ ) or pressure ( $P_c$ ) at which the traditional separation between gas and liquid phases no longer applies.[82] In a supercritical state, the particular properties of the gas or liquid become undetectable, thus preventing the liquefaction of the supercritical fluid by changes in temperature and pressure. Supercritical fluids exhibit characteristics similar to those of gases, such as diffusivity and viscosity, as well as solvation power and surface tension, making it suitable for the extraction of compounds in a short period of time with higher yields. A typical SFE system is composed of a tank containing a mobile phase, typically  $CO_2$ , a pressurised gas pump, a co-solvent pump and an extraction vessel. Additional meter types, such as flow meters and dry/wet gas meters, can also be integrated into the system. Finally, a controller is used to ensure the high pressure within the system is maintained [83].

Carbon dioxide has been identified as an optimal solvent for the synthesis of SFE. Its critical temperature ( $T_c$ ) of 31 °C is comparable to that of room temperature, while its low critical pressure of 74 bars provides the opportunity to operate at moderate pressures

(generally ranging from 100 to 450 bar) [84]. Its only disadvantage is its lack of polarity, which makes it suitable for lipids, fats and non-porous substances, but not suitable for most pharmaceutical or drug samples. To overcome this limitation, a chemical modifier has been used [85], and a small amount is usually considered sufficient to significantly increase carbon dioxide polarity. For instance, the addition of 0.5 mL of DCM can significantly improve the extraction, which is equivalent to 4 hours of hydro distillation [86]. The characteristics of the sample and the targeted compounds, as well as the prior experimental result, are the primary criteria for selecting the most suitable modifier.

The success of bioactive compounds extraction from plant materials depends on various parameters of SFE, and the most important are the parameters which can be tuned [87]. Precise control over these parameters is needed in order to maximize the advantages of this method. Extraction efficiency is primarily affected by several key factors, including temperature, pressure, particle size, moisture content in the feed material, extraction duration, CO<sub>2</sub> flow rate, and the solvent-to-feed material ratio [84].

The following are some of the advantages of using a supercritical fluid for bioactive compound extraction: Supercritical fluids exhibit a greater diffusion coefficient when contrasted with liquid solvents. This enables deeper penetration into the sample matrix and improves the transfer of mass. This reduces the extraction time significantly compared to conventional methods. Supercritical fluid can be repeated to the sample for complete extraction. This improves the selectivity of the fluid compared to liquid solvent. The effectiveness of a supercritical fluid in dissolving substances can be precisely adjusted by modifying either the temperature or pressure. With depressurisation of a supercritical fluid, it becomes a convenient way to separate the solute from the solvent, eliminating the need for the traditional solvent-based extraction process. The supercritical fluid operates at room temperature. Supercritical fluid extraction is well-suited for the extraction of thermally sensitive compounds, making it an ideal choice. SFE typically extracts a smaller sample quantity when compared to traditional solvent-based extraction methods. This saves time for the overall experiment. The use of SFE does not require large amounts of organic solvent. This is considered environmentally friendly. It's possible to connect SFE to chromatographic processes online, which is great for volatile compounds. Plus, you can recycle and reuse supercritical fluid, so you don't have to worry about waste. You can also set up a scale for SFE, from a few milligrams in the lab to kilos to tons of samples in industries. Finally, the SFE process gives idea about the extraction process and how it works, so one can adjust it to make it as efficient as possible [88].

The study of Saldaña and Verma demonstrated that SFE can be used to extract purine alkaloid substances (caffeine and theobromine) from the leaves of the herbal maté tea *Ilex Paraguariensis* at a temperature and pressure of 313–343 K and 14 to 24 Mega Pascal respectively [89,90]. Additionally, the study of Supercritical CO<sub>2</sub> (15 Wt.%) modified with ethanol yielded higher extraction yields of the flavonoid naringin (from *Citrus paradise*) than the pure supercritical CO<sub>2</sub> (9.5 Mega Pascal) at a temperature of 58.6 C [91]. Similarly, in 2004, Khorassani and Taylor found that SFE could be used to extract polyphenols and protcyanidins from grape seeds, with methanol as a modifier and CO<sub>2</sub> as a modifier (40%). Furthermore, the research demonstrated that by employing a 6.6 percent methanol solution for a duration of 40 minutes, over 79 percent of catechin from *Citrus Paradise* and epicatechin from grape seeds were successfully extracted using SFE [92].

## VII. CONCLUSION

The ongoing search for more convenient extraction techniques is driven by the consistent demand for bioactive compounds from plants. The improvement in chromatographic techniques and environmental consciousness are two major drivers towards non-conventional extraction techniques. Comprehending all facets of unconventional extraction processes is vital, as these methods frequently depend on distinctive mechanisms, and improving the extraction outcomes stems from this comprehensive understanding. Hybrid approaches should also be incorporated and developed keeping the plant material properties in mind, and chemical selection should be in line with that. As of now, many of the new approaches lack sufficient experimental data, so research should be carried out to overcome those gaps. Anticipated developments in extraction technologies, driven by the growing importance of bioactive motifs and compounds in both scientific research and the economy, will likely result in more sophisticated extraction methods in the future.

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