**EXTRACTION METHOD OF BIOACTIVE COMPOUNDS FROM PLANTS**

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**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 mode and method of experimental procedures should be well-established and documented. Extraction is the first step in isolating and purifying [bioactive compounds](https://www.sciencedirect.com/topics/food-science/bioactive-compound) 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, drying of the plant material, chopping, and grinding of the plant material to obtain a homogenous matrix of samples which improves the kinetics of extraction by increasing the contact of the solvent with the sample surface [Altemimi 2017, Sasidharan et al., 2011]. Next comes the solvent selection part. Indeed, extracting solvent is not selected arbitrarily; its selection depends on the specific nature of the targeted bioactive compound(s). A number of different solvents or mixtures of solvent systems are available to extract the desired bioactive compounds from medicinal plants. Hydrophilic compounds can be extracted by using polar solvents such as acetone, ethyl-acetate, methanol, ethanol or water; on the other hand, chloroform, dichloromethane or a mixture of dichloromethane/methanol in the ratio of 1:1 is used for the extraction of lipophilic compounds. Extraction of phenolic acids and flavonoids often becomes difficult because of their insoluble nature. Conventional extraction methods include reflux, Soxhlet, percolation, maceration etc., which are well-known procedures for the extraction of [bioactive compounds](https://www.sciencedirect.com/topics/food-science/bioactive-compound) and the equipment involved in these techniques are distinct from one another. Now one question may arise. What is an appropriate extraction technique? An appropriate extraction technique that balances the quality of the product, efficiency of the process, costs of production, and environmentally acceptable methods should be used for the extraction of 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 high [hydrostatic pressure](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/hydrostatic-pressure) (HHP), ultrasound (US), [pulsed electric field](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/pulsed-electric-field) (PEF), [supercritical fluid](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/supercritical-fluid) (SF) etc., are rapidly replacing the conventional methods. The use of novel new techniques increases the extent of extraction, thereby increasing yields along with increased extraction rates. This extraction accounts for lower impurities in the resultant extract, preserves thermo-sensitive compounds, uses different inorganic solvents, and consumes low energy. In this review, our main purpose will be to discuss different conventional and novel technologies involved in the extraction process of bioactive compounds from medicinal plants [Jah, 2022, Azmer 2013].

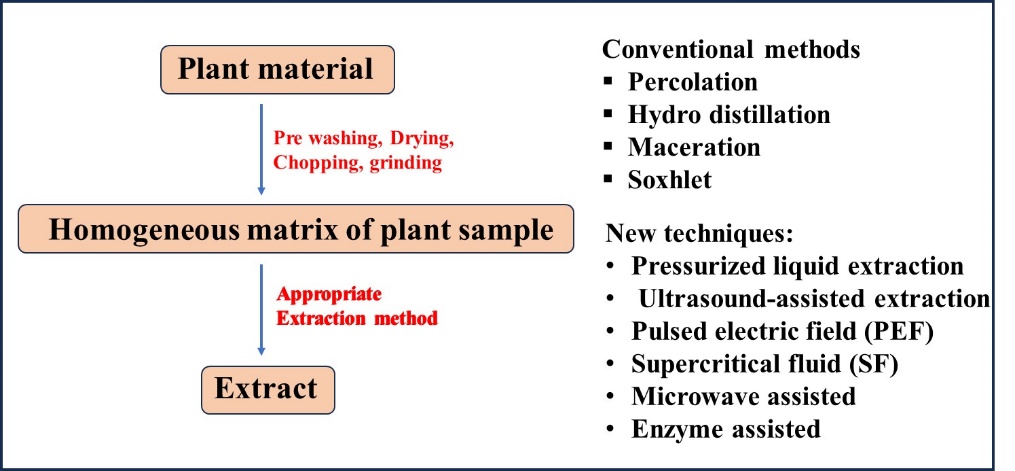


Fig. 1: Schematic representation of extraction protocol

**Bioactive compounds present in plants**

The biological system of the plant is composed of primary and secondary metabolites. The primary metabolites are carbohydrates, amino acids and proteins and are used extensively throughout the development and maturing of plant tissues [Azmir, 2013]. The secondary metabolites are created during the development cycle to help plants survive and overcome natural obstacles. Bioactive compounds are found in a wide range of plant products and can be classified into different classes such as terpenoids; alkaloids; nitrogen-containing compound; organosulphur compound; and phenolic compound [Altemimi, 2017]. The most well-known class of terpenoids are tocotrienols, which include: Morphine, Strychnine, Atropine, Colchicine, Ephedrine, Quinine, Nicotine, Acridine, Carotenoids, Isoquinoline, Lycopodium, Pyrrolidine, Quinoline, Quinolizid, Phytosterols etc. [Dillard & German, 2000]. 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. [Zhang et al., 2018].

**Selection of appropriate extraction protocol**

Bioactive compounds can not only be derived from plant sources but also from other sources which includes microorganism, animals and marine organism [Swamy & Akhtar, 2019]. But in this review, we will restrict our discussion to plants only. The quantity of bioactive compounds present in plant material is low enough and all plant parts, such as leaves, stems, roots, barks, tuber roots, woods, gums or oleoresin, exudates, fruits, flowers, rhizomes etc. produces bioactive chemicals in fairly low quantities and at different concentrations. And for this selection of the right extraction process is very much essential to maximize the extract from plants [Joana Gill-Chavez et al., 2013]. The extractability is dependent on various elements like extraction technique, plant component, matrix properties of plant materials, extracting solvent, temperature, pressure, and time [Drosou et al., 2015]. In the last couple of decades, researchers have focused more towards novel extraction techniques that are environment-friendly [Belwel et al. 2020]. They have also tried to reduce the use of organic solvents, shortening the operational time and obtaining superior-quality of 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 [Putnik et al., 2018].

The extractability of any conventional method depends mainly on the choice of solvents [Cowan, 1999]. The solvent is chosen in such a way that it matches the polarity of the targeted compound. In addition to this molecular affinity between solute and solvent, transfer of mass, role of co-solvent, environmental issues, human toxicity and financial feasibility should also be considered while selecting the solvent for bioactive compound extraction. Following table documents some examples of bioactive compound extraction using the choice of solvents in the conventional way.

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| --- | --- |
| **Solvents** | **Class of compounds** |
| Water | Anthocyanins, Tannins, Saponins, Terpenoids, Carbohydrates |
| Ethanol | Tannins, Polyphenols, Flavonol, Terpenoids, Alkaloids |
| Methanol | Anthocyanin, Terpenoids, Saponins, Tannins, Flavones, Polyphenols |
| Chloroform | Terpenoids, Flavonoids |
| DCM | Terpenoids |
| Ether | Alkaloids, Terpenoids |
| Acetone | Flavonoids |

**Properties of some common extracting solvents**

(i) Water. With a polarity index of 1.000, water is the most polar solvent and is used to extract a variety 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. [Das 2010, Tiwari 2011]

(ii) Alcohol. Alcohol has a polar character (Polarity index of methanol is 0.762 and ethanol is 0.654), is miscible with water, and has the ability to extract polar secondary metabolites. At a concentration greater than 20%, it becomes self-preservative. Low concentrations are harmless, and only a little heat is needed to concentrate the extract. Its disadvantage is that it is volatile and combustible, and it does not dissolve wax, gums, or fats. [Das 2010, Tiwari 2011]

(iii) Chloroform. It works as a nonpolar solvent (Polarity index is 0.259) for extracting substances like terpenoids, flavonoids, lipids, and oils. Advantages. It is colourless having sweet smell and soluble in alcohols. It is also efficiently digested and absorbed by the body. Main disadvantage is that both sedative and carcinogenic. [Tiwari 2011, Pandey 2014]

(iv) Ether. It is 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 low boiling solvent, is miscible with water, and has no taste. Additionally, it is a very stable substance that does not react with metals, acids, or bases. Its disadvantage is that it has a significant degree of volatility and flammability. [Tiwari 2011, Pandey 2014]

(v) 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 is a widely used oil extraction material due to its ease of oil recovery, low boiling point (63-69 °C), and high solubility. [Liu 2005]

(vi) Ionic liquid (green solvent). This particular extraction solvent is very polar and incredibly heat stable. Even at 3,000°C, it can maintain a liquid condition and is suitable in high-temperature applications. It is extremely miscible with water and other solvents and works well for polar chemical extraction. The main advantage is that it is suitable for microwave-assisted extraction because it contains superior solvent that draws and transmits microwaves. It is extremely polar, non-flammable, and useful for liquid-liquid extraction. Although it is not the best method for making tinctures. [Bhan 2017]

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.

**Conventional extraction techniques**

Traditional extraction procedures can be used to extract bioactive chemicals from plant sources. The majority of these approaches rely on the extraction power of the various solvents in use, as well as the use of heat and/or mixing. The known conventional procedures for extracting bioactive chemicals from plants are (1) Soxhlet extraction, (2) Maceration, (3) Hydro distillation, and (4) Percolation. The Soxhlet extractor was invented by German scientist Franz Ritter von Soxhlet in 1879 [Soxhlet 1879]. It was created primarily for lipid extraction; however, it is no longer restricted to this use. Soxhlet extraction has been widely utilized to extract important bioactive compounds from various natural sources. It is used as a model for comparing novel extraction methods. A thimble is usually filled with a small amount of dry material. The thimble is then put in a distillation flask containing the solvent of interest. A siphon is used to aspirate the thimble-holder solution when it has reached an overflow level. Siphon returns the solution to the distillation flask. The extracted solutes are carried into the bulk liquid by this solution. The solute remains in the distillation flask while the solvent returns to the plant's solid bed. The procedure is repeated until the extraction is finished.

Maceration has traditionally been utilised in the manufacture of tonic. It quickly became a popular and low-cost source of essential oils and bioactive substances. Maceration on a small scale usually consists of multiple phases. Plant materials are ground into minute particles to improve the surface area for optimal solvent mixing. Second, a suitable solvent known as menstruum is introduced to a closed vessel in the maceration procedure. Finally, the liquid is strained, but the marc, the solid residue of this extraction process, is pressed to recover many occluded solutions. Filtration is used to extract contaminants from the strained and squeeze out liquids. Occasional shaking in maceration aids extraction in two ways: (a) it increases diffusion, and (b) it removes concentrated solution from the sample surface, allowing the additional solvent to enter the menstruum and boost extraction yield.

The extraction of bioactive components and essential oils from plants by hydro distillation is a conventional process. It does not need the use of organic solvents and can be carried out prior to the dehydration of plant materials. Water distillation, water and steam distillation, and direct steam distillation are the three methods of hydro distillation [Vankar, 2004]. First, the plant components are packed in a still compartment; second, appropriate water is added and then heated to a boil. Direct steam is also introduced into the plant sample. Hot water and steam are the most critical elements in releasing bioactive chemicals from plant tissue. Water condenses the vapour combination of water and oil during indirect cooling. The condensed mixture passes from the condenser to the separator, where oil and bioactive chemicals separate from the water automatically [Silva et al., 2005]. Hydro distillation entails three major physicochemical processes: hydro-diffusion, hydrolysis, and heat degradation. Some volatile components may be lost at high extraction temperatures. This limitation limits its use in thermolabile chemical extraction.

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 saturated solvent is continually replaced by a fresh solvent. In a study conducted by Zhang et al., percolation was compared with refluxing and other extraction methods 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 not significantly affected by either method. The study was conducted in the context of Goupil Patch, a compound Chinese medicinal product composed of 29 Chinese medicinal products. Fu et al. employed the total alkaloid content as the index, with the ethanol percolating method being optimized by soaking the medicine for 24 hours with 55% alcohol, then percolating 12 times the same amount with 55% alcohol. 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% ethanol, followed by percolating 20 times the amount with 70% ethanol; the transfer rates for these two substances were 78.23% and 76.92% respectively.

**Non-conventional extraction techniques**

Longer extraction times, the need for expensive and high-quality solvent, evaporation of a large volume of solvent, limited extraction selectivity, and heat breakdown of thermolabile chemicals are the key problems of traditional extraction [Luque de Castro and Garcia-Ayuso, 1998]. New and promising extraction strategies are developed to address the limitations of traditional extraction methods. These are known as non-conventional extraction methods. Ultrasound aided extraction (UAE), enzyme-assisted extraction, microwave-assisted extraction, pulsed electric field-assisted extraction, supercritical fluid extraction, and pressured liquid extraction are some of the most promising approaches. Some of these approaches are termed ''green techniques,'' since they meet requirements established by the Environmental Protection Agency in the United States. Less hazardous chemical synthesis includes designing safer chemicals, safe solvent auxiliaries, designing for energy efficiency, using renewable feedstock, reducing derivatives, catalysis, designing to prevent degradation, atom economy, and time analysis for pollution prevention, and inherently safer chemistry for accident prevention [Awad et al. 2021].

**Ultrasound assisted extraction (UAE)**

Ultrasound is a form of sound wave that is above the range of human hearing. In chemistry, it is usually between 20 kHz and 100 MHz. It, like other waves, travels through a material by compressing and expanding. This process causes cavitation, which is the formation, growth, and collapse of bubbles. The transfer of kinetic energy of motion into heating the contents of the bubble can provide a huge quantity of energy. Bubbles, according to Suslick and Doktycz (1990), have a temperature of around 5000 K, a pressure of 1000 atm, and a heating and cooling rate of more than 1010 K/s. UAE was created on the basis of this premise. Cavitation occurs only in liquids and liquids containing solids. The primary advantage of UAE may be seen in solid plant samples because ultrasonic energy increases the leaching of organic and inorganic chemicals from plant matrix [Herrera and Luque de Castro, 2005]. The most likely mechanism is ultrasound-enhanced mass transfer and quicker solvent access to plant cell components. The ultrasonic extraction technique incorporates two major physical phenomena: (a) diffusion over the cell wall and (b) washing the contents of the cell after shattering the walls [Mason et al., 1996]. Sample moisture content, grinding degree, particle size, and solvent are all critical aspects in achieving efficient and successful extraction. Furthermore, the controlling parameters for ultrasonic action include temperature, pressure, frequency, and period of sonication. UAE has also been integrated with different traditional ways since they are said to improve the efficiency of a traditional system. An ultrasonic device is positioned in an optimal position in a solvent extraction unit to improve extraction efficiency [Vinatoru et al., 1998]. The benefits of UAE include reduced extraction time, energy consumption, and solvent use. Ultrasound energy for extraction also allows for more effective mixing, faster energy transfer, reduced thermal gradients and extraction temperature, selective extraction, smaller equipment size, faster response to process extraction control, quick start-up, increased production, and process step elimination [Chemat et al., 2008]. UAE is proved to be an efficient extraction process for extracting bioactive compounds from herbal plants. Rostagno et al. (2003) demonstrated the extraction efficiency of four isoflavone derivatives from soybean using a mix-stirring technique with varying extraction periods and solvents, namely daidzin, genistin, glycitin, and malonyl genistin. The authors discovered that depending on the solvent used, ultrasound can boost extraction yield. Herrera and Luque de Castro (2004) developed a semiautomatic technique based on ultrasounds to extract phenolic chemicals such as rutin, naringin, naringenin, quercetin, ellagic acid, and kaempferol from straw berries at 0.8 s duty cycle for 30 seconds. Li et al. (2005) discovered that UAE under optimal conditions (70% methanol, 20:1 solvent, sample ratio, and 30 min duration) recovered more chlorogenic acid from fresh leaves, fresh bark, and dried bark of *Eucommia ulmodies* Oliv. than traditional extraction procedures. Yang and Zhang (2008) 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. Ionic liquid-based UAE has been shown to be particularly successful in extracting three alkaloids from Catharanthus roseus (vindoline, catharanthine, and vinblastine) [Yang et al., 2011]. Anthocyanins and phenolic compounds were extracted from grape peel using UAE, and the extraction method was adjusted in terms of solvent, extraction temperature, and extraction duration [Ghafoor et al., 2011, 2009]. Phenolcarboxylic acids, carnosic acid, and rosmarinic acid were extracted from Rosmarinus officinalis utilizing an Ionic liquid-based UAE methodology that showed to be more efficient and faster than standard extraction procedures [Zu et al., 2012].

**Pulses electric field extraction**

Pulsed electric field treatment has been identified in the last 10 years as a useful process for enhancing the press, dry, extract, and diffusion of plant material. The principle of the PEF treatment is to destroy the matrix of the cell membrane to increase the extraction. During the suspension of the living cell in the electric field, the electric potential passes through the cell membrane of the cell. [Barsotti and Cheftel, 1998; Angersbach et al., 2000; Vorobiev et al., 2005; Vorobiev and Lebovka, 2006]. Due to the dipole character of the membrane molecules, the electric potential separates the molecules according to the charge of the membrane molecules. After reaching a critical value (about 1 V of trans membrane potential) of trans membrane potential, the repulsion of charge carrying molecules causes the formation of pores in weak regions of the membrane causing a drastic increase in permeability [Bryant and Wolfe, 1987]. PEF treatment for plant materials is usually performed using a simple circuit of exponential decay pulses. The treatment chamber consists of two electrodes and the plant material is placed in the treatment chamber. Depending upon the design of the treatment chamber, the PEF process may operate in continuous mode or batch mode [Puértolas et al., 2010]. The efficiency of PEF treatment is highly dependent on the process parameters such as field strength, specific power input, pulse number and treatment temperature, and properties of the material to be treated [Heinz et al., 2003]. PEF can enhance mass transfer during the extraction by destroying the membrane structure of the plant material for enhanced extraction and decreased extraction time. PEF has been used to improve the release of the intracellular compound from plant tissue by increasing the cell membrane permeability. PEF treatment at a medium electric field (500-1000 V/cm; 104-102s) is shown to damage the cell membrane of plant material with little temperature increase. Therefore, PEF can minimise the degradation of heat-sensitive compounds. PEF is also used on plant material as a pretreatment procedure before conventional extraction to reduce the extraction effort [López et al., 2009]. Pulsed electric field (PEF) treatment (1 kV/cm, low energy consumption = 7 kJ/ kg) in the solid-liquid extraction process of beetroots to extract betanin showed the highest degree of extraction when compared to freezing and mechanical pressing. Guderjan et al., 2005, showed that phytosterols recovered by 32.4 % from maize increased by PEF treatment. Isoflavonoids recovered by 20–21 % from soybeans when PEF treatment was used as a pretreatment process. Corralesa et al., 2008, extracted bioactive compound(s) like anthocyanins(s) from grape by-products using various techniques. They found better extraction of Anthocyanin Mono Glucosides by using PEF. [López et al., 2008]. The application of PEF on grape skin prior to the maceration step reduces the maceration time and improves bioactive stability (anthocyanins, polyphenols, etc.) during vinification. Pulsed electric field treatment of Merlot skin improves the permeability of Merlot skin [Delsart et al., 2012].

**Enzyme assisted extraction**

Enzyme-assisted extraction is a method of extracting organic compounds from plant matrices through the seed cell wall hydrolysis. This method is known as Enzymatic Pre-treatment and has been found to be a novel and effective way to release bound compounds, thus increasing overall yield [Rosenthal et al., 1996]. 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 EAAE due to the lack of Polysaccharide-Protein Colloid in this system. Adding certain enzymes such as Cellulase, a-Amylase, and Pectinase during the extraction process further enhances recovery by hydrolysing the Structural Polysaccharides and Lipid Bodies [Singh et al., 1999]. 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 [Latif and Anwar, 2009]. According to the research conducted by Dominguez and colleagues (1995), 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. The EAE process is recognised as an environmentally friendly technology for the extraction of organic compounds and oil, as it utilises water as a solvent rather than organic chemicals [Puri et al., 2012]. Meyer et al., 1998, 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. Landbo et al., 2001, demonstrated an improvement in the exfoliating power of various enzymes when using EAAE to extract phenolic compounds (pectinyl acid, non-antichyanoid flavonoids, and phenocyanins). Li et al., 2006, extracted the total phenolic content of five Citrus Peels (Yen Ben Lemon, Meyer Lemon, Grapefruit, Mandarin and Orange) using various EAAE enzymes, with the highest recovery rate being achieved with the use of Celluzyme MX (Cellulolytic enzyme). Another important finding from this study was that the extraction of the phenolic antioxidants was significantly improved with higher enzyme concentrations [Maier et al., 2008]. Finally, Laroze et al. 2010, demonstrated an increase in the extraction of Phenolic Antioxidant from raspberry Solid Waste by application of Enzyme in Hydro-alcoholic Extraction, compared to non-enzymetic control. Enzymes may be used as an alternative to bioactive compounds to extract phenolic compounds from agri-industrial byproducts. In 2012, Gómez-Garcia 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.

**Microwave assisted extraction**

Microwave-assisted extraction (MAE) is a novel technique for the extraction of soluble products from a broad range of materials by the use of microwave energy [Paré et al., 1994]. 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 materials [Letellier and Budzinski, 1999]. Heat is generated by the conversion of electromagnetic energy to heat, which is achieved through the use of ionic and dipole rotational mechanisms [Jain, 2009]. During the ionic process, heat is generated due to the resistance of the medium-to-flow ion. Conversely, ions maintain their direction along the field signs, which are frequently altered. Microwave-assisted extraction (MAE) is a selective extraction technique that involves the frequent changing of directions of molecules, resulting in collisions between them and the generation of heat. The mechanism of MAE extraction is proposed to involve three steps: first, solute separation from the active sites of the sample matrix under high temperature and pressure; second, solvent diffusion across the sample matrix; and third, solute release from the sample matrix to the solvent [Alupului, 2012]. 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 [Cravottoa et al., 2008]. 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., 2009, 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. Hui et al. 2009, 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. Chiremba et al. 2012, used MAE for releasing bound phenolic acids in sorghum and corn fractions of different hardness. Asghari, et al., 2011, used the MAE process 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 [Hui et al., 2009].

**Pressurized liquid extraction**

Richter et al., 1996, coined the term "Pressurized Liquid Extraction" (PLE). 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) [Nieto et al., 2010]. The principle of PLE is to apply high pressure to a solvent liquid beyond its normal boiling point, which facilitates the extraction of the solvent. Automation techniques have been the primary driving force for the development of PLE techniques, as they reduce the extraction time and solvent requirements. PLE technique necessitates the use of small quantities of solvents due to the combination of the high pressure and temperatures, which results in a more rapid extraction. Furthermore, the higher extraction temperature can improve the solubility of analytes by increasing both the solubility and the mass transfer rate and decreasing the viscosity of solvents and surface tension, thus improving the extraction rate [Ibañez et al., 2012].

Compared to the conventional soxhlet, PLE significantly reduced time consumption and solvent consumption (Richter et Al., 1996). Nowadays, PLE is used to extract polar com pounds, and is also seen as an alternative to supercritical fluid extractions [Kaufmann and Christen, 2002]. PLE is also used to extract organic pollutants from environmental matrixes that are stable at high temperature [Wang and Weller, 2006]. 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 small amount of organic solvent use [Ibañez et al., 2012].

The use of PLE has been demonstrated to be effective in the extraction of bioactive compounds 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 [Rostagno et al., 2004]. Shen and Shao, 2005, compared ASE for the extraction of Terpenoids and Sterols from tobacco with the use of Soxhlet extraction, as well as Ultrasonically Assisted Extraction (SAE). PLE has been proposed as an alternative to conventional methods, due to its faster process and reduced solvent consumption. 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 ºC water solvent [Howard et al., 2008]. 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, Catechin, Epicatechin, Gallate, Caffeic Acid, Chlorogenic 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) [Erdogan et al., 2011].

**Supercritical fluid extraction**

The use of supercritical fluid in extraction applications began with the discovery of Hannay in 1879 by Hogarth and Hannay-Hogarth (1879). However, Zosel (1964) was the first to patent a technique for decaffeinating coffee using SFE. Since that time, the technique has generated widespread scientific interest and has been utilised in environmental, pharmaceutical, polymer, and food analysis applications [Zougagh et. al. 2004]. Several industrial sectors have been utilizing this technique for a long time, particularly in the decaffeinated coffee production industries [Ndiomu and Simpson, 1988].

The three basic states of all earthly substances are Solid, Li quido and Gas. A supercritical state is a distinct state that can only be achieved when a substance is exposed to a temperature and pressure greater than its critical point. A critical point is defined as a temperature (Tc) or pressure (Pc) threshold above which distinct gas and liquid phases cease to exist [Inczedy et al. 1998]. 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. The supercritical fluid has gas-like properties, such as diffusion and viscosity, as well as surface tension and solvation power, 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 CO2, a pressurised gas pump, a co-solvent pump and an extraction vessel. Other types of meters may also be connected to the system, such as flow meter and dry / wet gas meter. Finally, a controller is used to ensure the high pressure within the system is maintained [Sihvonen et al., 1999].

Carbon dioxide has been identified as an optimal solvent for the synthesis of SFE. Its critical temperature of 31ºC 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) [Temelli et al., 2005]. 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 [Lang et al., 2001], and a small amount is usually considered sufficient to significantly increase carbon dioxide polarity. For instance, the addition of 0.5 mL of CH2Cl2 can significantly improve the extraction, which is equivalent to 4 hours of hydrodistillillation [Hawthorne, 1994]. 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 [Raverchon et al., 2006]. Precise control over these parameters is needed in order to maximize the advantages of this method. The main parameters that influence the extraction efficiency are the temperature, the pressure, the size of the particles and the moisture content of the feed material, the extraction time, the flow rate of the CO2, and the ratio of the solvent to the feed material [Temelli et al., 2005].

The following are some of the advantages of using a supercritical fluid for bioactive compound extraction: Supercritical fluid has higher diffusion coefficient compared to a liquid solvent. This allows for more penetration to the sample matrix and better mass transfer. This reduces the extraction time significantly compared to conventional methods. Supercritical fluid can be repeated to the sample for complete extraction. This improves selectivity of the fluid compared to liquid solvent. The solvation power of the supercritical fluid can be fine-tuned either by changing the temperature or/or the pressure. The process of separating the solute from the solvent in a conventional extraction method can be easily bypassed by the use of a depressurizing supercritical fluid. The supercritical fluid operates at room temperature. This makes the supercritical fluid ideal for thermolabile compound extraction. Small amount of sample is extracted in SFE compared to solvent extraction methods. This saves time for the overall experiment. The use of SFE does not require large amounts of organic solvent. This is considered environment 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 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 [Lang and Wai, 2001].

The study of Saldaña (1999) and Verma (2008) demonstrated that SFE can be used to extract purine alkaloid substances (caffeine and theobromine) from the leaves of the herbal maté tea *Ilex Paraguaryensis* at a temperature and pressure of 313–343 K and 14 to 24 MPa respectively. Additionally, the study of Supercritical CO2 (15 Wt.%) modified with ethanol yielded higher extraction yields of the flavonoid naringin (from *Citrus paradise*) than the pure supercritical CO2 (9.5 MPa) at a temperature of 58.6 C [Giannuzzo, 2003]. 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 CO2 as a modifier (40%). Furthermore, the study showed that SFE was able to release more than 79 percent of catechin (from Citrus Paradise) and epicatechin (from grape seed) using 6.6 percent methanol for 40 minutes.

**Conclusion**

The ever-increasing demand for plant bioactive substances drives the ongoing search for more simple extraction techniques. The improvement of chromatography and environmental consciousness are two major drivers in the creation of most non-conventional extraction techniques. However, knowing every part of the non-traditional extraction process is critical since most of these approaches are based on distinct mechanisms and extraction improvement is the consequence of diverse processes. Hybrid approaches should also be incorporated and developed in light of plant material properties and chemical selection. Some of the available approaches still require sufficient experimental data. The selection of standard procedures has an impact on the assessment of extraction efficiency. On the other hand, the growing economic importance of bioactive compounds and bioactive compound-rich commodities may lead to the development of more complex extraction technologies in the future.

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