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Modified Mechanical and Methodological Attributes Interplay for Isolating Antioxidant Concomitant in Herbal Materials: An Innovative Concept

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Abstract

The plant products and materials are believed to have numerous health benefits. The Asian continent is recognized as a hub for medicinal plants. As on today, a large populace relies on medicinal plants and their associated products for the treatments of ailments. The popularity of the medicinal plants is continuously increasing because they are assumed to impose no health risk and side effects unlike synthetic therapeutants. Edible vegetals and agroindustrial residues are considered to be abundant and promising sources of natural antioxidants. The extraction method, of crucial importance for both technical and economic reasons, ideally should be non-destructive, time efficient and suitable for producing high quantities of extracts, which should be processed by selective techniques to yield concentrates of enhanced antioxidant capacity. The extraction methods, solvent system used and sample nature greatly effects the final bioactive compounds yield and efficacy. This article briefly summarized the basics of extraction and separation of bioactive compounds and improvisation in further downline applications.

1. Introduction

In addition to their traditional usage as fodder crops, medicinal plants can also be used to treat a wide range of human diseases and health issues such as diabetes and cancer. Plants' ability to aid in the development of drugs is primarily based on the secondary metabolites that protect plants from harmful environmental circumstances that play a key role in their drug development properties. Bioactive chemicals, which are found in all living systems, including humans, make up the primary metabolite's secondary constituents. Antioxidants, flavonoids, and chemicals that protect DNA from harm are examples of bioactive substances. There are numerous hidden stories that phytochemicals and bioactive components like antioxidants, flavonoids, tannin, saponin, and others can testify to in the herbal material's crude extract (Joshi *et al.,* 2012). The visualisation of the extract's colouring can be used to assess its apparent bio-efficacy. There is a direct correlation between the yield of the extract and its biochemical composition and mineral profiling, and these factors have a significant impact on these outcomes (Meena *et al.,* 2020). Separating bioactive principles from plant products is largely dependent on the choice of solvent system and extraction process. Extraction methods include both traditional and non-conventional approaches to obtaining bioactive chemical extractions. Any conventional method's extraction effectiveness is heavily influenced by the solvent used. Longer extraction durations, the need for expensive and high-purity solvent, the evaporation of large amounts of solvent, low extraction selectivity, and the heat breakdown of thermolabile chemicals are the key problems of traditional chromatography. New and promising extraction strategies have been devised to overcome the drawbacks of classic extraction methods. Non-conventional extraction techniques are the name given to these kinds of methods. Ultrasound assisted extraction, pulsed electric field, enzyme assisted extraction, microwave assisted extraction, pressurized liquid, supercritical fluid and pressurized low-polarity water extraction and molecular distillation are some of the most promising approaches described in this article.

2. Rationale of Extraction of Bioactive Principles from Plant Source

Plant processing industry by-products are a low-cost source of bioactive chemicals, in particular antioxidants. Plant by-products provide several inexpensive antioxidants. By-product anti-oxidants such as these are critical for the development of functional foods that consumers will consume. Higher concentrations of antioxidants can be extracted using commercially available techniques. When compared to

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synthetic approaches, these ones did not appear to be any more carcinogenic or dangerous. When it comes to biorefineries, the idea proposed by Bozell and Petersen (2010) takes antioxidant generation and recovery into consideration.

Many indigenous medical systems rely heavily on the use of medicinal plants, and this trend is continuing in modern culture as people seek alternatives to synthetic pharmaceuticals. Herbal medicines have a number of advantages over synthetic drugs, including being less expensive and easier to obtain, as well as having less side effects (Dey and De, 2015). Botanical, phytochemical, biological, and molecular techniques are all being used in new medical plant drug research activities (Balunas and Kinghorn, 2005). Bioactive components are responsible for medicinal plants' therapeutic capabilities, such as hypoglycemia and anti-diabetic effects. In addition to medicinal plant therapeutic capabilities like analgesic and antidiabetic activities, antioxidant and anti-microbial properties, these compounds are also responsible for the molecules in the food we eat. The bioactive components of many diabetic-fighting herbs, for example, Chikezie *et al.* (2015), have been extensively studied elsewhere. Pure molecules or standardized extracts from medicinal plants offer unlimited possibilities for novel therapeutic leads due to the abundance and chemical variety of bioactive components found in the plant world. People and animals are susceptible to toxins that come from a range of chemical sources, including those found in plants. This is according to a study called Chemo diversity. There are numerous poisonous compounds found in plants, such as phytohaemagglutinines or lectins (Tadele, 2015), neurotoxic amino acids such as N-Oxalyl-L-and-diaminopropionic acid (ODAP), cyanogenic glycosides (FSANZ, 2004), myristicin (Lee *et al.,* 2005), and phyto solubility, process conditions, and extraction method, all of which influence extractive yield and antioxidant (Selvamuthukumaran and Shi, 2017). Through processes like polymerization and breakdown, these variables may have an effect on the antioxidant content and quality of the extracts. Using an effective extraction, the most bioactive compounds can be extracted with the least degradation and the least amount of non-antioxidant components (such as sugars and organic acids). In many plant materials, polyphenols are present in varying amounts and types depending on the extraction process, chemical composition, particle size, and other variables. Contamination and storage conditions can also have an impact on polyphenols (Suwal and Marciniak, 2018). Some have low levels of phenolic acids, anthocyanins, and phenylpropanoids, whereas others have high concentrations of these compounds (Balasundram *et al.,* 2006; Tsao, 2010). Other insoluble high-MW phenolics, proteins, and carbohydrates may also interact with them to form complexes (Herrero *et al.,* 2012). Phenolic chemicals are often taken from plants in a crude state to avoid interference with pigments, terpenes, lipids, and wax during the extraction process. In order to deal with this, an extraction process must be tweaked. Solid-phase extraction, fractionation, and acidity-based purification are common procedures for removing interferences. Because of this, it must be ensured that the extraction efficiency of polyphenols from natural sources depends on a number of critical variables such solvent, material nature, and the amount of time spent on the extraction (Herrero *et al.,* 2012).

2.1 Conventional Extraction Techniques

Traditionally, phenolic chemicals are extracted by a variety of methods including maceration, decoction, percolation, infusion, digesting, serial exhaustive extraction, and soxhlet. Due to the availability of other more practical procedures, the maceration process is currently underutilized. Extraction by maceration is a straight forward procedure that involves immersing a pulverized sample in a suitable solvent in a closed system and agitating the mixture at room temperature on a regular or irregular basis (Olejar *et al.,* 2015). Solids are extracted from the solvent and then separated using some sort of separation procedure after that. In general, this is accomplished using one of three methods: filtration, decantation, or clarifying. Even though this method is straight forward, it comes at the cost of efficiency in that it takes a long time and consumes a lot of solvent (Alara *et al.,* 2018a, Alara *et al.,* 2018b). The plant material is combined with a condensed solvent in a soxhlet apparatus for a solid-liquid extraction procedure. It is advantageous to use soxhlet since it is repetitious in contacting the solid matrix and does not require any filtration after the operation is completed. Nevertheless, it has drawbacks, such as a high demand for the solvents (Garcia-Vaquero *et al.,* 2020) and consequent evaporation/ concentration process; no stirring during the process; and possible thermal degradation of the compounds because the process is usually carried out at the boiling point of the solvents for an extended period of time (Garcia-Vaquero *et al.,* 2020; Alara *et al.,* 2018a, Alara *et al.,* 2018b). No one knows which solvent is better for a certain raw material because there are so many studies on extraction yield in literature. Ethanol and water are the best solvents for the food business because of their GRAS (generally recognized as safe).

2.2 Non-Conventional Extraction Techniques

2.2.1 Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is a technique that uses supercritical fluids as the extraction solvent to separate an extractant from a matrix. There are many advantages of using supercritical fluid extraction (SFE) over other extraction processes, including efficiency, environmental friendliness, and selectivity. During the SFE process, the fundamentals and the most critical parameters must be taken into account. Additional SFE advances are shown to keep readers up-to-date on the current applications in these fields where SFE has made a significant impact on the extraction of bioactive compounds from intriguing natural sources such as plants, marine goods, and food and agricultural by-products. When compared to conventional organic solvent extraction, SFE is a more environmentally friendly technique since it uses super critically heated fluids as solvents rather than large quantities of hazardous solvents, which are unsuitable for use in the food business. The process also decreases degradation because it is done in the absence of light and oxygen. It is easy to separate extracted solutes from carbon dioxide, which is the most often employed solvent to achieve supercritical conditions. It is also compatible with foods and can be utilized when low temperatures are required. For the extraction of lipophilic plant components like lipids, essential oils, and aroma compounds, SFE with carbon dioxide is an efficient substitute for standard solvent extraction procedures.

2.2.2 High Hydrostatic Pressure (HHP) and Pressurized Liquid Extraction (PLE)

To boost the mass transfer rate between solid matrices and extraction solvent, pressure is employed in both HPP and PLE processes. This leads to more cost-effective and time-efficient extraction procedures for active chemicals. High pressure, on the other hand, can improve extraction efficiency, but at a higher cost. In order to use HHP, a mixture of raw materials and solvents must be hermetically sealed in a package and then delivered into an enclosed vessel containing a pressure-transmitting medium (*e.g*., water, hydrophilic and lipophilic organic solvents at different concentrations).

Because of high pressures (3.33-20.30 MPa) and temperatures (40-200 °C) used in the PLE process, the material/ solvent mixture can be extracted in short periods of time (3-20 minutes). Above their boiling temperatures, pressurized solvents stay liquid, enabling high-temperature extraction. The term "accelerated solvent extraction" refers to the use of pressurized liquid extraction. It's one of the newest methods for getting at phytochemicals, having only recently been invented. It necessitates the utilization of high temperatures and pressures. When high pressures of 3.3-20.3 MPa are used in conjunction with high temperatures between 40-200 °C, it helps with molecular desorption and solubility in solvents. It was discovered by Nieto *et al*. (2010) that the PLE extraction method is more advanced than conventional methods since it ensures a quick extraction process while using a less volume of solvents. In addition, because of the high temperature, it stimulates the greater use of water as an extraction solvent, such as the so-called subcritical water extraction. The purity of the extracts obtained with the PLE method is one of the methods' acknowledged benefits, according to numerous researchers. Since no purification steps are required, such as liquid chromatography mass spectrometry analyses, they are unnecessary. However, PLE's major drawbacks are its poor analyte selectivity during extraction, the presence of interferents during the extraction process, the high levels of extract dilution, particularly when using multiple cycles, and the necessity of specialized instrumentation, which is an expensive process to implement. Despite these drawbacks, pressurized liquid extraction is a widely used extraction method for obtaining polyphenols from various sources (Liazid *et al.,* 2014). By using pressurized liquid extraction in conjunction with solid-phase extraction, the desired phenolic chemicals can be isolated more effectively. By combining an extraction cell with an on-line dispersing agent, it is possible to place a solid phase inside an extraction cell alongside an extracted material that has been specifically targeted for dispersion. Prior to chemical analysis, this process improves sample purity. For the extraction of phenolic compounds, researchers have used the off-line and in-line PLE-SPE approach in several investigations (Plaza and Turner, 2015). The concentration of proanthocyanins in malt was quantified using other commercial online platforms (Prior and Liwei, 2005). If an automated device is used, the extracts from the PLE collection bottle will be transferred directly to the SPE apparatus.

2.2.3 Ultrasound-Assisted Extraction (UAE)

In the UAE method, ultrasonic waves are used to break apart the target molecules in a combination of liquids and solids. As a result of a combination of mixing effects and the physical effects of ultrasound on raw material (*e.g.,* fragmentation and erosion), the mass transfer rate improves. This could explain the improved extraction performance of this technology (Shirzad *et al.,* 2017). In the long run, it reduces extraction time, solvent consumption, and energy consumption. It is also possible to combine ultrasound with other techniques such as supercritical fluid extraction, microwave extraction, and soxhlet elution. Ultrasound-assisted extraction, for example, has been shown to be more effective than conventional extraction approaches in extracting rosmarinic and carnosic acid. Latest research shows highest yields of 13.20 mg g^{-1} dry weight from spruce wood bark using the UAE approach (Ghitescu *et al.,* 2015). According to Cai *et al.* (2016), the use of the UAE increased anthocyanin output from purple sweet potato. The use of ultrasound-assisted extraction, according to these studies, increases the solubility of compounds in the extraction solvent, resulting in a reduction in the amount of solvent necessary for complete phytochemical recovery. According to these reports, this approach appears to be less costly for the following reasons: A smaller amount of solvent is used, resulting in a larger test sample volume and shorter extraction time. Low temperatures and shorter sonication times improve polyphenol extraction and maintain thermolabile chemicals, it is also acknowledged.

2.2.4 Microwave-Assisted Extraction (MAE)

Microwaves are another environmentally friendly extraction technique that relies on the chemicals' polarity to affect them directly. By utilizing MAE, energy may be rapidly delivered to a large volume of solvent or solid matrix, and then heated uniformly and efficiently across the entire system. It allows for a reduction in extraction time and solvent volume, and can be used in either open or closed system applications. Solvent and sample are kept separate in sealed jars at a constant temperature and pressure. This is the preferred method. This reduces extraction time while also increasing extraction efficiency since closed vessels allow solvent temperatures to climb above the boiling point. However, this method's efficiency can be drastically reduced when working with non-polar or volatile solvents. So, polar solvents like ethanol, methanol, and water should be employed.

In extracting short-chain polyphenols such as phenolic acids and flavonoids, the MAE has been proven to be beneficial. Microwave-assisted extraction can damage polyphenols with many hydroxyl-type substituents and heat-sensitive ones like anthocyanins; hence it's used sparingly when studying polymeric polyphenols like anthocyanins and tannins. When using MAE, the extraction temperature varies with the amount of time and power (in watts), but it is inversely proportional to the mass of the solvent sample and its heat capacity. Higher temperatures and smaller sample volumes in the microwave-assisted extraction technique favour solvent diffusion and extraction kinetics. Many phytochemicals, such as polyphenols, have been extracted using the MAE technique, which appears to offer a high yield of polyphenols in less time while using less solvents. When utilizing this method for polyphenol extraction, you must take into account a number of factors, including the type of sample you're working with as well as the solvent type and purity, the amount of power and time the microwave has to work, and the operating temperature. Because it has an impact on everything from target component solubility to overall process efficiency, the solvent type is crucial. Since the solvent is critical to the process, it must be chosen with care, taking into account both its affinity for the target chemicals and its ability to absorb microwave energy. It's possible that transparent solvents like hexane or dichloromethane won't work in MAE since they can't get hot enough when exposed to microwave radiation. There are other solvents with strong microwave absorption capacity that may easily heat up and so reduce the amount of time that microwave power needs to be applied. These other solvents should be utilized in the procedures instead of ethanol or water. Aside from that, these solvents have little to no effect on thermolabile substances.

2.2.5 Enzyme-Assisted Extraction (EAE)

Current technology uses the enzymes' ability to break down cell wall compartments to ensure the passage of cytoplasmic content into an extraction fluid such as water for the extraction process. Cell walls can be broken and weakened by the enzymes used during EAE, allowing the cellular material to be extracted. Polyphenols can be extracted from these samples more easily this way. Due to hydrogen or hydrophobic bonding, the vast majority of plant phytochemicals are inaccessible because they are linked to lignin (a tough component of the plant cell wall). As a result, the effective release of bound phytochemicals will necessitate an enzymatic pre-treatment procedure.

Enzymes such as cellulases, hemicellulases, pectinases, and others are used to aid the extraction of phytochemical components from plant materials. Hydrolysis of cell wall lignin to improve extraction efficiency can be accomplished with these enzymes. A number of studies have shown that EAE has a good impact on polyphenol extraction when used as a pretreatment. When it came to the recovery of polyphenols from grape wastes, researchers found that pretreatment enzymes such as celluclast®, pectinex® and novoferm® produced a high polyphenol yield (Gómez-Garca *et al.,* 2012). According to other research, EAE should be regarded an alternate technique for improved extraction of carbohydrate-bonded phenolics from wine-making wastes (de Camargo, 2016). Enzyme activity was observed as cell wall components were degraded to improve bioactive chemical extraction efficiency in these investigations. Water is used as a solvent instead of organic solvents, making this a greener approach to utilize. Apart from that, it is one of the most up-to-date extraction methods that have attracted a lot of attention recently as part of a movement to adopt environmentally friendly laboratory techniques.

3. Factors Affecting of Antioxidant Extraction

Analyte degradation, oxidation, and solubilization can all occur simultaneously in the same sample at different temperatures and times. Through an increase in both the mass transfer rate and solubility, a greater extraction temperature promotes increased soluble of the analyte. More heat reduces surface tension and viscosity of the solvent, enhancing extraction rates since the solvents can access sample matrices easier. The hydrolyzation and oxidation of many phenolic compounds, on the other hand, is a serious problem. In order to reduce the phenolic yield in the extracts, higher extraction temperatures and longer extraction durations increase the possibilities of oxidation occurring. According to one study, typical extraction and concentration methods commonly use temperature ranges of 20-50 °C when working with anthocyanin, while temperatures above 70 °C have been shown to rapidly damage the pigment's antioxidant properties. As a result, the choice of extraction procedures and technologies is crucial, as is maintaining the phenolic compound's stability.

4. Purification and Fractionation

Since carbohydrates and lipoidal components make up the majority of plant crude extract phenolic contents, these concentrations are typically modest. The use of SPE and liquid-liquid partitioning or successive extraction techniques based on acidity and polarity to concentrate and obtain polyphenol-rich fractions before analysis leads to this. It is common to wash the crude extract with non-polar solvents (*e.g.*, hexane) to remove the dichloromethane or chloroform and the lipoidal material that makes up the mixture.

SPE is commonly used to remove polar and non-phenolic substances (such as sugar and organic acids). Due to its affordability, speed, and sensitivity, the SPE technique has been widely used. It also allows the use of a wide range of sorbents *via* various cartridges and discs. Additionally, this technique now has the ability to be automated. C18 cartridges are the most widely used method of phenolic compound separation because of their versatility. Aqueous samples should be run through preconditioned C18 cartridges, and the cartridges should be washed with acidified water and other water-soluble ingredients such as organic acids and sugar. After that, either 100% methanol or aqueous acetone will be used to elute the polyphenols.

5. Characterization of Antioxidants

There are various methods for evaluating total phenolic content, but none of them is perfect because of the variability of natural phenolics and the likelihood of contamination from oxidized compounds found in plant materials. In the past, procedures including Folin-Ciocalteu, Folin-Denis, ferric salt colorimetry, permanganate titration, and UV absorbance have been tried. The F-C technique has consistently outperformed the alternatives in the vast majority of circumstances.

6. Processing for Stabilization

With capsule structures (from the nanometer to the micrometer range), stabilization is often achieved by producing a core and wall material made of nanometer to micrometer capsule structures. There are two parts to capsules: a core and a coat. The core is comprised of food-grade materials, while the coat is made of the external layers that cover it and are referred to as the coating or shell.

7. Conclusion

In the food sector, antioxidants derived from natural sources are valuable bioactive chemicals with well-documented benefits. For functional food products, their usage as an alternative to synthesized chemicals has been studied as a means of increasing product stability and preventing oxidation-induced degradation during processing and storage. Natural antioxidants derived from agricultural byproducts and underutilized plant materials are being used in the framework of a circular economy. Natural antioxidants have already been studied from extraction to application at every stage. When it comes to extraction, the best approaches depend on the sort of molecule you're trying to recover. To avoid the use of large volumes of solvent in traditional solvent extraction methods, newer, more environmentally friendly techniques have been investigated. Although non-conventional technologies have begun to take the place of conventional ones, progress must be made in terms of scale. Spray drying has been the most widely utilized stabilization procedure following extraction because of its ease of operation and scaling up, producing encapsulated antioxidants in the form of powder microparticles and allowing for easy manipulation and doses. Due to their natural origin, however, it is necessary to consider the doses and probable toxicological effects of these chemicals when applying them to food products. Furthermore, some natural substances have detrimental effects on sensory qualities, such as flavour and taste, which must be addressed. As a result, more people will buy foodstuffs rich in natural antioxidants, which will lead to lower pricing for those products.

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