Recent Advances in Extraction of Bioactive Ingredients from Medicinal Plants

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Abstract: Ecofriendly production of chemical compounds and organic or inorganic materials are generally accompanied by the concept of saving resources by optimizing conditions and/or introducing new process technologies. All conventional methods require more extraction time, less yield as compared to novel extraction techniques. Various novel techniques including supercritical fluid extraction, microwave assisted extraction, ultrasound assisted extraction and pressurized fluid extraction have been developed for the extraction of active ingredients or active ingredients from plants in order to shorten the extraction time, decrease the solvent consumption, increase the extraction yield, and enhance the quality of extracts. This review gives a theoretical background of above novel extraction methods and brief about which parameters should be considered while selecting an extraction technique is also been discussed.

Keywords: Extraction of Bioactive compounds, Microwave assisted extraction, Novel extraction techniques, Pressurized fluid extraction, Supercritical fluid extraction, Ultrasonic assisted extraction.

1. Introduction

Extraction involves the separation of medicinally active portions of plant or animal tissues from the inactive components by using selective solvents in standard extraction procedures. The products so obtained from plants are meant only for oral or external use and which are relatively impure liquids, semisolids or powders. The purposes of standardized extraction procedures for crude drugs are to attain the menstruum. The extract thus obtained may be ready for use as a medicinal agent in the form of tinctures and fluid extracts, it may be further processed to be incorporated in any dosage form such as tablets or capsules, or it may be fractionated to isolate individual chemical entities such as ajmalicine, hyoscine and vincristine, which are modern drugs [1]. Thus, standardization of extraction procedures contributes significantly to the final quality of herbal drug.

There are various methods for extraction of medicinal plants viz., pressurized solvent extraction, ultrasonic assisted extraction, microwave assisted extraction, accelerated solvent extraction, supercritical fluid extraction, counter current extraction, infusion, percolation, decoction, maceration, etc. Novel extraction techniques have decreased the solvent consumption, increased the extraction yield and enhanced the quality of product.

2. Supercritical fluid extraction (SFE)

Supercritical fluid extraction is the process of separating one component from another (solid matrix) using supercritical fluids as the extracting solvent. SFE can be used to extract compounds from plants at ambient temperature. Supercritical fluid extraction is an alternative sample preparation method with an aim of reduced use of organic solvents and increased sample throughout. SFE is used for extraction of volatile or aroma compounds, such as essential oils, and caffeine from the plant materials. The extraction is carried out in high-pressure equipment in batch or continuous manner. In both the cases, the supercritical solvent is put in contact with the material from which a desirable product is to be separated. More commonly cylindrical extraction vessels are used for sample preparation. In batch processing, solid is placed into the extraction vessel and the supercritical solvent is fed in until the target extraction conditions are reached. In semi-batch processing, the supercritical solvent is fed continuously through a high pressure pump at a fixed flow rate, to precipitate the solute from supercritical solution. Supercritical state of a substance is achieved when the temperature and the
pressure of a substance is raised over its critical value.

2.1. Supercritical Fluids (Extracting solvent)

This particular behavior of substances was first observed in 1822 by French engineer and physicist, Charles Cagniard de La Tour [2]. It was then defined as supercritical fluid by Irish chemist, Thomas Andrews.

The supercritical fluid has characteristics of both gases and liquids. Some factors must be considered for successful SFE like selection of supercritical fluid, modifiers (cosolvent) addition, extraction conditions, temperature, pressure and flow. Selection of supercritical fluids is a critical step for the development of SFE process. Two parameters which are of prime importance when considering the selection of supercritical fluid are critical pressure and critical temperature. The dissolving power of a supercritical fluid solvent depends on its density, which is highly adjustable by changing the pressure or/and temperature. The supercritical fluid has a higher diffusion coefficient, lower viscosity and surface tension than a liquid solvent, leading to more favorable mass transfer [3].

Supercritical fluids are suitable as a substitute for organic solvents in a range of industrial and laboratory processes. Supercritical fluids have properties between those of a gas and liquids. CO2 and water are most widely used as the extracting fluid. With reduction in price of carbon dioxide and restrictions in the use of other organic solvents, carbon dioxide has begun to move from some marginal applications to being the major solvent for supercritical fluid extraction. CO2 is most widely used as extracting fluid because it has critical temperature and pressure of only 304 K and 7.3MPa respectively. It is chemically inert, non toxic and also safe. It is non-flammable. CO2 is available at high purities and at low cost. It is non-corrosive and odorless. CO2 leaves no solvent residue in the product.

It has some disadvantages also, polarity limitations is major amongst all. Solvent polarity is important when extracting polar solutes and when strong analytic-matrix interactions are present. Organic solvents are frequently added to the carbon dioxide extracting fluid to alleviate the polarity limitations. Many nutraceuticals such as phenolic, alkaloid and glycosidic compounds are poorly soluble in carbon dioxide and hence are not extractable. Addition of modifiers to the supercritical fluid CO2 increases the solubility of polar compounds. Among all the modifiers including methanol, ethanol, acetonitrile, acetone, water, ethylether and dichloromethane, methanol is the most commonly used as it is very effective polar modifier and is miscible up to 20% with CO2 [3]. The best modifier usually can be determined based on preliminary experiments. One disadvantage of using a modifier is that it can cause poor selectivity. Argon can also be used as extracting fluid as it is cheaper and more inert than CO2. The component recovery rates generally increase with increasing pressure or temperature. The highest recovery rates with use of argon are obtained at 500 atm and 150° C. Several other solvents can be used for SFE such as hexane, pentane, butane, nitrous oxide, sulfur hexafluoride and fluorinated hydrocarbons, etc.

2.2. Principle

The system must contain a pump for the CO2, a pressure cell to contain the sample, a mean of maintaining pressure in the system and a collecting vessel. The liquid is pumped to a heat exchanger, where it is heated to supercritical conditions. It is then passed to the extraction vessel, where it is rapidly diffused into the solid matrix and dissolves the material to be extracted. The dissolved material is swept from the extraction vessel into a separator at lower pressure, and the extracted material settles out. The CO2 can then be cooled, re-compressed and recycled or discharged to atmosphere.

2.3. Advantages

SFE has been demonstrated to be more effective than other conventional techniques for the extraction of antimicrobial compounds. This is an environment friendly extraction technique. Low viscosity of supercritical fluid allows more selective extractions. This extraction process is fast and hence is useful where the process time is one of the factors. It offers better diffusivity as compared to the other processes. As there is no solvent residue it reduces the burden
of storage. The extraction of constituents at low temperature strictly avoids damage from heat and some organic solvents. It is suitable for extraction and purification of compounds having low volatility present in solid or liquid. There is complete separation of solvent from extract and raffinate. It also has low cost of handling. Solvent recovery is easy as compared to other processes. Thus supercritical solvent extraction is versatile and efficient.

2.4. Limitations

The extraction process has some limitations such as solute diffusion from solid into the SCF takes time. The requirement of high pressure increases the cost compared to conventional liquid extraction. Carbon dioxide is non-polar and has limited dissolving power, so cannot be always used as a solvent on its own, particularly for polar solutes. Modifiers have to be used along with carbon dioxide for extraction of polar solutes.

2.5. Applications

SFE finds extensive application in the extraction of pesticides, environmental samples, foods and fragrances, essential oils, polymers and natural products [4]. Since the residual solvent present in the extracted material is of critical importance in the pharmaceutical industry, carbon dioxide as supercritical fluid has found several applications. The extraction of vitamin E from soybean oil and its purification method is another application of this technique. Supercritical fluid extraction has been proposed as an alternative technique for soil remediation and activated carbon regeneration. Over 99% of organics can be removed from contaminated soil. It has also been used to extract plant materials especially lipids. SFE can prevent the oxidation of lipids. It is [5] found that the contents of free fatty acids, sterols, triacylglycerols and tocopherols in the hazelnut oil extracted by SFE were comparable with those obtained with n-hexane extraction. SFE can achieve higher yields and quality of essential oils, flavors and natural aromas than conventional steam distillation.

3. Microwave assisted extraction (MAE)

MAE is a simple, environment friendly and economical technique for the extraction of biologically active compounds from different plant materials. The use of microwave energy as a heating source in analytical laboratories started in the late 1970s and was applied to acid digestions [6]. The development of microwave assisted extractions was first reported by Ganzler and co-workers [7, 8].

3.1. Principle

Microwaves are electromagnetic radiations with a frequency from 0.3 to 300 GHz. Domestic and industrial microwaves generally operate at 2.45 GHz, and occasionally at 0.915 GHz in the USA and at 0.896 GHz in Europe. Microwaves are transmitted as waves, which can penetrate biomaterials and interact with polar molecules such as water in the biomaterials to create heat. Consequently, microwaves can heat a whole material to penetration depth simultaneously.

Microwaves possess electric and magnetic fields which are perpendicular to each other. The electric field causes heating via two simultaneous mechanisms, namely, dipolar rotation and ionic conduction. Dipolar rotation is due to the alignment on the electric field of the molecules possessing a dipole moment in both the solvent and the solid sample. This oscillation produces collisions with surrounding molecules leading to liberation of thermal energy into the medium. With a frequency of 2.45 GHz, this phenomenon occurs 4.9×10⁷ times faster and thus the resulting heating is very fast. Indeed, larger the dielectric constant of the solvent more rapid the heating is. Consequently, unlike classical conductive heating methods, microwaves heat the whole sample simultaneously. In the case of extraction, the advantage of microwave heating is the disruption of weak hydrogen bonds promoted by the dipole rotation of the molecules [9].

Components of the sample absorb microwave energy in accordance to their dielectric constants [10]. When plant material is immersed inside a microwave transparent solvent, the heat of microwave radiation directly reaches to the solid without being absorbed by the solvent, resulting in instantaneous heating of the residual moisture in the solid. Heating causes the moisture to evaporate and creates a high vapor pressure that breaks the cell wall of substrate and releases the content into solvent. Solvents employed for most MAE operations are those with a high dielectric constant and capacity to strongly absorb microwave energy, however, the extraction selectivity, and the ability of the medium to interact with microwaves can be modulated by using mixtures of solvents. It is not uncommon to use binary mixture of solvents, with only one solvent capable of absorbing microwave. Though polar solvents are usually believed to be better than non-polar ones [11] Addition of water to the solvent may lead to increased yields. Microwave transparent solvents like acetone proved to be best for extraction of phenolic compounds.
3.2. Instrumentation

There are two types of commercially available MAE systems: closed extraction vessels under controlled pressure and temperature, and focused microwave ovens at atmospheric pressure [9]. These technologies are named as pressurized microwave assisted extraction (PMAE) and focused microwave assisted extraction (FMAE), respectively [12].

3.3. Pressurized microwave assisted extraction

This is a closed MAE system generally used for extraction under drastic conditions such as high extraction temperature. In closed vessel system the solvent may be heated much above their atmospheric boiling point. Both extraction speed and efficiency are enhanced in this procedure [9]. In closed vessels the temperature may be elevated by simply applying the correct pressure. The closed vessel system is most suitable for volatile compounds.

Figure 2. Schematic diagram of closed vessel system for microwave assisted extraction.

Figure 2 shows a schematic diagram of a closed vessel system from CEM Corporation. In order to overcome the nonhomogeneity of the field, the cells are placed on a rotating carousel as in a domestic oven. The solvents can be varied, and the pressure in the vessels essentially depends on the volume and boiling point of the solvents used. The temperature can be set at a fixed value by adjusting the microwave power. Typically, the cells are made of Teflon. In closed vessel systems, the maximal power delivered is about 600–1000 W [13, 14], but the chosen power has to be set correctly to avoid excessive temperatures leading to possible solute degradation and overpressure problems. The vessel must be cooled to room temperature before opening: this is particularly important in the presence of volatile solutes which can partition into the headpace, but this step can considerably increase the overall extraction time. Furthermore, an additional filtration or centrifugation step is necessary in order to remove the solid residue.

3.4. Focused microwave assisted extraction

The above diagram shows the schematic diagram of focused microwave assisted extraction. The system works at atmospheric pressure, and the maximum temperature is determined by the boiling point of the solvent used [15, 16]. The solvent is heated and refluxed through the sample and in this case the microwaves are focused on the sample placed into the vessel allowing homogeneous and very efficient heating [17]. The sample to be extracted can be placed into a Soxhlet-type cellulose cartridge in order to avoid filtration steps, or may be directly dipped into the solvent. Compared to closed vessel extractions, open cells offer increased safety in sample handling and, furthermore, they allow larger samples to be extracted.

Figure 3. Schematic diagram of focused microwave assisted extraction.

3.5. Advantages

MAE has been considered as a potential alternative to traditional solid-liquid extraction for the extraction of metabolites from plants. It has been used to extract nutraceuticals for several reasons: (1) reduced extraction time (2) reduced solvent usage and (3) improved extraction yield. MAE is also comparable to other modern extraction techniques such as supercritical fluid extraction due to its process simplicity and low cost. By considering economical and practical aspects, MAE is a strong novel extraction technique for the extraction of nutraceuticals. MAE is highly effective for obtaining extracts under mild conditions. MAE has improved purity of the extract in comparison to Soxhlet extraction. Microwaves have been reported to cause little or no quality deterioration when applied to substances of plant origin such as ascorbic acid. The
ability of microwave radiation to heat solid material effectively can be used for obtaining essential oils. However, compared to SFE, an additional filtration or centrifugation is necessary to remove the solid residue during MAE. Furthermore, the efficiency of microwaves can be very poor when either the target compounds or the solvents are non-polar, or when they are volatile.

3.6. Applications

MAE can extract nutraceutical products from plant sources in a faster manner than conventional solid–liquid extractions. The majority of the applications concern the extraction of pollutants from environmental matrices. A higher microwave temperature and a short extraction time are more effective in extracting antioxidative phenolic compounds from tomato using MAE. A system has been developed simultaneously to saponify and extract ergo sterol by MAE. MAE technique has been developed to extract thymol from seeds of *Trachyspermum ammi* [18].

4. Ultrasonic assisted extraction (UAE)

UAE involves application of high-intensity, high-frequency (>20 kHz) sound waves and their interaction with materials. UAE is a potentially useful technology as it does not require complex instruments and is relatively low-cost. UAE instrument is shown in Fig 4. It can be used both on small and large scale [19].

UAE involves ultrasonic effects of acoustic cavitations. Under ultrasonic action solid and liquid particles are vibrated and accelerated and, because of that solute quickly diffuses out from solid phase to solvent [20]. Several probable mechanisms for ultrasonic enhancement of extraction, such as cell disruption, improved penetration, and enhanced swelling, capillary effect, and hydration process have been proposed [21]. If the intensity of ultrasound is increased in a liquid, then it reaches at a point at which the intramolecular forces are not able to hold the molecular structure intact, so it breaks down and bubbles are created, this process is called cavitation. Collapse of bubbles can produce physical, chemical and mechanical effects which result in the disruption of biological membranes to facilitate the release of extractable compounds and enhance penetration of solvent into cellular materials and improve mass transfer [20, 22]. The beneficial effects of sound waves on extraction are attributed to the formation and asymmetrical collapse of micro cavities in the vicinity of cell walls leading to the generation of micro jets rupturing the cells. The pulsation of bubbles is thought to cause acoustic streaming which improves mass transfer rate by preventing the solvent layer surrounding the plant tissue from getting saturated and hence enhancement of convection. Skin of external glands of plant cell wall is very thin and can be easily destroyed by sonication, and this facilitates release of essential oil contents into the extraction solvent, thus resulting in reduced extraction time and increased extraction efficiency [23]. Two general designs of ultrasound-assisted extractors are ultrasonic baths or closed extractors fitted with an ultrasonic horn transducer. The mechanical effects of ultrasound induce a greater penetration of solvent into cellular materials and improve mass transfer. Ultrasound in extraction can also disrupt biological cell walls, facilitating the release of contents. Therefore, efficient cell disruption and effective mass transfer are cited as two major factors leading to the enhancement of extraction with ultrasonic power [24]. Scanning electron micrographs (SEM) have provided evidence of the mechanical effects of ultrasound, mainly shown by the destruction of cell walls and release of cell contents.

4.1. Advantages and disadvantages

Ultrasound-assisted extraction is an inexpensive, simple and efficient alternative to conventional extraction techniques. The main benefits of use of ultrasound in solid–liquid extraction include the increase of extraction yield and faster kinetics. Ultrasound can also reduce the operating temperature allowing the extraction of thermo labile compounds. Compared with other novel extraction techniques such as microwave-assisted extraction, the ultrasound apparatus is cheaper and its operation is easier. Furthermore, the ultrasound-assisted extraction, like Soxhlet extraction, can be used with any solvent for extracting a wide variety of natural compounds.

However, the effects of ultrasound on extraction yield and kinetics may be linked to the nature of the
plant matrix. The presence of a dispersed phase contributes to the ultrasound wave attenuation and the active part of ultrasound inside the extractor is restricted to a zone located in the vicinity of the ultrasonic emitter. Therefore, those two factors must be considered carefully in the design of ultrasound-assisted extractors.

4.2. Applications

Ultrasound-assisted extraction has been used to extract nutraceuticals from plants such as essential oils and lipids, dietary supplements. The application of ultrasonic assisted extraction (UAE) in food processing technology is of interest for enhancing extraction of components from plant and animal materials. Ultrasonic assisted extraction of oil from Rice Bran using response surface methodology is efficient [25]. Ultrasound-assisted extraction was considered as an efficient method for extracting bioactive compounds from Solvia officinalis, Hibiscus flower, antioxidants from Rosmarinus officinalis steroids and triterpenoids from Chresta spp. [26].

5. Pressurized fluid extraction (PFE)

Pressurized Fluid Extraction (PFE) or Pressurized Liquid Extraction (PLE) is a new sample extraction method that employs liquid solvents at elevated temperatures and pressures to prepare samples for analysis by either gas chromatography or liquid chromatography. Pressurized liquid extraction is similar to Soxhlet extraction, except that during the extraction process the solvent condition inside the PLE cell approaches the supercritical region which results in more efficient extractions. The elevated temperature allows the sample to become more soluble and achieve a higher diffusion rate while the elevated pressure keeps the solvent below its boiling point. At elevated pressures and temperatures solvents can penetrate solid samples more efficiently which reduces solvent usage. A pressurized liquid extraction when compared to a traditional Soxhlet extraction shows a reduction in extraction time to 22 minutes from 18 hours and a decrease of total organic solvent consumption to 80 mL or less of organic solvent from 300 mL.

To perform a pressurized liquid extraction, between 5 grams and 100 grams of sample is mixed with sodium sulfate, loaded in the extraction cell and capped with two filtration end fittings. The PLE™ system then automatically starts pressurizing and heating the samples. The pressure is maintained at 1500-3000 PSI, at a temperature of 70-200 °C. The extracted solvent containing the target analyts is then automatically transferred to a concentration/evaporation vessel where it is brought to final volume directly in a gas chromatography (GC) or liquid chromatography (LC) vial. The vial can then be transferred to the analytical instrument for final analysis.

5.1. Advantages

Solvent is available at low cost. The main advantage of this process is that it has reduced the extraction time. Maintenance and cleaning up is easy. No extra labors are needed due to automation.

5.2. Applications

The use of the pressurized fluid extraction technique (PLE) is mainly focused on the extraction of environmental pollutants present in soil matrices, sediments, and sewage sludge. PLE coupled with analytical techniques is used for the quantification and detection of contaminants and toxic substances in foods. Recently, a method based on pressurized fluid extraction (PFE) was developed for measuring microplastics in environmental samples [27].

6. Conclusion

This paper was aimed to discuss and compare different extraction techniques along with their basic mechanism for extracting bioactive compounds from medicinal plants. The use of bioactive compounds in different commercial sectors such as pharmaceutical, food, cosmetics and chemical industries signifies the need of the most appropriate and standard method to extract these active components from plant materials. Along with conventional methods, many novel methods have been established but till now no single method is regarded as standard for extracting bioactive compounds from plants. Limitations of conventional soxhlet extraction were discussed and are individually taken care in novel techniques like supercritical fluid extraction, pressurized fluid extraction, ultrasonic and microwave assisted extraction. We conclude that novel technique requires cheaper solvents, reduces extraction time and are easy to maintain.

7. References


