



Techniques for extraction of bioactive compounds from plant materials: A review

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ARTICLE INFO

Article history:

Available online 23 January 2013

Keywords:

Extraction method
Bioactive compounds
Medicinal plant
Mechanism of extraction process
Non-conventional extraction

ABSTRACT

The use of bioactive compounds in different commercial sectors such as pharmaceutical, food 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, numerous new methods have been established but till now no single method is regarded as standard for extracting bioactive compounds from plants. The efficiencies of conventional and non-conventional extraction methods mostly depend on the critical input parameters; understanding the nature of plant matrix; chemistry of bioactive compounds and scientific expertise. This review is aimed to discuss different extraction techniques along with their basic mechanism for extracting bioactive compounds from medicinal plants.

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1. Introduction

The qualitative and quantitative studies of bioactive compounds from plant materials mostly rely on the selection of proper extraction method (Smith, 2003; Sasidharan et al., 2011). Extraction is the first step of any medicinal plant study, plays a significant and crucial role on the final result and outcome. Extraction methods are sometimes referred as “sample preparation techniques”. Most of the time, this part of study is neglected and done by non-trained research personnel (Hennion et al., 1998), though two-third of effort of an analytical chemist account for sample preparation techniques. A study conducted by Majors (1999) showed that the most of researchers believe in the importance of sample preparation during any analytical study.

It is true that development of modern chromatographic and spectrometric techniques make bioactive compound analysis easier than before but the success still depends on the extraction methods, input parameters and exact nature of plant parts (Poole et al., 1990). The most common factors affecting extraction processes are matrix properties of the plant part, solvent, temperature, pressure and time (Hernández et al., 2009). The increased understanding about dynamic chemical nature of the diverse bioactive molecules is pioneer fuel for the progress of bioactive analysis during past decade (Torssell, 1997). As a result of these huge technological and technical improvements; pharmaceuticals, food additives even on natural pesticides sectors have become inter-

ested in bioactive molecules from natural sources (Anklam et al., 1998; Ambrosino et al., 1999). Characteristically, bioactive compounds remain together with other compounds present in plants. Bioactive compounds can be identified and characterized from various plant parts such as leaves, stem, flower and fruits.

Extraction of plant materials can be done by various extraction procedures. Non-conventional methods, which are more environmental friendly due to decreased use of synthetic and organic chemicals, reduced operational time, and better yield and quality of extract, have been developed during the last 50 years. To enhance overall yield and selectivity of bioactive components from plant materials, ultrasound (Vinatoru et al., 1997; Ghafoor et al., 2011), pulsed electric field (Toepfl et al., 2006), enzyme digestion (Gaur et al., 2007), extrusion (Lusas and Watkins, 1988), microwave heating (Kaufmann and Christen, 2002), ohmic heating (Lakkakula et al., 2004), supercritical fluids (Marr and Gamse, 2000; Lang and Wai, 2001; Meireles and Angela, 2003; Wang et al., 2008; Ghafoor et al., 2010, 2012), and accelerated solvents (Kaufmann and Christen, 2002; Smith, 2002) have been studied as non-conventional methods. At the same time conventional extraction methods, such as Soxhlet is still considered as one of the reference method to compare success of newly developed methodology. Substantial number of scientific reports, book chapters and monographs exist where non-conventional methods were extensively reviewed (Jennings and Rapp, 1983; Moldoveanu and David, 2002; Szumski and Buszewski, 2002; Majors, 2003; Smith, 2003; Wang and Weller, 2006). These writings are emphasizing the use of extraction methods in term of nutraceuticals, food additives and many other sectors but lack in herbal plant's bioactive

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compounds extraction. The present paper is aimed to provide a comprehensive review of different techniques for extraction of bioactive compounds from medicinal plants.

2. History and definition of bioactive compounds

The history of plant's used for mankind is as old as the start of humankind. Initially, people used plants for their nutritional purposes but after the discovery of medicinal properties, this natural flora became a useful source of disease cure and health improvement across various human communities. Egyptian papyruses showed that coriander and castor oil were useful for medicinal applications, cosmetics and preservatives through thousands of recipes (Vinatoru, 2001). During Greek and Roman period, a thousand of therapeutic uses of herbal plants were described by several scholars namely Hippocrates, Theophrastus, Celsus, Dioscorides and many others (Paulsen, 2010). Romanians are known for their use of medicinal herbs since very long. For example, Herodotus (5th century B.C) mentioned *Leonurus cardiaca* (Mother wort) was used by the people living north of the Danube river in his writings. In 19th century Romanian pharmacopoeia introduced herbal products and in 1904 the first institute of medicinal herbs was established in Cluj city (Vinatoru, 2001). The use of herbal plants in the ancient time actually illustrates the history of bioactive molecules. In the past, people had no idea about bioactive molecules but the use of these compounds was sufficiently diverse in different prospect.

Typically, bioactive compounds of plants are produced as secondary metabolites (Bernhoft, 2010). Every living body, from one cell bacterium to million cell plants, processes diverse chemical compounds for their survival and subsistence. All compounds of biological system can be divided into two broad arenas. One is primary metabolites, which are the chemical substances aimed at growth and development, such as carbohydrates, amino acids, proteins and lipids. Another is secondary metabolites, which are a group of compounds other than primary metabolites believed to help plant to increase their overall ability to survive and overcome local challenges by allowing them to interact with their surroundings (Harborne, 1993). In different words, secondary metabolites are those metabolites which are often produced in a phase of subsequent to growth, have no function in growth (although they may have survival function), are produced by certain restricted taxonomic groups of microorganisms, have unusual chemical structures, and are often formed as mixtures of closely related members of a chemical family (Martin and Demain, 1978). The production of secondary metabolites in different species is mainly selected through the course of evaluation and the particular need of that species. For example, synthesis of aroma by floral species to attract insect for their pollination and fertilization, and synthesis toxic chemical has evolved toward pathogens and herbivores for suppressing the growth of neighboring plants (Dudareva and Pichersky, 2000). Among secondary metabolites some of these substances have effect on biological systems which are considered as bioactive. Thus a simple definition of bioactive compounds in plants is: secondary plant metabolites eliciting pharmacological or toxicological effects in human and animals (Bernhoft, 2010).

3. Classification and synthesis of bioactive compounds

Classification of bioactive compounds in different categories is still inconsistent rather it depends upon the intention of the particular classification. For example, biosynthetic classifications which serve the simplicity of the description of biosynthetic pathways that will not match the scope of pharmacological classification. According to Croteau et al. (2000) bioactive compounds of plants

are divided into three main categories: (a) terpenes and terpenoids (approximately 25,000 types), (b) alkaloids (approximately 12,000 types) and (c) phenolic compounds (approximately 8000 types). General structures of different categories of bioactive compounds are given in Fig. 1.

The majority of bioactive compounds belong to one of a number of families, each of which has particular structural characteristics arising from the way in which they are built up in nature (biosynthesis). There are four major pathways for synthesis of secondary metabolites or bioactive compounds: (1) Shikimic acid pathway, (2) malonic acid pathway, (3) Mevalonic acid pathway and (4) non-mevalonate (MEP) pathway (Tiaz and Zeiger, 2006). Alkaloids are produced by aromatic amino acids (come from shikimic acid pathway) and by aliphatic amino acids (come from tricarboxylic acid cycle). Phenolic compounds are synthesized through shikimic acid pathway and malonic acid pathway. Through mevalonic acid pathway and MEP pathway terpenes are produced. Simplified illustrations of different pathways for the production of three major groups of plant bioactive compounds are shown in Fig. 2.

4. Extraction of bioactive compounds

Considering the great variations among bioactive compounds and huge number of plant species, it is necessary to build up a standard and integrated approach to screen out these compounds carrying human health benefits. Farnsworth et al. (1985) reported an integrated approach showing sequence of medicinal plant study, which started from name collection of frequently used plants and ended at industrialization. Works of particular order for medicinal plant study and the position of extraction techniques are shown by a flow chart in Fig. 3.

It is only possible to conduct further separation, identification, and characterization of bioactive compounds followed by an appropriate extraction process. Different extraction techniques should be used in diverse conditions for understanding the extraction selectivity from various natural sources. Different techniques, many of them remain almost same through hundreds of years; can also be used to extract bioactive compounds. All these techniques have some common objectives, (a) to extract targeted bioactive compounds from complex plant sample, (b) to increase selectivity of analytical methods (c) to increase sensitivity of bioassay by increasing the concentration of targeted compounds, (d) to convert the bioactive compounds into a more suitable form for detection and separation, and (e) to provide a strong and reproducible method that is independent of variations in the sample matrix (Smith, 2003).

5. Conventional extraction techniques

Bioactive compounds from plant materials can be extracted by various classical extraction techniques. Most of these techniques are based on the extracting power of different solvents in use and the application of heat and/or mixing. In order to obtain bioactive compounds from plants, the existing classical techniques are: (1) Soxhlet extraction, (2) Maceration and (3) Hydrodistillation.

Soxhlet extractor was first proposed by German chemist Franz Ritter Von Soxhlet (1879). It was designed mainly for extraction of lipid but now it is not limited for this only. The Soxhlet extraction has widely been used for extracting valuable bioactive compounds from various natural sources. It is used as a model for the comparison of new extraction alternatives. Generally, a small amount of dry sample is placed in a thimble. The thimble is then placed in distillation flask which contains the solvent of particular interest. After reaching to an overflow level, the solution of the thimble-holder is aspirated by a siphon. Siphon unloads the solution back into the distillation flask. This solution carries extracted

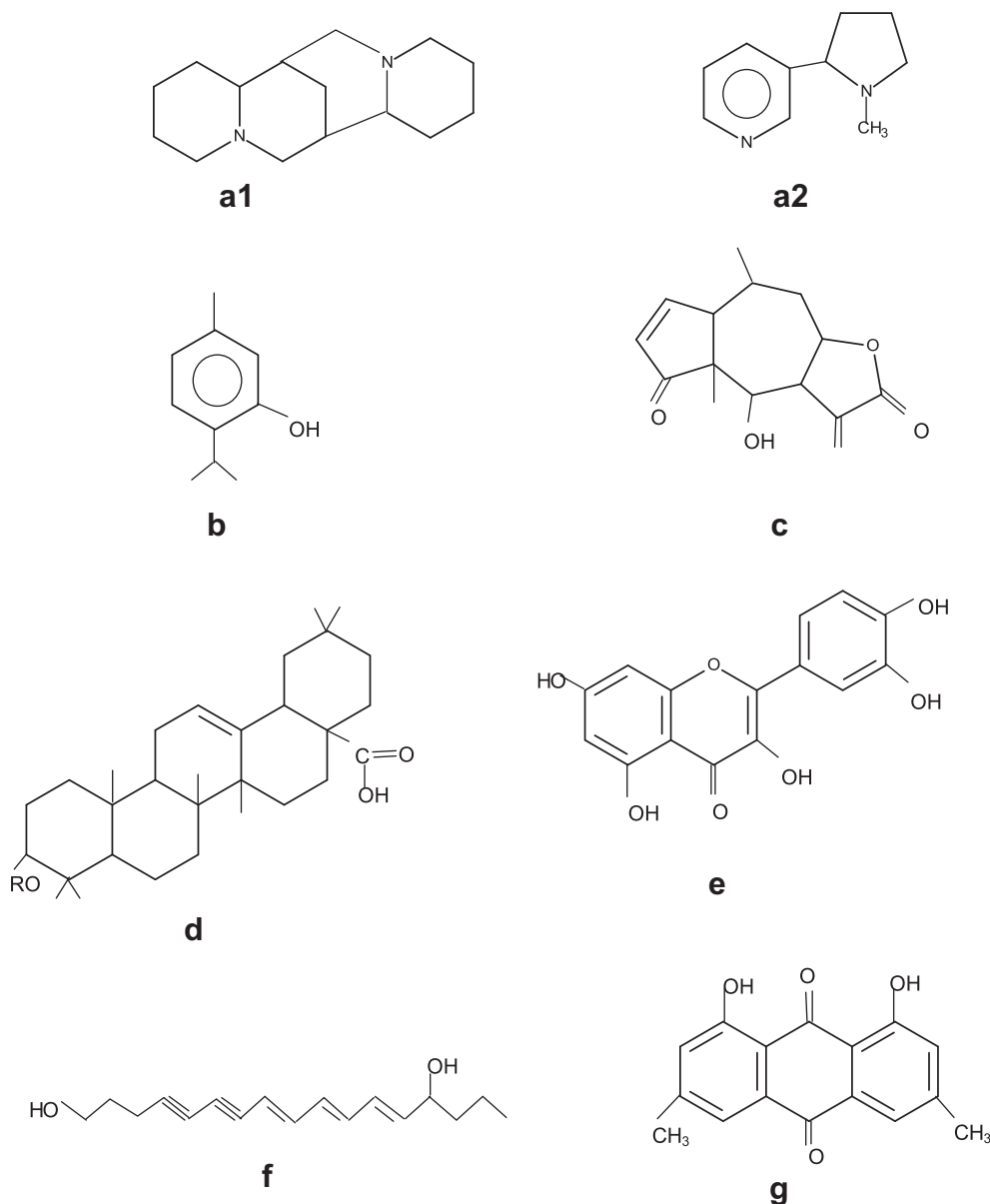


Fig. 1. General structures of different categories of plant bioactive compounds: alkaloids (a1 and a2), monoterpenes (b), sesquiterpenes (c), triterpenes, saponins, steroid (d), flavonoids (e), polyacetylenes (f), polyketides (g) (Wink, 2003).

solutes into the bulk liquid. Solute is remained in the distillation flask and solvent passes back to the solid bed of plant. The process runs repeatedly until the extraction is completed.

Maceration was used in homemade preparation of tonic from a long time. It became a popular and inexpensive way to get essential oils and bioactive compounds. For small scale extraction, maceration generally consists of several steps. Firstly, grinding of plant materials into small particle is used to increase the surface area for proper mixing with solvent. Secondly, in maceration process, appropriate solvent named as menstruum is added in a closed vessel. Thirdly, the liquid is strained off but the marc which is the solid residue of this extraction process is pressed to recover large amount of occluded solutions. The obtained strained and the press out liquid are mixed and separated from impurities by filtration. Occasional shaking in maceration facilitate extraction by two ways; (a) increase diffusion, (b) remove concentrated solution from the sample surface for bringing new solvent to the menstruum for more extraction yield.

Hydrodistillation is a traditional method for extraction of bioactive compounds and essential oils from plants. Organic solvents are not involved and it can be performed before dehydration of plant materials. There are three types of hydrodistillation: water distillation, water and steam distillation and direct steam distillation (Vankar, 2004). In hydrodistillation, first, the plant materials are packed in a still compartment; second, water is added in sufficient amount and then brought to boil. Alternatively, direct steam is injected into the plant sample. Hot water and steam act as the main influential factors to free bioactive compounds of plant tissue. Indirect cooling by water condenses the vapor mixture of water and oil. Condensed mixture flows from condenser to a separator, where oil and bioactive compounds separate automatically from the water (Silva et al., 2005). Hydrodistillation involves three main physicochemical processes; Hydrodiffusion, hydrolysis and decomposition by heat. At a high extraction temperature some volatile components may be lost. This drawback limits its use for thermo labile compound extraction.

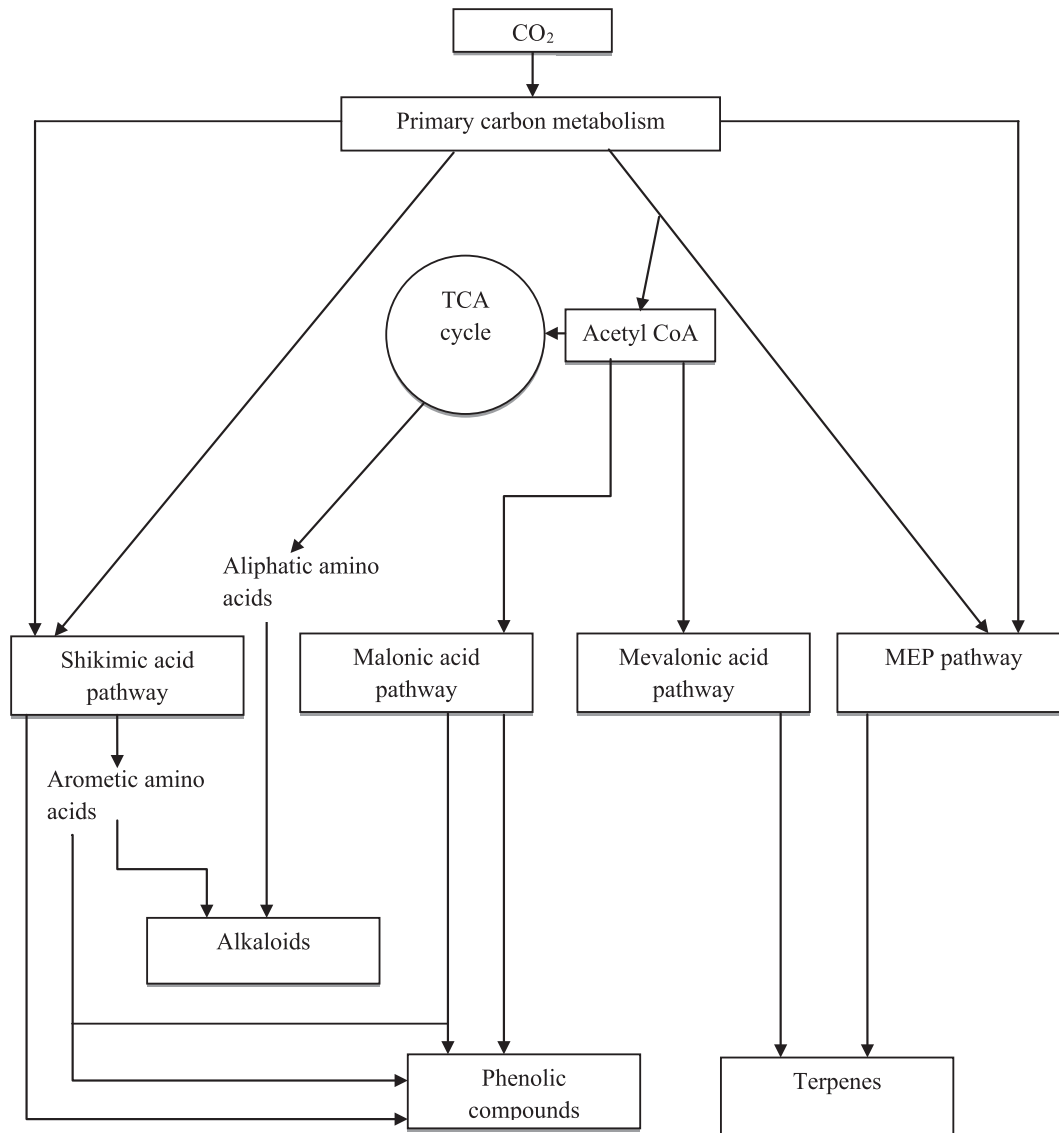


Fig. 2. A simplified view of pathways for production of three major groups of plant bioactive compounds (adapted from Tiaz and Zeiger (2006)).

Extraction efficiency of any conventional method mainly depends on the choice of solvents (Cowan, 1999). The polarity of the targeted compound is the most important factor for solvent choice. Molecular affinity between solvent and solute, mass transfer, use of co-solvent, environmental safety, human toxicity and financial feasibility should also consider in selection of solvent for bioactive compound extraction. Some examples of bioactive compound extracted using different solvents are given in Table 1.

6. Non-conventional extraction techniques

The major challenges of conventional extraction are longer extraction time, requirement of costly and high purity solvent, evaporation of the huge amount of solvent, low extraction selectivity and thermal decomposition of thermo labile compounds (Luque de Castro and Garcia-Ayuso, 1998). To overcome these limitations of conventional extraction methods, new and promising extraction techniques are introduced. These techniques are referred as non-conventional extraction techniques. Some of the most promising techniques are ultrasound assisted extraction, enzyme-assisted extraction, microwave-assisted extraction, pulsed electric field as-

sisted extraction, supercritical fluid extraction and pressurized liquid extraction. Some of these techniques are considered as “green techniques” as they comply with standards set by Environmental Protection Agency, USA (http://www.epa.gov/greenchemistry/pubs/about_gc.html). These include less hazardous chemical synthesis; designing safer chemicals, safe solvents auxiliaries, design for energy efficiency, use of renewable feedstock, reduce derivatives, catalysis, design to prevent degradation, atom economy, and time analysis for pollution prevention and inherently safer chemistry for the prevention of accident.

6.1. Ultrasound-assisted extraction (UAE)

Ultrasound is a special type of sound wave beyond human hearing. Usually, in chemistry it is 20 kHz to 100 MHz. Like other waves, it passes through a medium by creating compression and expansion. This process produces a phenomenon called cavitation, which means production, growth and collapse of bubbles. A large amount of energy can produce from the conversion of kinetic energy of motion into heating the contents of the bubble. According to Suslick and Doktycz (1990), bubbles have temperature about

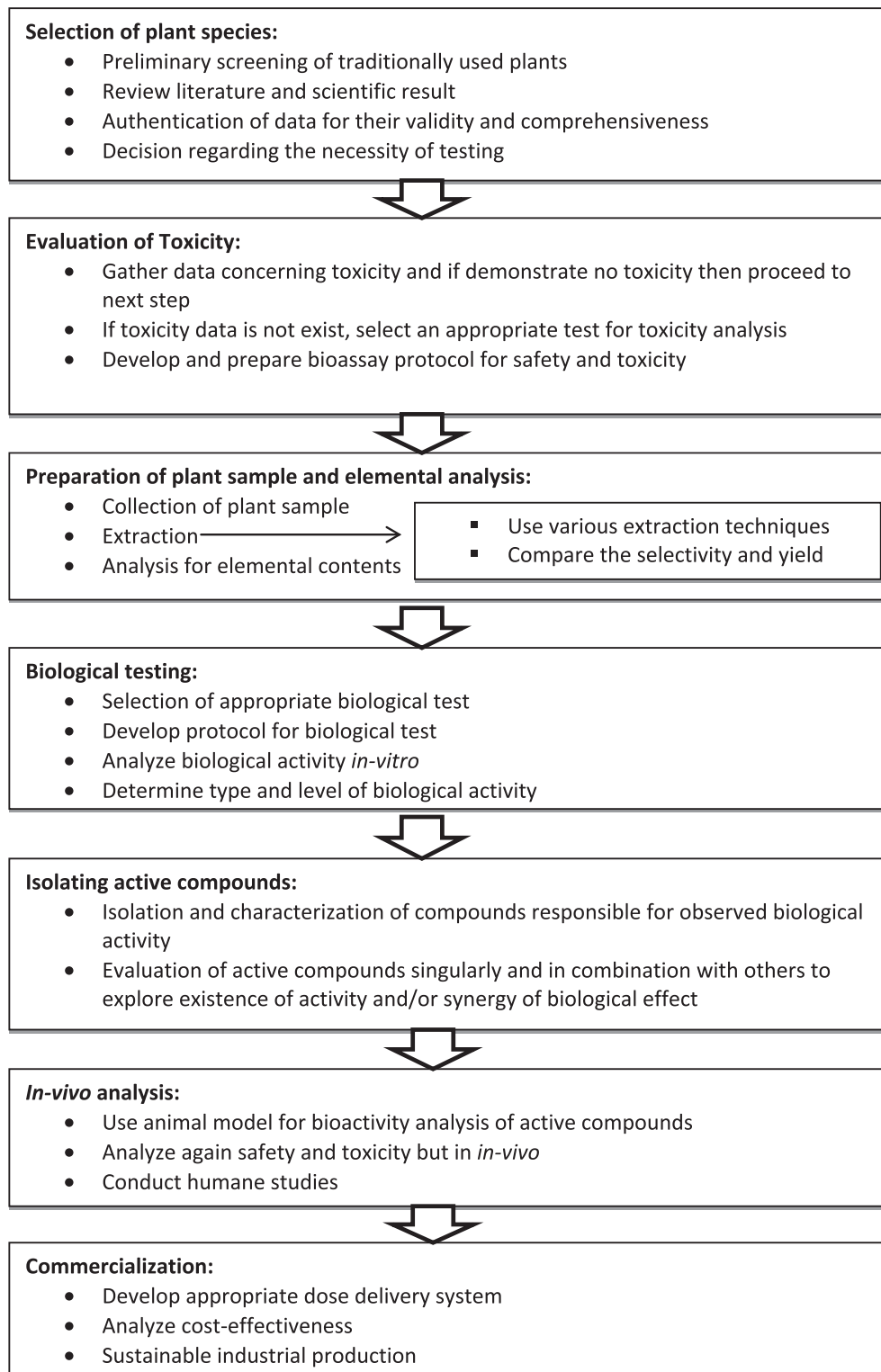


Fig. 3. The flow chart of medicinal plant study and position of extraction techniques (adapted from Farnsworth et al. (1985)).

5000 K, pressure 1000 atm and, heating and cooling rate above 10^{10} K/s. Based on this principle, UAE has been developed. Only liquid and liquid containing solid materials have cavitation effect. The main benefit of UAE can be observed in solid plant sample because ultrasound energy facilitates organic and inorganic compounds leaching from plant matrix (Herrera and Luque de Castro, 2005). Probable mechanism is ultrasound intensification of mass

transfer and accelerated access of solvent to cell materials of plant parts. The extraction mechanism by ultrasound involves two main types of physical phenomena, (a) the diffusion across the cell wall and (b) rinsing the contents of cell after breaking the walls (Mason et al., 1996). Moisture content of sample, milling degree, particle size and solvent are very important factors for obtaining efficient and effective extraction. Furthermore, temperature, pressure,

Table 1
Example of some extracted bioactive compounds by different solvents (adapted from Cowan (1999)).

Water	Ethanol	Methanol	Chloroform	Dichloromethanol	Ether	Acetone
Anthocyanins	Tannins	Anthocyanin	Terpenoids	Terpenoids	Alkaloids	Flavonoids
Tannins	Polyphenols	Terpenoids	Flavonoids		Terpenoids	
Saponins	Flavonol	Saponins				
Terpenoids	Terpenoids	Tannins				
	Alkaloids	Flavones				
		Polyphenols				

frequency and time of sonication are the governing factors for the action of ultrasound. UAE have also been incorporated along with various classical techniques as they are reported to enhance the efficiency of a conventional system. In a solvent extraction unit, an ultrasound device is placed in an appropriate position to enhance the extraction efficiency (Vinatoru et al., 1998).

The advantages of UAE include reduction in extraction time, energy and use of solvent. Ultrasound energy for extraction also facilitates more effective mixing, faster energy transfer, reduced thermal gradients and extraction temperature, selective extraction, reduced equipment size, faster response to process extraction control, quick start-up, increased production and eliminates process steps (Chemat et al., 2008).

UAE is seemed to be an effective extraction technique for bioactive compound extraction from herbal plants. Rostagno et al. (2003) showed extraction efficiency of four isoflavone derivatives namely, daidzin, genistin, glycitin and malonyl genistin from soybean with mix-stirring method using different extraction times and solvents. Authors found that ultrasound can improve the extraction yield depending on solvent use. Herrera and Luque de Castro (2004) extracted phenolic compounds such as rutin, naringin, naringenin, quercetin, ellagic acid and kaempferol from strawberries using 0.8 s duty cycle for 30 s by developing semiautomatic method based on ultrasounds. Li et al. (2005) found better recovery of chlorogenic acid from fresh leaves, fresh bark and dried bark of *Eucommia ulmoides* Oliv. by UAE at optimized condition (70% methanol, 20:1 solvent, sample ratio and 30 min time) than classical extraction techniques. Yang and Zhang (2008) applied optimized sonication condition to extract bioactive compounds called rutin and quercetin from *Euonymus alatus* (Thund.) Sieb and concluded that ultrasonic method had better extraction efficiency than conventional methods. Ionic liquid based UAE have been regarded as very effective for extracting three alkaloids (vindoline, catharanthine and vinblastine) from *Catharanthus roseus* (Yang et al., 2011). Anthocyanins and phenolic compounds were extracted from grape peel using UAE and the extraction process was optimized with reference to solvent, extraction temperature and time (Ghafoor et al., 2011, 2009). Phenolcarboxylic acids, carnosic acid and rosmarinic acid were extracted from *Rosmarinus officinalis* using Ionic liquid based UAE technique which was proved to have high efficiency and shorter extraction time than conventional extraction methods (Zu et al., 2012).

6.2. Pulsed-electric field extraction (PEF)

The pulsed electric field (PEF) treatment was recognized as useful for improving the pressing, drying, extraction, and diffusion processes during the last decade (Barsotti and Cheftel, 1998; Angersbach et al., 2000; Vorobiev et al., 2005; Vorobiev and Lebovka, 2006). The principle of PEF is to destroy cell membrane structure for increasing extraction. During suspension of a living cell in electric field, an electric potential passes through the membrane of that cell. Based on the dipole nature of membrane molecules, electric potential separates molecules according to their charge in the cell membrane. After exceeding a critical value of approximately

1 V of transmembrane potential, repulsion occurs between the charge carrying molecules that form pores in weak areas of the membrane and causes drastic increase of permeability (Bryant and Wolfe, 1987). Usually, a simple circuit with exponential decay pulses is used for PEF treatment of plant materials. It has a treatment chamber consisting of two electrodes where plant materials are placed. Depending on the design of treatment chamber PEF process can operate in either continuous or batch mode (Puértolas et al., 2010). The effectiveness of PEF treatment strictly depends on the process parameters, including field strength, specific energy input, pulse number, treatment temperature and properties of the materials to be treated (Heinz et al., 2003).

PEF can increase mass transfer during extraction by destroying membrane structure of plant materials for enhancing extraction and decreasing extraction time. PEF has been applied to improve release of intracellular compounds from plant tissue with the help of increasing cell membrane permeability (Toepfl et al., 2006). PEF treatment at a moderate electric field (500 and 1000 V/cm; for 10^{-4} – 10^{-2} s) is found to damage cell membrane of plant tissue with little temperature increase (Fincan and Dejmek, 2002; Lebovka et al., 2002). Due to this reason, PEF can minimize the degradation of heat sensitive compounds (Ade-Omowaye et al., 2001). PEF is also applicable on plant materials as a pretreatment process prior to conventional extraction to lower extraction effort (López et al., 2009).

PEF treatment (at 1 kV/cm with low energy consumption of 7 kJ/kg) in a solid liquid extraction process for extraction of betanin from beetroots showed highest degree of extraction compared with freezing and mechanical pressing (Fincan et al., 2004). Guderjan et al. (2005) showed that the recovery of phytosterols from maize increased by 32.4% and isoflavonoids (genistein and daidzein) from soybeans increased by 20–21% when PEF was used as pretreatment process. Corrales et al. (2008) extracted bioactive compound such as anthocyanins from grape by-product using various techniques and found better extraction of anthocyanin monoglucosides by PEF. The application of a PEF treatment on grape skin before maceration step can reduce the duration of maceration and improve the stability of bioactives (anthocyanin and polyphenols) during vinification (López et al., 2008). The permeabilization of Merlot skin by a pulsed electric field treatment resulted in increased extraction of polyphenols and anthocyanins (Delsart et al., 2012).

6.3. Enzyme-assisted extraction (EAE)

Some phytochemicals in the plant matrices are dispersed in cell cytoplasm and some compounds are retained in the polysaccharide-lignin network by hydrogen or hydrophobic bonding, which are not accessible with a solvent in a routine extraction process. Enzymatic pre-treatment has been considered as a novel and an effective way to release bounded compounds and increase overall yield (Rosenthal et al., 1996). The addition of specific enzymes like cellulase, α -amylase, and pectinase during extraction enhances recovery by breaking the cell wall and hydrolyzing the structural polysaccharides and lipid bodies (Rosenthal et al., 1996; Singh

et al., 1999). There are two approaches for enzyme-assisted extraction: (1) enzyme-assisted aqueous extraction (EAAE) and (2) enzyme-assisted cold pressing (EACP) (Latif and Anwar, 2009). Usually, EAAE methods have been developed mainly for the extraction of oils from various seeds (Hanmoungjai et al., 2001; Rosenthal et al., 1996, 2001; Sharma et al., 2002). In EACP technique, enzymes are used to hydrolyze the seed cell wall, because in this system polysaccharide-protein colloid is not available, which is obvious in EAAE (Concha et al., 2004). Various factors including enzyme composition and concentration, particle size of plant materials, solid to water ratio, and hydrolysis time are recognized as key factors for extraction (Niranjan and Hanmoungjai, 2004). Dominguez et al. (1995) reported that the moisture content of plant materials is also an important factor for enzymatic hydrolysis. Bhattacharjee et al. (2006) described EACP as an ideal alternate for extracting bioactive components from oilseed, because of its nontoxic and nonflammable properties. The oil extracted by enzyme-assisted methods was found to contain higher amount of free fatty acids and phosphorus contents than traditional hexane-extracted oil (Dominguez et al., 1995). The EAE is recognized as eco-friendly technology for extraction of bioactive compounds and oil because it uses water as solvent instead of organic chemicals (Puri et al., 2012).

EAE of phenolic antioxidants from grape pomace during wine production was tested by Meyer et al. (1998) who found a correlation between yield of total phenols and degree of plant cell wall breakdown by enzyme. Landbo and Meyer (2001) showed improved release of phenolic compounds from *Ribes nigrum* pomace using various enzymes. Li et al. (2006) extracted total phenolic contents from five citrus peels (Yen Ben lemon, Meyer lemon, grapefruit, mandarin and orange) by EAAE using different enzymes and the recovery was highest with cellulzyme MX. Another important finding of that study was the extraction of phenolic antioxidants improved significantly with higher enzyme concentration. Maier et al. (2008) used mixture of pectinolytic and cellulolytic enzyme in the ratio of 2:1 to extract bioactive compounds (phenolic acids, non-anthocyanin flavonoids and anthocyanins) from grape pomace where obtained yields were higher compared with sulfite-assisted extraction. Extraction of phenolic antioxidant from raspberry solid wastes was increased by application of enzyme in hydro-alcoholic extraction compared with non-enzymatic control (Laroze et al., 2010). Gómez-García et al. (2012) extracted phenolic compounds from grape waste using different types of enzymes, celluclast, pectinex and novoferm in EAE and found that novoferm had the strongest effect on phenolic release from grape waste. The authors illustrated enzyme technology as an alternative to extract bioactive compounds from agro-industrial byproducts.

6.4. Microwave assisted extraction (MAE)

The microwave-assisted extraction is also considered as a novel method for extracting soluble products into a fluid from a wide range of materials using microwave energy (Paré et al., 1994). Microwaves are electromagnetic fields in the frequency range from 300 MHz to 300 GHz. They are made up of two oscillating fields that are perpendicular such as electric field and magnetic field. The principle of heating using microwave is based upon its direct impacts on polar materials (Letellier and Budzinski, 1999). Electromagnetic energy is converted to heat following ionic conduction and dipole rotation mechanisms (Jain, 2009). During ionic conduction mechanism heat is generated because of the resistance of medium to flow ion. On the other hand, ions keep their direction along field signs which change frequently. This frequent change of directions results in collision between molecules and consequently generates heat. The extraction mechanism of microwave-assisted extraction is supposed to involve three sequential steps

described by Alupului (2012): first, separation of solutes from active sites of sample matrix under increased temperature and pressure; second, diffusion of solvent across sample matrix; third, release of solutes from sample matrix to solvent. Several advantages of MAE have been described by Cravotto et al. (2008) such as quicker heating for the extraction of bioactive substances from plant materials; reduced thermal gradients; reduced equipment size and increased extract yield. MAE can extract bioactive compounds more rapidly and a better recovery is possible than conventional extraction processes. It is a selective technique to extract organic and organometallic compounds that are more intact. MAE is also recognized as a green technology because it reduces the use of organic solvent (Alupului, 2012).

For polyphenols and caffeine extraction from green tea leaves, MAE achieved higher extraction yield at 4 min than any extraction methods at room temperature for 20 h (Pan et al., 2003). Ginsenosides extraction yield from *ginseng* root obtained by 15 min using focused MAE technique was better than conventional solvent extraction for 10 h (Shu et al., 2003). Dhobi et al. (2009) showed increased extraction efficiency of MAE by extracting a flavolignin, silybinin from *Silybum marianum* compared with the conventional extraction techniques like Soxhlet, maceration. Asghari et al. (2011) extracted some bioactive compounds (E- and Z-guggolsterone, cinnamaldehyde and tannin) from various plants under optimum conditions and showed that, MAE is faster and easier method in comparison to conventional extraction processes. MAE was applied to release bound phenolic acids from bran and flour fractions of sorghum and maize of different hardness by Chiremba et al. (2012). MAE process from Chinese quince (*Chaenomeles sinensis*) was optimized for solvent concentration, extraction time and microwave power using designed experiments to maximize recoveries of flavonoids and phenolics and to enhance electron donating ability of the extracts (Hui et al., 2009).

6.5. Pressurized liquid extraction (PLE)

In 1996, Richter et al. first described PLE. This method is now known by several names; pressurized fluid extraction (PFE), accelerated fluid extraction (AFE), enhanced solvent extraction (ESE), and high pressure solvent extraction (HSPE) (Nieto et al., 2010). The concept of PLE is the application of high pressure to remain solvent liquid beyond their normal boiling point. High pressure facilitates the extraction process. Automation techniques are the main reason for the greater development of PLE-based techniques along with the decreased extraction time and solvents requirement. PLE technique requires small amounts of solvents because of the combination of high pressure and temperatures which provides faster extraction. The higher extraction temperature can promote higher analyte solubility by increasing both solubility and mass transfer rate and, also decrease the viscosity and surface tension of solvents, thus improving extraction rate (Ibañez et al., 2012).

In comparison to the traditional soxhlet extraction PLE was found to dramatically decrease time consumption and solvent use (Richter et al., 1996). Now a days, for extraction of polar compounds, PLE is also considered as a potential alternative technique to supercritical fluid extraction (Kaufmann and Christen, 2002). PLE is also useful for the extraction of organic pollutants from environmental matrices those are stable at high temperatures (Wang and Weller, 2006). PLE has also been used for the extraction of bioactive compounds from marine sponges (Ibañez et al., 2012). Applications of PLE technique for obtaining natural products are frequently available in literature (Kaufmann and Christen, 2002). Additionally, due to small amount organic solvent use PLE gets broad reorganization as a green extraction technique (Ibañez et al., 2012).

PLE has been successfully applied to extract bioactive compounds from different plant materials. Using optimized condition isoflavones were extracted from soybeans (freeze-dried) without degradation by PLE (Rostagno et al., 2004). Shen and Shao (2005) compared ASE for extraction of terpenoids and sterols from tobacco with Soxhlet extraction and ultrasonically assisted extraction. In consideration of yield, reproducibility, extraction time, and solvent consumption, PLE has been considered as an alternate to conventional methods due to faster process and lower solvent use. Flavonoids extracted from spinach by PLE using a mixture of ethanol and water (70:30) solvent at 50–150 °C were more effective than water solvent at 50–130 °C (Howard and Pandjaitan, 2008). Luthria (2008) showed temperature, pressure, particle size, flush volume, static time, and solid-to-solvent ratio parameters have influence on the extraction of phenolic compounds from parsley (*Petroselinum crispum*) flakes by PLE. PLE was optimized for extraction of lycorine and galanthamine (*Amaryllidaceae* alkaloids) from *Narcissus jonquilla* and an optimized PLE method was more effective than hot-solvent extraction, MAE, and UAE (Mroczek and Mazurek, 2009). Individual phenolic compounds such as galocatechin (GCT), catechin, epicatechin gallate, caffeic acid, chlorogenic acid, and myricetin and total phenolic contents were recovered from various parts of *Anatolia propolis* using PLE at optimum condition (40 °C, 1500 psi for 15 min) (Erdogan et al., 2011).

6.6. Supercritical fluid extraction (SFE)

The application of supercritical fluid for extraction purposes started with its discovery by Hannay and Hogarth (1879) but the credit should also be given to Zosel who presented a patent for decaffeination of coffee using SFE (Zosel, 1964). Since this beginning, supercritical fluid technique has attracted wide scientific interest and it was successfully used in environmental, pharmaceutical and polymer applications and food analysis (Zougagh et al., 2004). Several industries have been using this technique for many years, especially, decaffeinated coffee preparation industries (Ndiomu and Simpson, 1988).

Every earthly substance has three basic states namely; Solid, Liquid and Gas. Supercritical state is a distinctive state and can only be attained if a substance is subjected to temperature and pressure beyond its critical point. Critical point is defined as the characteristic temperature (T_c) and pressure (P_c) above which distinctive gas and liquid phases do not exist (Incedy et al., 1998). In supercritical state, the specific properties of gas and/or liquid become vanish, which means supercritical fluid cannot be liquefied by modifying temperature and pressure. Supercritical fluid possesses gas-like properties of diffusion, viscosity, and surface tension, and liquid-like density and solvation power. These properties make it suitable for extracting compounds in a short time with higher yields (Sihvonen et al., 1999). A basic SFE system consists of the following parts: a tank of mobile phase, usually CO_2 , a pump to pressurize the gas, co-solvent vessel and pump, an oven that contains the extraction vessel, a controller to maintain the high pressure inside the system and a trapping vessel. Usually different type of meters like flow meter, dry/wet gas meter could be attached to the system. A symmetric diagram of typical SFE instrumentation is given in Fig. 4.

Carbon dioxide is considered as an ideal solvent for SFE. The critical temperature of CO_2 (31 °C) is close to room temperature, and the low critical pressure (74 bars) offers the possibility to operate at moderate pressures, generally between 100 and 450 bar (Temelli and Güçlü-Üstündag, 2005). The only drawback of carbon dioxide is its low polarity which makes it ideal for lipid, fat and non-polar substance, but unsuitable for most pharmaceuticals and drug samples. The limitation of low polarity of carbon dioxide has been successfully overcome by the use of chemical modifier

(Lang and wai, 2001; Ghafoor et al., 2010). Usually a small amount of modifier is considered as useful to significantly enhance the polarity of carbon dioxide. For example, 0.5 ml of Dichloromethane (CH_2Cl_2) can enhance the extraction which is same for 4 h hydrodistillation (Hawthorne et al., 1994). The properties of sample and targeted compounds and the previous experimental result are main basis for selection of the best modifier.

The successful extraction of bioactive compounds from plant materials rely upon several parameter of SFE and most importantly these parameter are tunable (Raverchon and Marco, 2006). These parameter need to be precisely controlled for maximizing benefits from this technique. The major variables influencing the extraction efficiency are temperature, pressure, particle size and moisture content of feed material, time of extraction, flow rate of CO_2 , and solvent-to-feed-ratio (Temelli and Güçlü-Üstündag, 2005; Ibañez et al., 2012).

The advantages of using supercritical fluids for the extraction of bioactive compounds can be understood considering following points (Lang and wai, 2001): (1) The supercritical fluid has a higher diffusion coefficient and lower viscosity and surface tension than a liquid solvent, leading to more penetration to sample matrix and favorable mass transfer. Extraction time can be reduced substantially by SFE in compared with conventional methods. (2) The repeated reflux of supercritical fluid to the sample provides complete extraction. (3) The selectivity of supercritical fluid is higher than liquid solvent as its solvation power can be tuned either by changing temperature and/or pressure. (4) Separation of solute from solvent in conventional extraction process can easily be bypassed by depressurization of supercritical fluid, which will save time. (5) SFE is operated at room temperature, so an ideal method for thermo labile compound extraction. (6) In SFE, small amount of sample can be extracted compared with solvent extraction methods which will save time for overall experiment. (7) SFE uses little amount of organic solvent and considered as environment friendly. (8) On-line coupling of SFE with chromatographic process is possible which is useful for highly volatile compounds. (9) The recycling and reuse of supercritical fluid is possible and thus minimizing waste generation. (10) SFE scale can be arranged on specific purpose from few milligram samples in laboratory to tons of sample in industries. (11) SFE process provides information regarding extraction process and mechanism which can be manipulated to optimize extraction process.

Saldaña et al. (1999) extracted purine alkaloids (caffeine, theobromine, and theophylline) from *Ilex paraguayensis* (herbal maté tea) using SFE at 313–343 K temperature and pressure from 14–24 MPa. Supercritical CO_2 modified with ethanol (15 wt.%) gave higher extraction yields of naringin (flavonoid) from *citrus paradise* than pure supercritical carbon dioxide at 9.5 MPa and 58.6 °C (Giannuzzo et al., 2003). Polyphenols and procyanidins were extracted from grape seeds using SFE, where methanol was used as modifier and methanol modified CO_2 (40%) released more than 79% of catechin and epicatechin from grape seed (Khorassani and Taylor, 2004). Verma et al. (2008) used optimized condition of SFE to extract indole alkaloids from *Catharanthus roseus* leaves and best recoveries for catharanthine were at 25 MPa and 80 °C using 6.6% methanol as modifier for 40 min.

7. Concluding remarks

The ever growing demand to extract plant bioactive compounds encourages continuous search for convenient extraction methods. The chromatography advancement and awareness about environment are two important factors for the development of most non-conventional extraction processes. However, understanding of every aspect of non-conventional extraction process is vital as

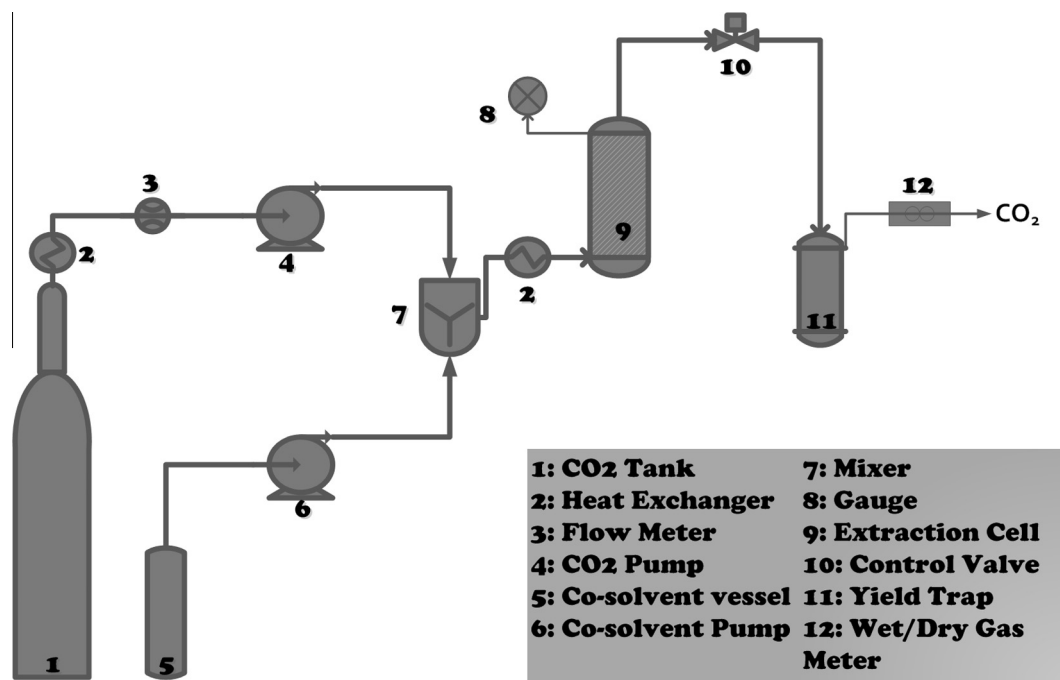


Fig. 4. A symmetric diagram of SFE apparatus.

most of these methods are based on different mechanism and extraction enhancement is resulted from different process. Incorporation and development of hybrid methods should also be investigated considering plant material characteristics and choice of compounds. Sufficient experimental data is still lacking in some of the existing methods. Proper choice of standard methods also influences the measurement of extraction efficiency. On the other hand, the increasing economic significance of bioactive compounds and commodities rich in these bioactive compounds may lead to find out more sophisticated extraction methods in future.

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