A REVIEW OF NATURAL PRODUCTS FROM PLANTS USING CARBON DIOXIDE SUPERCRITICAL FLUID EXTRACTION

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ABSTRACT

Supercritical fluid extraction (SFE) is the most effective and efficient way to extract valuable constituents from plants. SFE is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids that is CO_2 as the extracting solvent. It is a well-established process for recovery of different organics, mainly nonpolar substances from various solid matrixes. CO_2 is the most common used extraction solvents for botanicals. Extraction conditions for supercritical CO_2 are above its critical temperature of 31 °C and critical pressure of 74 bar. Supercritical fluids are highly compressed gases, which have combined properties of gases and liquids in an intriguing manner. Supercritical fluids can lead to reactions which are difficult or even impossible to achieve in conventional solvents. In addition, supercritical fluid can be separated from analytes by simply releasing pressure, leaving almost no trace and yields a pure residue. The use of supercritical carbon dioxide is a simple, inexpensive, fast, effective and virtually solvent–free sample pre-treatment technique. In this research, a complete study on supercritical carbon dioxide extraction of natural products from plants is carried out to examine the principal of this method, the bioactive compounds that can be extracted and also the effect of process variables on extraction yield. Finally, SFE also has been compared with other conventional and non-conventional extraction methods in terms of selectivity, rapidity, cleanliness and possibility of manipulating the composition of the extract.

Keywords: Supercritical fluid extraction; carbon dioxide; Natural products.

1.0 INTRODUCTION

Plant extracts have been used for various purposes and in demand as alternative medicines and food preservatives. Biological properties of plant extracts such as antimicrobial and antioxidant ability gives significant importance in our daily life (Cos, *et al.,* 2006). From the analysis of World Health Organization (WHO), large population is dependent on folk medicine for their dominant healthiness demand. In Asia, the interaction of human with environment was exhibited through the use of herbal medicines in daily basis. Plants that contain substances that are able to defeat chronic and infectious diseases are always used as traditional medicine (Duraipandiyan, *et al.,* 2006). Secondary metabolites in plant material offered diverse medical properties such as antitumor, antifungal, antibacterial, anti-litholitic and antioxidant. These secondary metabolites are normally in the form of composite molecules with functional structures (Lahlou, 2013). According to the biosynthetic origins of secondary metabolites, they are able to be categorized into three major classes which are phenolic compounds, terpenoids and nitrogen-containing alkaloids.

Phenolic compounds in plant material are a group of antioxidants composed by various subclasses such as phenolic acids, stilbenes, lignans and others. In recent times, due to their potential health effects, an increased interest in extraction of phenolic compounds has arisen. Phenolic compounds contain phytonutrients with strong antioxidant properties. The principle role of antioxidants is to prevent the initiation and propagation of oxidizing chain reactions by free radicals. In consequence, it may decelerate the oxidation processes of other molecules and weaken oxidizing harm (Lobo, *et al.,* 2010). Hence, antioxidants are typically utilized on daily life as their consumption represent a central role in the avoidance of many syndromes which related to oxidative stress like cancer, cognitive and cardiovascular diseases (Uttara, *et al.,* 2009).

Essential oils have been contemplated as characteristic constituents of aromatic plants. They are composed of a group of volatile organic compounds that stimulate flavour and fragrance of a plant (Ercioglu, Velioglu and Boyaci, 2015). Furthermore, due to their comparatively safe status, their wide acknowledgement by users and exploitation for potential multi-functional use, essential oils are gaining more interest in the recent years. (Lin, *et al.,* 2009). One of the important components in essential oils is terpenoids, which are the most diverse natural compounds found in many plants. Terpenoids are biologically active and are employed for treatment of malaria, cancer, inflammation and infectious diseases. Alkaloids can be described as a group of organic particles with composite structures that consist of a heterocyclic nitrogen ring. They can be discovered mainly in plants specifically in particular families of flowering plants. Alkaloids have been extensively utilized for various aspects in favour of their numerous pharmacological characteristics. Even though alkaloids are commonly detected in organisms, including animals and microbes, normally the higher plants also consist of different array of these compounds. Some of the alkaloids are advantageous for medicinal appliances but some others are poisonous and potentially harmful to humans (Murphy, 2017).

Extract from plants are complex mixtures of constituents and the method for extraction, sample clean up, separation and detection for each extract is distinctive. For the analysis of medicinal plants, the preliminary step is extraction since it is required to conduct the separation of intended bioactive compounds from the plants in order to isolate and carry out investigation. The main procedures for extraction include pre-washing, dehydrating of plant materials and milling to collect a homologous sample. During the process, the analyte extraction kinetics needs to be enhanced regularly and also enlarge the surface of contact between sample and solvent system so that extraction can be carried out efficiently. Some precautions and proper manners are necessary to assure that no active constituents will be damaged (Fabricant and Farnsworth, 2001).

The options for solvent system commonly relies on the particular character of bioactive compounds that being extracted. For the extraction process of bioactive compounds from plants, different solvent systems are accessible. Since bioactive compounds are thermally labile and could be varied from polar to non-polar, the applicability of the techniques of extraction need to be studied (Sasidharan, *et al.,* 2011). In general, the extraction methods used are solid–liquid extraction (SLE), solid-phase extraction (SPE) or liquid–liquid extraction (LLE). However, the latest extraction techniques include ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE) or microwave-assisted extraction (MAE), which each of these methods own certain benefits and have received a great interest for overcoming the drawbacks of conventional solvent extraction. These techniques are benefit in exclusion of extra cleaning up of sample and concentration procedures before the analysis of chromatographic, deduction in usage of organic solvent and in sample degradation, increase in selectivity of extraction, extraction performance and kinetics. Their practises for the extraction of bioactive compounds from plant materials are preferred due to the simplicity and automation of these techniques (Huie, 2002).

From the end of the 1970s, the interest in supercritical techniques and supercritical fluids used to isolate natural products are getting more and more high (Brunner, 2005). This interest is showed in the recent years in the form that large quantity of scientific reviews concerns with supercritical fluid extraction (SFE) being announced. In addition, industrial utilization of SFE has achieved an aggressive evolution in terms of patents since the early 1990s in (Schutz, 2007). In the review, the main focus is on extraction of bioactive compounds from plants using carbon dioxide as main solvent. Carbon dioxide (CO_2) with critical temperature 30.9 °C and pressure 73.8 bar, has benefits in terms of cost, less harmful to the environment and acknowledged as secure by FDA and EFSA. Besides CO_2 also has high diffusivity and adjustable solvent strength. Moreover, the characteristic of CO_2 remains as gaseous phase under room temperature and pressure makes extraction process much easier and it also offers solvent-free analytes (Herrero, *et al.,* 2006).

Supercritical fluid extraction (SFE) using carbon dioxide is an advanced technology with higher efficiency alternative i.e. short in time, high purity and lower temperature (to avoid temperature induce degration) compared to the conventional methods for example like soxhlet extraction or hydrodistillation (Manjare and Dhingra, 2019). The extraction of bioactive compounds by supercritical carbon dioxide extraction has been extensively studied due to its benefits over conventional extractions based on organic solvents. Its advantages include the consumption of non-toxic solvent that is obtainable in high purity but inexpensive and least solvent residues in final products. Additionally, SFE process can be performed at relatively low temperature, preventing degradation of heat-induced product. The capability of SFE using carbon dioxide to be conducted at low temperature is essential for sample preparation of natural products because it permits the extraction of thermal sensitive or easily oxidized compounds with the use of a non-oxidant medium (Schutz, 2007).

Comprehension of the character of plant matrix, chemical properties of bioactive compounds and also input parameters are very important since they will directly affect the efficiency of non-conventional and conventional extraction techniques. Extraction optimization is considered as one of the major aspects in SFE. By utilizing the optimum values for every process parameters, the efficiency of SFE extraction and yield of targeted bioactive compound could be significantly improved (Ruan, *et al.,* 2016). Normally, the performance of SFE is altered by various factors including process temperature, pressure, duration of extraction, existence of modifier and CO_2 flow rate.

2.0 BIOLOGICAL ACTIVE COMPOUNDS

Based on the reason of their wide range of structural variety and pharmacological activities, plants are acknowledged in various aspects especially in pharmaceutical industry. Phytochemistry defines the substantial amount of secondary metabolic compounds exist in plants. These phytochemicals are used as sources of direct pharmaceutics medium. They can be derived out of distinct parts from plants like roots, leaves, seed coat, seed, pulps, barks and flowers (Krishnananda, *et al.,* 2017).

Originally, human beings consumed plants in the interest of nutritional reason. After the exploration of their pharmaceutic abilities, this natural plants turn into an effective medicine for disease treatment and also useful for health improvement (Azmir, *et al.,* 2013). Therefore, natural sources such as extract from plants, open up a new horizon for the revelation of new medicinal agents. In most developing countries, traditional drugs and medicinal plants are used as a formal basis for the support of good health and about 80 % of the community has been noticed depends on herbal medicines. This is because plants consist a lot of chemical compounds that are suitable for chronic treatment in addition to infectious diseases. They discovered precisely more than thousands of phytochemicals are valuable and contain biological properties for example wound healing, antioxidant, anticancer, analgesic and antimicrobial (Krishnananda, *et al.,* 2017).

The comprehension regarding dynamic chemical properties of various bioactive components is the forerunner for the development of bioactive determination from the last decade (Torssell, 1997). Subsequently of these significant technological advancements, different industries have become more concerned in bioactive compounds from natural materials such as food additives, pharmaceuticals and even on natural pesticides sectors (Azmir, *et al.,* 2013). Generally, biological active compounds exist together with some other components in plants. They can be mainly classified into two categories which are primary metabolites and secondary metabolites. Secondary metabolites can be described as metabolites that are usually formed in a stage of consequent to growth. They consist of uncommon chemical structures and also existed as combinations of mutually related members from one family group (Martin and Demain, 1978).

The generation of secondary metabolites is principally chosen by the way of assessment and the certain demand of particular species. For instance, the purpose of fragrance by flowering plants is to draw attention from insect to help them in fertilization and pollination, and synthesis of poisonous compound has been utilized against herbivores and pathogens for restraining the development of neighbouring species. In the middle of these secondary metabolites, some of the compounds are useful for biological organisms which are treated as bioactive. Hence a common explanation of bioactive compounds in plants can be defined as secondary metabolites evoking toxicological and pharmacological properties in both animals and human (Azmir, *et al.,* 2013). Plant secondary metabolites can be classified into three main categories. These three categories include phenolic compounds, nitrogen-containing compounds and terpenoids. The examples of terpenoids are sterols, plant volatiles, carotenoids, and cardiac glycosides; phenolic compounds are tannins, phenolic acid, coumarins, flavonoids, stilbenes and lignans; nitrogen-containing compounds include alkaloids, non-protein amino acids and cyanogenic glucosides (Croteau *et al.,* 2000).

2.1 Phenolic Compounds

Phenolic compounds (PCs), is the largest group of secondary metabolites in plants and they can be found ubiquitously in daily life. In other words, a huge amount of medicinal plants have been proved to be abundant in PCs. These plants involve Ginkgo biloba L., Salvia miltiorrhiza Bge., Myristica fragrans Houtt., Boehmeria nivea (L.) Gaudich. and Cimicifuga foetida L. Other than that, in our daily diets, vegetables, fruits, cereals and spices are also popular sources of PCs especially polyphenols. PCs possess a particular chemical structure composing an aromatic ring hydroxyl substituents that are able be classified into few groups. With the expanding acknowledgement of medicinal properties of PCs, they have been proved to aid in reducing the risk of various chronic disorders. The major groups of PCs include phenolic acids, tannins, flavonoids, lignans and stilbenes. As many reviews published, PCs give pharmacological properties such as anti-carcinogenic, anti-microbial, anti-inflammatory, antioxidant, estrogenrelated prevention of cancers, diabetes, cardiovascular diseases and ailments related with oxidative stress (Xu, *et al.,* 2017). Hence, these newly exposed characteristics of PCs have been utilized in the development of cosmetics, nutraceuticals foods.

Chemical structure of phenolic compounds varied from simple phenols to high molecular weight and long chain polymers such as condensed tannins (C_6–C_3–C_6)n and lignins (C_6–C_3)$_n$. Intermediate molecular weights of phenolic compounds include flavonoids (C_6–C_3–C_6) and stilbenes (C_6–C_2–C_6) (Ejaz, *et al.,* 2017). Among PCs, flavonoids represented a crucial group of compound with great importance because they can be used by human body to fight against diseases. Flavonoids are composed by 15 carbon atoms which organized in three rings (C_6–C_3–C_6) labelled as A, B, and C, respectively; where A and B are two aromatic rings, and C is a three-carbon bridge heterocyclic ring (Xu, *et al.,* 2017). They are well known to have anti-hypertensive properties. Flavonoid compounds are a type of extraordinarily efficient natural antioxidants and less harmful than synthetic antioxidants. These highly reactive molecules are normally present in biological systems and can lower the chance of degenerative diseases like cancer, heart disease or dermal disorders. Several reviews have showed that flavonoids can be consumed to avoid certain prevalent diseases like atherosclerosis, cancers and ameliorate muscle wasting (Ouédraogo, *et al.,* 2018).

PCs exert a great structural diversity and comprise a major class of plant secondary metabolites which are extensively spread in the domain of plant. Their stability alter significantly and are neither consistently distributed in the plant. This properties indeed make their extraction and isolation processes more difficult, which means that a single standardized procedure is not applicable for all kinds of phenolic compounds or plant materials. Thus, the procedures need to be amended based on the objective of the study, the targeted analytes and importantly the properties of sample (Xu, *et al.,* 2017). Basically, the amount of hydroxyl groups in PCs will affect its lipophilicity. Due to the polar region of the lipid bilayer, the interaction between phenolic compounds and lipids such as membranes of lipid cell are restricted. Phenolic compounds are normally more hydrophilic than lipophilic due to their phenolic nature. Hence, phenolic compounds can be readily separated by supercritical carbon dioxide extraction method with the aid of modifiers such as methanol, ethanol, water and etc.

2.2 Alkaloids

Alkaloids, non-protein amino acids and cyanogenic glucosides are the examples of plant secondary metabolites which contain nitrogen compound as part of their structure. They are nitrogen-containing compounds that normally modified from amino acids like tryptophan, tyrosine, aspartic acid and lysine. These compounds existed in the form of secondary metabolites in around 20 % of plant materials and presented as defensive role to defeat the attacks of herbivores and pathogen (Hegnauer, *et al.,* 1988). An alkaloid is a compound has at least one nitrogen atom in an amine-type structure. Nearly all alkaloids contain nitrogen atoms as part of a ring of atoms, which usually named as cyclic system. Alkaloids are generally categorized depending on their chemical structure. Such as, alkaloids that consist of a ring system (indole) are named as indole alkaloids. Based on this principal, the major groups of alkaloids are divided into pyrrolidines, pyridines, tropanes, pyrrolizidines, isoquinolines, indoles, quinolines, terpenoids and steroids.

Alkaloids have different physiological influences on both human being and faunas. Some famous alkaloids are strychnine, ephedrine, morphine, nicotine and quinine. Alkaloids are discovered mainly in plant materials and are exclusively popular in particular families of flowering plants. For example, all plants from poppy family (Papaveraceae) are found to have alkaloids. The Amaryllidaceae (amaryllis), Solanaceae (nightshades) and Ranunculaceae (buttercups) are examples of other prominent alkaloid-containing families of plants.

The pharmaceutic abilities of alkaloids are relatively diverse. For example, morphine as an effective narcotic employed to reduce ache, even though its obsessive characteristics restrict its suitability for usage. Besides, codeine which is derivative from methyl ether of morphine discovered in the opium poppy, is an outstanding analgesic drug that is comparatively non-addictive. Meanwhile some other alkaloids can be acted as cardiac or respiratory stimulants. From plants of the genus Cinchona, quinidine can be obtained for treatment of arrhythmias or irregular rhythms of the heartbeat. In this aspect, lobeline that come from Lobelia inflata is harmless and thus is more useful. Besides, Ergonovine obtained from the fungus of Claviceps purpurea and ephedrine from Ephedra species can be used as blood-vessel constrictors. Ephedrine is also frequently consumed to cure the anxiety of common colds, sinusitis and bronchial asthma.

Particular techniques have been advanced for isolating valuable alkaloids. In most conditions, aqueous solutions of the alkaloids can be attained by processing plant tissue. Those alkaloids are later separated from the solution using method of extraction, which include dissolving some elements of the mixture with reagents. Dissimilar alkaloids are then can be divided and decontaminated from the mixture.

2.3 Terpenoids

Terpenoids is a subdivision of the prenyllipids (terpenes, prenylquinones and sterols), which are widespread kind of natural products and symbolize the oldest class of small molecular substances produced by plants. Terpenoids, which methyl groups are taken out or extra oxygen atoms joined can be expressed as modified terpenes. All terpenoids may be described as a group of molecules where their chemical structure is depend on a variety but definite amount of isoprene units. The biosynthesis of terpenoids begins from basis of two general five carbon (C_5) pioneers which are isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The two basic prenyl units are utilized precisely to compose hemiterpenes or polymerized in increased number of five carbon units through the expansion of IPP to develop prenyl diphosphates with varying lengths of chains. These prenyl diphosphates are the universal forerunners to primary terpenes that can be discover in plant materials, such as monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), triterpenes (C_{30}), carotenoids (C_{40}) and polyprenols ($C > 45$). Terpenoids served as the biggest and most diverse group of chemicals among the myriad compounds obtained by plants. Plants utilize terpenoid metabolites for primary activities in growth and development but also consume most of the terpenoids for protection in the abiotic and biotic environment and for more particularly chemical interactions. Generally, terpenoids from plants have been consumed by community in the respect of food, chemical industries, pharmaceutical industry and currently have been used in the development of biofuel products. Various researches have confirmed proved that this kind of compounds shows a wide spectrum of essential pharmacological features. The diverse compilation of terpenoid structures and properties have exasperated more interest for their commercial usage resulting in some with conclusively proved medical utilizations being registered as drugs on the market. Some of the terpenoids are more biologically active and are effective in healing malaria, inflammation, cancer and infectious disorders. However, several composites from this group exhibit poisonous properties lead to central nervous system manifestations and gastrointestinal problems among others.

There are different kinds of mono-, sesqui-, di-, tri-, tetra- terpene compounds have been universally reviewed. These components are discovered throughout natural sources and are found in most plants. Most of the monoterpenes are acknowledged for their impressive aromas and high volatility which are used to attract insects for pollination of flowering plants. For instance, essential oils extracted from rose, peppermint, lemon and lavender have various oxygenated monoterpenes, aliphatic and aromatic compounds that offer these oils their specially refreshing fragrance. Meanwhile, other higher molecular weight terpenes and non-aromatic terpenes can use as protective compounds by helping the plant to push away possibly harmful predators. Hydrophobicity of terpenes which allows them to cross the lipid membranes of invading cells effortlessly can be described as another protective characteristic. Even though terpenes are commonly not harmful for large organisms such as humans and animals, they can be useful against a lot of environmental threats.

Terpenes and terpenoids are the main components of essential oils which obtained from different species of medicinal plants. Essential oils are often utilized as fragrances in perfumery and substitute medicines such as aromatherapy. Artificial derivatives and variations of natural terpenes and terpenoids also largely expand the diversity of aromas which utilized in flavors used in food additives such as Vitamin A is a terpenoid. Due to the wide variety of structures that terpenes can compose, the techniques for their extraction process will vary case-by-case, mainly based on the nature properties of the targeted terpene, and quantity of the raw material and the accessibility of instruments and reagents used (Jiang, Kempinski and Chappell, 2016).

3.0 EXTRACTION TECHNIQUES

The analysis of bioactive compounds from plants mainly depends on the choice of suitable extraction technique. Extraction is the primary procedure of all medicinal herbs research, it presents an important and critical role on the outcome and final results. Extraction methods are often used as sample preparation step for analytical purposes and to strip unwanted material or obtain desired material from a product. A research carried out by Majors (1999) indicated that numerous researchers have admit the significance of sample preparation step for research project (Azmir, *et al.,* 2013).

Extraction of bioactive compounds from plant materials and their quantitative and qualitative assessment is essential for the analysis of new discovered biomolecules. These biomolecules are utilized by agrochemical and pharmaceutical industry directly or used as a lead molecule to produce more potent molecules. Extraction from the plant is a practical exercise as various solvents are used at different conditions such as pressure, temperature and duration of extraction. In addition, in the extraction process, further the separation of targeted analytes from co-extractives compounds is important. Further fractionation of extracted compounds can be accomplished on the basis of their molecular size, acidity or polarity (Krishnananda, *et al.,* 2017).

Extraction of plant materials can be conducted by different extraction techniques. Firstly is the non-conventional methods, which have been developed during the last 50 years, is more eco-friendly since it required low usage of artificial and organic solvents in shorter process time with greater yield and quality of product. For instance, to increase overall extraction yield and selectivity of bioactive compounds, pulsed electric field, extrusion, enzyme digestion, ultrasound, supercritical fluids, accelerated solvents, ohmic heating and microwave heating have been considered as non-conventional methods. Meanwhile, for conventional extraction methods, for example Soxhlet extraction is yet studied as the reference to compare with advanced technique. Generous amount of book chapters, monograms and scientific reports be present where non-conventional methods were widely studied (Azmir, *et al.,* 2013).

To proceed with a further separation, analysis and interpretation of bioactive compounds, it is necessary to conduct a successful extraction process. Various extraction methods could be carried out in different settings for the consideration of extraction selectivity from different natural materials.

3.1 Conventional Extraction Techniques

Extraction of bioactive compounds from plant materials can be done by numerous traditional extraction methods. Most of these methods are depend on the solvating power of solvent used and the application of heat or mixing. Some common extraction methods used to collect bioactive compounds from plants are Soxhlet extraction, Maceration and Hydrodistillation.

The first Soxhlet extractor was created 1879 by a chemist from Germany Franz Ritter Von Soxhlet. This extractor was created primarily for the extraction of lipids but presently this method is not restricted only for this purpose. Soxhlet extraction has been extensively practiced for the extraction of bioactive compounds from natural sources. It is utilized as a reference method and alternatives of new extraction techniques. Typically, at first a little quantity of sample matrix is located in a thimble and then the thimble is situated in distillation flask which consists of solvent. The solution of the thimble-holder is aspirated by a siphon once it overflows. The siphon discharges the solution back into the distillation flask. This solution transfers extracted solutes into the bulk liquid. After that, solute is remain in the distillation flask whereas solvent flows back to the solid bed of sample. This procedure operates continually until the extraction is finished (Azmir, *et al.,* 2013).

Soxhlet extraction has been extensively utilized for both initial and bulk extraction. The main advantage of this method is that the sample can be extracted continuously. The solvent saturated in solubilized metabolites discharges into the flask, fresh recondensed solvent then re-extracts the material in the thimble. Soxhlet extraction use shorter process time and less solvent-consuming than others such as maceration and percolation. However, it has a main drawback which it is not suitable for thermolabile compounds or it can initiate the degradation as the extract is continually heated at the boiling point of the solvent used (Seidel, 2012).

Maceration is mainly employed in the preparation step for production of tonic long times ago. Recently, it has been developed to a well-known and inexpensive method to obtain bioactive compounds and essential oils from natural sources. For a smaller scale of maceration extraction, it commonly made up of numerous procedures. Initially, for proper mixing with solvent, crushing of plant samples into smaller particle is carried out to increase the surface area. Next is the addition of suitable solvent named as menstruum into a sealed container in maceration process. Thirdly, the solution is strained off but the solid residue is hard-pressed to recover more occluded solutions. The press out liquid and obtained strained and are then mixed together and eventually purified from contaminations by the way of filtration. In maceration, irregular shaking further extraction by two means which are enhance the diffusion and remove saturated solution from the sample surface for transporting fresh solvent to the menstruum for greater yield of extraction (Azmir, *et al.,* 2013). Maceration is applicable for both initial and bulk extraction of plant materials. However, exhaustive bulk extractions require longer processing time which takes from a few hours up to several weeks. Besides, large volumes of solvent may be consumed for this method (Seidel, 2012).

Hydrodistillation is another classical method to extract essential oils and valuable bioactive compounds from plant materials. However, organic solvent is not required in this technique and it can be carried out before the dehydration of plants. Hydrodistillation can be divided into three types which are water distillation, water and steam distillation and direct steam distillation (Vankar, 2004). For the hydrodistillation process, the first step is the introduction of the plant sample in a fixed compartment. Next, some water is added and boiled. On the other hand, for direct steam hydrodistillation, steam is inserted into the plant materials. In this method, hot water and steam is the dominant aspects to separate bioactive compounds from plant cells. The vapour mixture of oil and water is then condensed by indirect cooling of water. The condensed mixture is then flows to a separator from condenser. The separator is where bioactive compounds and oil can be extracted spontaneously from the solution (Silva *et al.,* 2005). Besides that, hydrodistillation includes three fundamental physicochemical routes which are hydrodiffusion, hydrolysis and decomposition by heat. One of the limitation of this technique is particular volatile composites may possibly be damaged in the process since this is a high temperature extraction. This weakness restricts its applicability for thermo sensitive compound extraction (Azmir, *et al.,* 2013).

3.2 Non-Conventional Extraction Techniques

The main disadvantages of conventional extraction techniques are they normally time-consuming, require expensive and high concentration solvent, involve vaporisation of large quantity of organic solvent, low selectivity of extraction and not suitable for thermo labile compounds. To overcome these drawbacks, some promising and advanced extraction methods are presented. These methods are considered as non-conventional extraction techniques. These new techniques include supercritical fluid extraction (SFE), enzyme-assisted extraction, microwave-assisted extraction (MAE), ultrasound assisted extraction (UAE), pressurized liquid extraction (PLE) and pulsed electric field assisted extraction (PEF). All these methodology are also studied as ‘‘green techniques’’ since they satisfy the criteria of the Environmental Protection Agency, USA. These rules consist of low level of harmful chemical solvent, designing nontoxic substances, safe solvents auxiliaries, intention to promote energy efficiency, usage of renewable raw material, reduce by-products, plan to avoid degradation and so on (Azmir, *et al.,* 2013).

However, every non-conventional extraction techniques have their own significant advantages and limitations. Different extraction methods of bioactive compounds are illustrated and compared in Table 1.

Table 1: Comparison of Non-conventional Extraction Methods

Extraction Method	Advantages	Disadvantages
SLE (Microwave-assisted extraction)	Simple and easy to operate; wide adaptability.	Need to use substantial amounts of hazardous organic reagents; long extraction time; low efficiency.
UAE (Ultrasound-assisted extractions)	Simple and easy to operate; efficient; economical; wide adaptability.	Unsuitable for industrial production
MAE (Microwave-assisted extraction)	Consumes less extraction solvent and extraction time; increases the content of extracted antioxidants.	Degradation and oxidation will occus under such conditions
SFE (Supercritical fluid extraction)	Increase safety and selectivity; avoid sample oxidization in the presence of air.	Unsuitable for the extraction of polar PCs; high requirement of capital investments.
ASE (Accelerated solvent extraction)	Require small amounts of solvent; provides faster extraction processes.	The need of high temperature and pressure.
HHPE (High hydrostatic pressure extraction)	Efficient; consumes less extraction solvent and extraction time.	The need of high pressure and expensive equipment.

Microwave-assisted extraction is a technique by heating the solvents and plant tissue using microwave to increase the kinetic of extraction. This techniques commonly named as microwave extraction which combines the microwave and traditional solvent extraction. Microwaves is defined as the electromagnetic fields within the frequency range of 300 MHz to 300 GHz and are produced by two oscillating fields which are perpendicular to each other like magnetic field and electric field. The purpose of heating of dried plant material is to further reduce microscopic traces of moisture that exists in the cells of plant. Due to microwave effect, the heating up of this moisture inside the plant cell results in dehydration and produces high stress on the wall of cell. The cell wall is then pushed from inside and rupture the cell wall. Hence the exudation of active components from the ruptured cells happens and the produce of phytoconstituents increased (Krishnananda, *et al.,* 2017). MAE is acknowledged as one of the green technology since it lessen the usage of organic solvent. Some advanatges of MAE are proposed by Cravottoa et al. on 2008, which includes heating up for the extraction of bioactive compounds from plant samples in shorter time, lower thermal gradients throughout the process, smaller size of apparatus used and greater yield of extraction. This technique can extract bioactive compounds faster and a greater recovery can be achieved compared with conventional extraction techniques. MAE is a selective method to organometallic and organic compounds that are more complete (Alupului, 2012).

Pressurized Liquid Extraction (PLE) is an advanced extraction technique that make use of solvents at high pressures and temperatures used sample preparation steps for analysis by either liquid chromatography or gas chromatography. PLE is actually very much alike with Soxhlet extraction, except that the solvent used for PLE cell approaches to the supercritical region during the extraction process. This results in a more effective extractions of sample. The high temperature required in this method can increase the solubility of sample and allows greater rate of diffusion whereas high pressure ensure the solvent is always lower than its boiling point. This is because at high temperatures and pressures, solvents can penetrate into solid matrixes more easily and effectively thus lessen the use of solvent.

PLE was established to reduce time consumption and the usage of organic solvent as compared to soxhlet extraction technique. For the extraction of polar composites, this method is considered as a promising substitute method to supercritical fluid extraction. It is effective for the extraction of organic impurities from environments as well been utilized for the extraction of bioactive compounds from marine sponges (Ibanez *et al.,* 2012). The procedures of pressurized liquid extraction involves mixing of sample with sodium sulfate, loaded in the extraction cell and covered with two filtration end fittings. Then, the system will mechanically starts pressurizing and heating the samples. For PLE, the extraction pressure is always kept at a range of 1500 PSI to 3000 PSI and at a temperature of 70 ºC to 200 ºC. The extracted solution which consists of targeted analytes is now transported to an evaporation or concentration chamber where it is eventually transformed to final volume directly in a gas chromatography (GC) or liquid chromatography (LC) vessel. The vessel can formerly be moved to the analytical device for examination process. With the advantages of low volume solvent requirements, PLE provides a more practical and environment-friendly substitute method to conventional approaches. This method is the most suitable way for rapid and reproducible initial extraction of a large amount of samples (Seidel, 2012).

4.0 SUPERCRITICAL FLUID EXTRACTION TECHNIQUE (SFE)

Nowsaday, a dramatically growing of attention has been noted for supercritical fluids as a substitute solvents involved in the extraction of bioactive compounds from natural sources. The primary cause for this attention was the feasibility of conducting extraction processes at temperature close to ambient using supercritical fluid extraction, thereby avoiding the targeted components from inducing in thermal damage. After numerous researches and development, SFE is probably can be acknowledged as a well-established technique for extraction and separation of compounds. Furthermore, the design and operating principles of SFE are completely been understood, thus it is able to apply in the extraction of medicinal and aromatic plants (MAPs) commercially (Karale, *et al.,* 2011). The utilization of supercritical fluid intended for extraction begun with its innovation by Hannay and Hogarth on 1879, and Zosel who published a patent with title of decaffeination of coffee via SFE technique. Ever since this starting point, supercritical fluid technique has drawn extensive attention and it was favourably used various industries especially in pharmaceutical, food analysis, environmental and polymer sectors.

Efficiency of extraction for any technique mostly relies on the appropriate selection of solvents (Cowan, 1999). The most important factor for solvent choice is the polarity of targeted bioactive compounds. Besides that, there are some other factors that should be considered in the selection of solvents for successful extraction such as molecular attractive force between solute and solvent, mass transfer, usage of modifier, environmentally friendly, human toxicity and commercial feasibility. (Azmir, *et al.,* 2013).

Some commonly solvents includes aliphatic and chlorinated hydrocarbons, esters and lower alcohols are used for extraction of particular bioactive compounds. Plants are complex matrices which containing an extent of secondary metabolites with different polarities and functional groups in it. Even though water is frequently employed as extracting solvent in lots of traditional procedures, organic solvents of different polarities are mostly chosen for new methods of extraction to exploit the varying solubilities of plant components (Seidel, 2012).

4.1 Supercritical Fluids (SFs)

Supercritical fluids (SFs) is a substance at supercritical condition, or defined as a dense gas which is a fluid at pressure and temperature above its critical point to a particular level. To achieve supercritical phase, the reduced temperature, Tr (T/TC) should not more than value of 1.3, meanwhile the reduced pressure, Pr (P/PC) is unlimited range and could be as high as allowed by technical restrictions. Under appropriate situations, any substance can achieve its supercritical phase. Nevertheless, only fluid with critical temperature around ambient temperature are suitable to be utilized as a substitute solvents for the extraction of bioactive compounds (Karale, *et al.,* 2011).

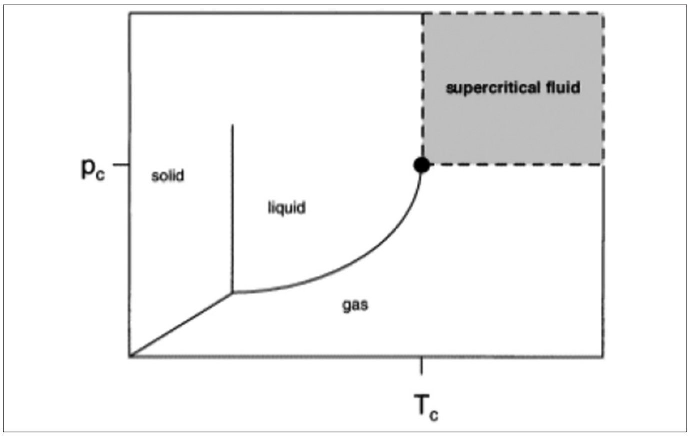

Figure 1: Phase diagram of a single substance (Karale, et al., 2011)

Figure 1 shows phase change of any substance by controlling its temperature and pressure. A graph of the solubility parameter for CO_2 against pressure is similar to that with parameter of density against pressure. The plot indicates that power of solvation for a supercritical fluid is directly connected to its density of fluid. The applicability of consuming supercritical fluids as extracting solvents is related to their fluid density. Once a fluid reaches critical phases, its density becomes nearer to that of in liquid phase. It is also acknowledged that expansion coefficient and compressibility of the fluid will become higher once it reaches critical point and thus even small alteration in the operating parameters can obviously change the density as well as the solvent power of supercritical fluid. Another attractive characteristic of supercritical fluids is that its behaviour lie between liquids and gases. Supercritical fluid possess densities like liquids, whereas the diffusivities and viscosities are more similar to gases. Therefore, a supercritical fluid is able to diffuse through a solid sample matrix more rapidly than a liquid, but still have a solvent ability to extract the solute from the solid sample matrix (Karale, *et al.,* 2011). The phase diagram for a pure compound at supercritical conditions is shown in Table 2.

Table 2: Critical Parameters for Selected Substances (Capuzzo, Maffei and Occhipinti, 2013)

Substance	T_c (°C)	P_c (bar)	ρ_c (g/cm^3)
Ar	-122.3	48.5	0.53
CH_4	-82.5	46.4	0.16
C_2H_6	32.4	48.8	0.20
C_2F_6	19.9	30.6	0.62
CHF_3	26.2	48.5	0.62
CO_2	31.1	73.8	0.47
C_2H_4	10.0	51.2	0.22
SF_6	45.6	37.2	0.73
NH_3	132.5	112.8	0.24
MeOH	240.6	79.9	0.27
EtOH	243.5	63.8	0.28
C_6H_6	289.0	48.9	0.30
H_2O	374.2	220.5	0.32

Supercritical fluids like methanol, ethane, pentane, propanol, ammonia, trifluoromethane and water are often employed in extraction process. Ghafoor *et al.* (2010), have developed SFE to extract phenolic compounds from grape seeds and the extract products obtained are examined, including gallic acid, protocatechuic acid and p-hydroxybenzoic acid. In the comparison with other extraction techniques, SFE utilize lower volume of hazardous organic reagents with shorter duration of extraction, increase selectivity and safety, and can prevent sample from oxidization in the existence of air. Anyhow, the main drawback of this process is the necessity of high capital investment cost (Xu, *et al.,* 2017). There are numerous compounds that applicable in supercritical techniques, however the most frequently used of solvent is supercritical carbon dioxide (SC-CO_2). Carbon dioxide (CO_2) is expressed as a perfect solvent used for SFE. One of the reason is that the critical temperature of CO_2 which is 31 °C, is near to ambient temperature and its low critical pressure of 74 bar allows the accessibility to be operated at moderate pressures, usually in the range of 100 bar to 450 bar. From the point of view of nutraceutical, food and pharmaceutical applications, SC-CO_2 is an ideal solvent since it is less hazardous, non-flammable, not expensive and ready to be separated from final product. It is more ecological friendly and acknowledged as safe by EFSA and FDA. In addition, SFE with SC-CO_2 using a non-oxidant medium is able to be conducted at low temperatures, which permits the extraction of temperature sensitive or easily oxidized compounds which is very essential for food and natural goods sample preparation (Karale, *et al.,* 2011).

On the other hand, the main limitation of CO_2 is its mild polarity that allows it only for extraction of lipid and non-polar compounds, but inappropriate for most of the pharmaceuticals compounds. Due to its mild-polar properties, it not suitable to be utilized for dissolution of polar molecules. However, this drawback of low polarity of CO_2 can be overcome completely by the addition of organic modifier (co-solvents) (Ghafoor, *et al.,* 2010). Generally only a little quantity of modifier is sufficient as helpful to increase the polarity of carbon dioxide significantly. For instance, only 0.5 ml of dichloromethane is needed to improve the efficiency of extraction which is same for 4 hours by hydrodistillation. The characteristics of sample matrix and targeted analytes and the reference experimental data are major basis for selection of the suitable modifier (Azmir, *et al.,* 2013). Besides, the milder processing conditions would leads to complication of system thermodynamics and increased capital costs (Karale, *et al.,* 2011).

In addition to CO_2, other supercritical solvents have been evaluated for agronomic applications. There are some reports about the choice of nitrous oxide (N_2O) as an extraction fluid. The chemical properties of this fluid make N_2O more suited for the extraction of polar compounds. However, in the presence of a high organic content, the gas can cause violent explosions. This drawback strongly limits its use. Even water was investigated as a possible supercritical fluid but, unlike CO_2, the high critical temperature and pressure together with the corrosive nature of H_2O under these conditions, has limited its practical applications. Even so, water was used in some cases as a co-solvent for the extraction of more polar compounds from aromatic plants. The presence of water as pretreatment of plant material or added to CO_2 at supercritical and subcritical state as a co-solvent has shown to influence the qualitative and quantitative composition of the extract. In addition, ethane, propane and dimethyl ether have been used as supercritical solvents for the extraction of bioactive compounds from plants. Beside critical points that are comparable with CO_2, these solvents have higher polarizability than CO_2, resulting in a stronger interaction with the more polar compounds and co-solvents. However, experimental results show that SC-CO_2 offers a wider versatility for the fractionation of extracted compounds using different operative pressures in the extractors or separators (Capuzzo *et al.,* 2013).

4.2 Mechanism of Supercritical Fluid Extraction

Supercritical fluid extraction has been practiced to a thousands of solid sample matrices. However, the extract or the extracted solid sample itself can be the final product. The advantage of consuming supercritical fluids in extraction technology is the simplicity of purification of the extracted products from supercritical fluid solvent. This separation process can be carried out by simple expansion. Moreover, supercritical fluids possess liquid-like density but they also have excellent mass transfer behaviour as contrasted with liquid solvents. This is due to their low surface tension and high diffusivity which allows diffusion into the permeable structure of solid sample matrix more easily to liberate the solute (Karale, *et al.,* 2011).

Extraction of solutes from solid matrices can be carried out by four different mechanisms. Firstly, the process is just a simple dissolution of solute into solvent that would not dissolve the solid matrix, when there are no connections between solute and the solid matrix. On the other hand, if interactions between the solute and solid matrix solute exists, the extraction process is labelled as desorption. The adsorption isotherm of the solute on the solid matrix with existence of solvent defines the equilibrium. Solids extraction process such as regeneration of activated carbon fall in this classification. The third mechanism is the swelling of solid matrix by solvent and extraction of the targeted solute by the first two mechanisms. The fourth mechanism of extraction is called reactive extraction, where the insoluble solute reacts with the solvent and the reaction products are soluble hence extractable (Mohamed *et al.,* 2008).

Extraction is usually complied with additional separation procedure which the extracted solute is separated from the solvent. One more considerable respect in supercritical fluid extraction involve to the interactions between solvent and solute. Typically the solid and solute interactions will define the simplicity of extraction, for example the intensity of the adsorption isotherm is defined by relations of adsorbent and the adsorbate. On the other hand, when supercritical fluids are employed, relations between the solute and solvent effect the adsorption properties because of large negative partial molar volumes and partial molar enthalpies in supercritical fluids (Karale, *et al.,* 2011). Generally, a fundamental SFE system consists of a few equipment such as a CO_2 mobile tank, a pump needed to pressurize the gas, container for modifier, an oven that attached with the extraction cell, a regulator to keep high pressure during whole process and a collecting chamber. Generally, various type of meters like flow meter or gas meter might be also equipped to the system for controlling. A schematic drawing of SFE equipment is shown in Figure 2.

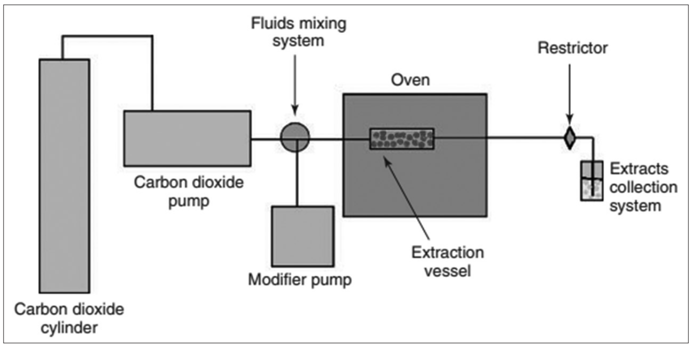

Figure 2: A Schematic Diagram of SFE System (Pourmortazavi, Rahimi-Nasrabadi & Mirsadeghi, 2014)

A common procedure for batch extraction are as follows. Firstly, natural resource feed is introduced in the extraction tank. To ensure that the extraction carried out at desired conditions, the tank is attached with temperature regulators and pressure control device at both ends of the tank. The extraction tank is then pressurized with a particular fluid with the assist of pumps. The pump is also required for the circulation of fluid. After that, the fluid and solubilized mixtures moved from the tank to separator. The salvation power of the fluid is reduced by elevating the temperature or lowering the pressure of supercritical fluid extractor. The final extract product is then collected through a valve placed at the bottom of the separator (Karale, *et al.,* 2011).

SFE procedure could be carried out by two principal modes of static and dynamic. In the static extraction, sample matrix is exposed to a fixed amount of SC-CO_2 for a definite time. In contrast, during the dynamic extraction, fresh CO_2 continuously passes through the sample matrix. In the most of

SFE experiments, a combination of both static and dynamic modes is employed. At first, a static mode is used to allow the penetration of SC-CO_2 to the plant matrix and solubilize the analytes, followed by a dynamic mode that sweeps the analytes from the extraction vessel, through the restrictor and into the collection system. After passing CO_2 and analytes through the restrictor, the CO_2 decompresses to the atmospheric pressure and loses its SCF properties such as solvating power. The analytes therefore are released into the trapping system (Pourmortazavi, Rahimi-Nasrabadi and Mirsadeghi, 2014).

5.0 EFFECT OF PROCESS PARAMETERS

The consumption of bioactive components in various commercial industries for example like pharmaceutical, chemical and food sectors represents the demand of the most suitable and typical technique to extract bioactive compounds from plant materials. Other than conventional methods, multiple advanced techniques have been identified but still not a process has been standardized for extracting different types of bioactive compounds from plant materials. The effectiveness of both non-conventional and conventional extraction methods are generally rely on the major process variables, comprehension about chemical properties of bioactive compounds, nature of plant matrix and previous experimental data (Azmir, *et al.,* 2013).

The first procedure in the SC-CO_2 extraction is to optimize the experimental settings to achieve a satisfactory extraction of the targeted analytes and prevent the co-extraction of other unwanted components. For the development of SC-CO_2 extraction technique, as various process variables possibly influence the extraction efficiency, the optimization of the operating settings is an important stage. The successful extraction of bioactive compounds from plant materials depend on various parameters of SFE and basically these parameter can be modified (Raverchon and Marco, 2006). Process parameter must be measured accurately in order to maximize advantages from this method. The main variables affecting the effectiveness of extraction are temperature, pressure, solubility of solute, extraction time, flow rate of CO_2 solvent and existence of modifier (Ibanez *et al.,* 2012).

Various parameters such as pressure, temperature, CO_2 flow rate and extraction time could affect the solubility of an analyte molecule in the CO_2 phase (Aghel *et al.,* 2004). The pressure and temperature of SCF influence the density of the fluid that determines the number of interactions between CO_2 and molecules of the analytes. Increasing these interactions provide enough cohesive forces between analyte molecules and SCF to conquer binding forces between analyte and sample matrix. In other words, the analyte molecules solubilized in the SCF. Therefore, the value of target molecules solubility in SC-CO_2 is dependent on the molecular weight of analyte compound and the level of interactions between SC-CO_2 and analyte molecules. On the other hand, the kinetics of SFE from plants matrices is variable due to the effect of various parameters on the extraction process. Commonly, the extraction of the first fraction of analytes is limited by their solubilities in fluid phase and the extraction rate of the rest analytes is limited by diffusion process. Meanwhile, the phase equilibrium is dependent on the interactions between analytes and the solid matrix (Pourmortazavi, Rahimi-Nasrabadi and Mirsadeghi, 2014).

5.1 Temperature

Figure 3 shows the typical extraction profiles from solid materials. In the plot, the extractor yield, which is the amount of targeted components extracted with respect to the overall quantity of components in the solid matrix, is constructed versus duration of extraction. From the profile, the higher the temperature, the steeper the plot. It is mainly consists of two parts. First, is a straight line corresponding to the extraction of the compounds readily extracted by supercritical CO_2 solvent and secondly an asymptotic curve describing the extraction of the part involved solid matrix. For the first situation, the extraction rate is restricted by solubility; meanwhile in the second case, the success of SFE is limited by mass transport (diffusion) properties. The consequence of process temperature are also defined in Figure 3 (Karale, *et al.,* 2011).

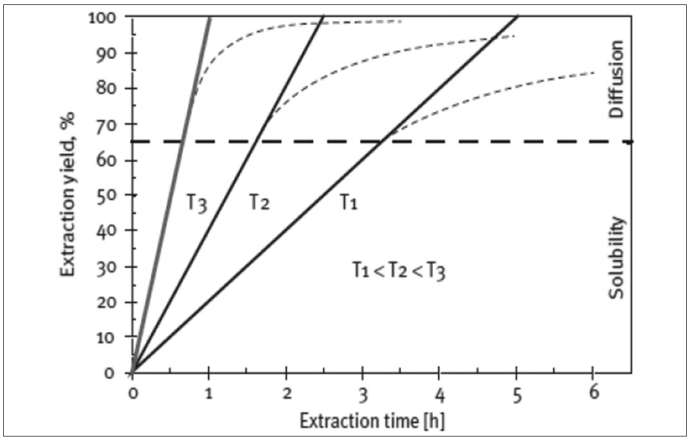

Figure 3: Extraction yield versus time at different temperatures (Karale, et al., 2011)

In view of considering consequence of temperature towards solubility of solid materials, there are two dissimilar effects. First is the improvement in solid volatility with increased temperature, results an increment in vapour pressure and secondly is the decline of density in solvent with higher temperature. The enhancement of solubility by temperature is reliant on which effect is more dominant. Higher process temperature causing a reduction in extraction rate by the reason of fluctuation in the solvent density and resultant diversity in solubility of solvent. However, a greater extraction rate can be accomplished by increasing process temperature in the case of solubility but under the situation that the limiting factor is not supercritical fluid (Karale, *et al.,* 2011).

A report published by Patil, *et al.,* in 2014 was aimed to study the flavonoids extraction process of wedelolactone from Wedelia calendulacea. by employing supercritical carbon dioxide extraction method. Wedelia calendulacea can be utilized as a treatment for numerous ailments. W. calendulacea is thought to own characteristics of main active constituent of flavonoids which is wedelolactone. Wedelolactone exhibits variety biological activities including antitumor, anti-inflammatory, antiosteoporotic, antivenom and hepatoprotective effects. In the report, the effect of extraction temperature on the extraction yield of wedelolactone was examined. As consider to the consequence of temperature on solid compounds solubility, there are relatively two distinctive impacts will be exposed. Firstly is the increase in solid volatility which leading to vapour pressure rise and another is lower solvent density in response to increased temperature. The increase of solubility by temperature is relying on which factor is dominant. In the study, the consequence of temperature on extraction yield at three different values which are 40 °C, 60 °C and 80 °C, was examined to advance the extraction process. From the result, it shows that the yield of wedelolactone was reduced with the increased of extraction temperature from 40 °C to 80 °C and the optimum yield of wedelolactone was collected at the condition of temperature 40 °C. This consequence of temperature may be caused by decrease in density of solvent and thus leads to a reduction solutes solubility in the supercritical fluid.

5.2 Pressure

Generally, the yield of targeted analytes with SC-CO_2 extraction is affected by extraction time, temperature, pressure, temperature and modifier. Elevated pressure can increase the density of fluid. Furthermore, the solubility of solid composites in supercritical fluid possibly will be also affected by the repulsive interaction of solute and fluid. It is proved that the solubility of supercritical CO_2 is influenced by its vapour pressure and density. Under constant temperature, the solutes solubility increases with respect to increment in density of supercritical CO_2 at higher pressures when the solubility of solutes is dominated mainly by density instead of vapour pressure. However, because of the decreased density of supercritical CO_2, the dissolving power will decrease at higher temperature under constant pressure (Al-Hamimi *et al.,* 2016).

The repulsive interaction between solute and fluid becomes stronger as pressure continues to increase. When pressure achieves a particular level for some complexes, the repulsive interaction of solute and fluid turn out to be stronger than the increase in the solubility which resulted from higher solvent density. Under this condition, the compounds solubility declines and hence leads to a reduction in extraction yield. The solubility of solute in supercritical fluid rely upon complicated balance among few aspects including density of fluid, solute vapour pressure and the repulsive interaction of solute and fluid which are limited by pressure and temperature. Normally, the density of fluid increase with elevated process pressure and hence varying the solubility of solute. At high pressure, a yield of volatile fractions and lower yield of non-volatile fractions can be attained. However, insignificant increment in recovery will be observed under higher pressure or flow rate than a certain value of pressure that guarantees high solubility of analyte in supercritical fluid (Barroso *et al.,* 2011). dl-Tetrahydropalmatine (dl-THP) is an alkaloid which frequently appear in plant. It has been intensively investigated due to its medicall properties. From the report that published by Liu, *et al.,* (2008), dl-THP in Corydalis yanhusuo was extracted through supercritical carbon dioxide extraction method. Four factors which are the dynamic duration of extraction, process temperature, pressure and various modifiers used in the extraction were studied. By using ANOVA, the impacts of the process variables on the dl-THP yield were studied.

In the study, three different pressures of SFE which are 200 bar, 300 bar and 400 bar were performed for analysis of the impacts of pressure on dl-THP yield. The mean yield of alkaloids varied from 1.07 mg/g at 200 bar to 1.02 mg/g at 400 bar, and this represents that the dl-THP yield was affected insignificantly by pressure. The difference of yield is about 0.05 mg between 200 bar and 400 bar. It is noticed that the effect of pressure is smaller than temperature on the yield of dl-THP when the preferred modifiers are used. The yield of the compound extracted does not improve with increasing pressure from 200 bar to 400 bar. From the study it showed that every variable showed an effect on the dl-THP yield in the specific limits.

5.3 Duration of Extraction

Extraction time is expressed as one of the dominant causes for exhausted extraction and is a critical index for determination of extraction effectiveness. The influence of extraction time must be considered in order to attain an optimum extraction yield. This is because shorter extraction time might lead to unfinished extraction but longer extraction time possibly will be time and solvent wasting. For SFE, the extraction time is usually less than 2 hours. The total extraction duration is actually counted from two stages which are static extraction and dynamic extraction. A example of the kinetics of the extraction practice at conditions of 40 °C and 90 bar and is shown in Figure 4. In the experiment, a dynamic extraction method was used which the extraction time is started from a duration of static pre-treatment with no CO_2 flow followed by a dynamic time which the flow rate of CO_2 is fixed. To examine the influence of dynamic extraction time on extraction efficiency of the natural sources, other variables must remained constant throughout the experiment. The dynamic extraction employing three different periods which include 20 minutes, 30 minutes and 60 minutes was carried out with 10 minutes of static pre-treatment. Figure 4 indicates that longer dynamic extraction time would improve the final extraction yield. However, the dependence between recovery and extraction duration gradually becomes flat. Hence a dynamic extraction time of 30 minutes was selection in following extractions. From the study, it can be concluded that the optimum time for experiment was 10 minutes for static extraction and 30 minutes for dynamic extraction (Herzi *et al.,* 2013).

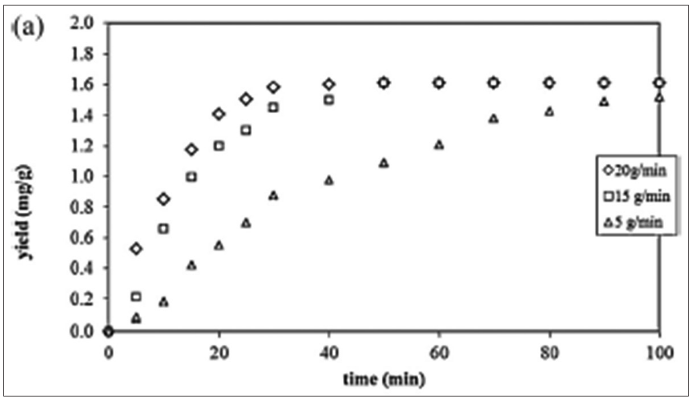

Figure 4: Extraction yield at different solvent flow rates as a function of the extraction time (Herzi et al., 2013)

5.4 Existence of Modifiers

The union between economic feasibility and safety are acquiring more consideration and indeed, safer and less harmful solvents that are easy to remove or recover, are gaining in popularity. More than 90% of supercritical fluid extractions have been performed with carbon dioxide as supercritical solvent because of the abovementioned practical reasons. Supercritical carbon dioxide (SC-CO_2) has a polarity comparable to liquid pentane and therefore, it is compatible for the solubilization of lipophilic compounds such as lipids and essential oils. However, this low polarity index makes SC-CO_2 hardly suitable for the extraction of polar compounds. To overcome this restriction, practical approaches involve the use of polar co-solvents (Capuzzo *et al.,* 2013).

Another typical exercise in SFE is to modify the supercritical fluid polarity and enhance their solvating power towards targeted analytes by using polar modifiers. For instance, the addition of comparatively low percentages of methanol which in the range from 1 % to 10 %, to carbon dioxide enlarges its extraction array to comprise analytes of high polarity. Based on the nature of solid matrix and the attractive forces of the targeted analyte, the modifier may effects the efficiency of extraction by few methods. Firstly, it increases the solubility of analytes in the supercritical fluid as a consequence of interactions between analyte and modifier in the fluid stage. Secondly, modifier can be used to promote desorption of analytes. The particles of polar modifiers allows interactions with the sample and compete with analyte for the active sites in the solid matrix. Lastly, it distorts the diffusion between solid matrix and analyte, thus further diffusion of solvent into the matrix by swelling (Casas *et al.,* 2007).

The effect of modifiers can be observed from the review published by Liu *et al.,* (2008). In the experiment, different types of modifiers including ethanol, methanol and 1,2-propanediol were employed to examine the influence of modifiers on final extraction yield of dl-THP. It can be seen in Figure 5 that the extraction yield is affected by different modifiers. The extraction yield fluctuates from 0.990 mg/g to 1.088 mg/g when 1,2-propanediol was used instead of ethanol. The extraction yield collected was highest when employing 1,2-propanediol as modifier with supercritical CO_2.

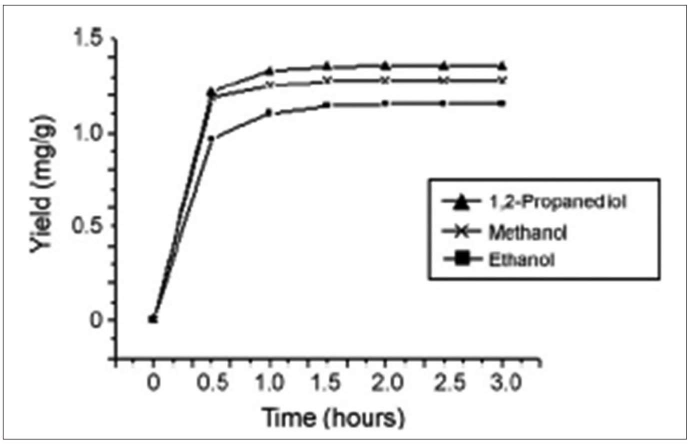

Figure 5: The cumulative yield of dl-THP using different modifiers (Liu et al., 2008)

5.5 Flow Rate of Solvent

Figure 6 was created to indicate the influence of solvent flow rate as a process variable. It represents that extraction yield increases with greater dioxide flow rate of supercritical carbon until it reaches the highest point and then declines with further increase in the flow rate. The outcomes are described as a trade-off between a diffusion process and a thermodynamic equilibrium condition. The interface gas phase concentration of the solute is a function of the mass transfer coefficient or flow rate of solvent, whereas the equilibrium state is always preferred by both high rate of diffusion and long residence time. At lower solvent flow rates, the resistance of mass transfer restricts the quantity of solute transfered into the bulk solvent and then supercritical carbon dioxide discharges from extractor unsaturated. By way of higher flow rate, resistance of mass transfer declines continuously till the leaving solvent is saturated and the equilibrium to be reached and hereafter the optimum extraction yield is achieved (Kumoro and Hasan, 2007).

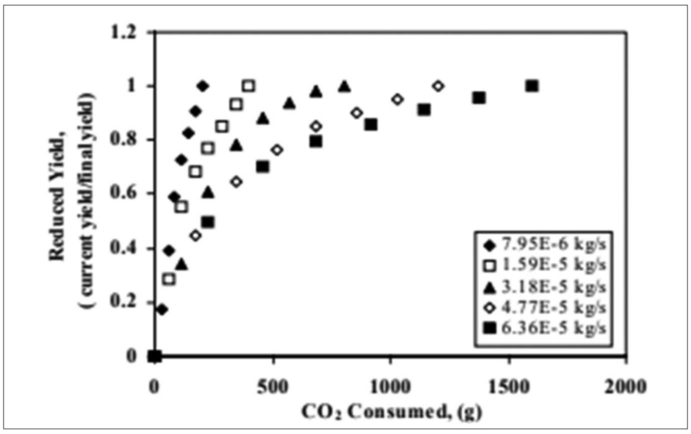

Figure 6: Effect of solvent flow rate on extraction yield as a function of CO_2 consumed (Kumoro and Hasan, 2007)

However, further increase of the solvent flow rate will decrease the residence time affecting the system to diverge from equilibrium and the solvent discharges from extractor unsaturated regardless of the high rate of mass transfer. This is caused by the quantity of solvent which is excess enters to the cellular structure of the samples, just bypassed the extractable samples (Saldana *et al.,* 2002). This performance showed the existence of a mass transfer resistance which is probably intra particle diffusion or an external film resistance. Hence in a semi batch supercritical fluid extraction, the optimal flow rate of solvent needs to be evaluated a priori. However, it should be noticed that the situation of optimal solvent flow rate be governed by the nature of the solvent – solute system, the geometry of the extractor and process temperature and pressure (Kumoro and Hasan, 2007).

In the work of Kumoro and Hasan (2007), a study to examine the consequence of flow rate of solvent towards the extraction rate was conducted and a few experiments was carried out at conditions of 313 K and 10 MPa, whereas the flow rate of supercritical CO_2 was adjusted from 7.95×10^{-6} kg/s to 6.36×10^{-5} kg/s. From Figure 6, it presented that the extraction yield improved with increment of solvent flow rate from 7.95×10^{-6} kg/s to 3.18×10^{-5} kg/s. However further increase of solvent flow rate limited the extraction yield. Consequently, an optimal flow rate of 3.18×10^{-5} kg/s was achieved for the system in experiment.

6.0 ADVANTAGES OF SUPERCRITICAL FLUID EXTRACTION

The advantages of using supercritical fluids for the extraction of bioactive compounds can be understood considering following points (Lang and wai, 2001):

i. 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 favourable mass transfer.

ii. Extraction time can be reduced substantially by SFE in compared with conventional methods.
iii. The repeated reflux of supercritical fluid to the sample provides complete extraction.
iv. The selectivity of supercritical fluid is higher than liquid solvent as its solvation power can be tunable.
v. The separation of solute from solvent can easily be bypassed by depressurization of supercritical fluid, which will save time.
vi. SFE is operated at room temperature so it is an ideal method for thermo labile compound extraction.
vii. Small amount of sample can be extracted compared with solvent extraction methods which will save time.
viii. SFE uses little amount of organic solvent and considered as environment friendly.
ix. The recycling and reuse of supercritical fluid is possible and thus minimizing waste generation.
x. SFE scale can be arranged on specific purpose from few milligram samples in laboratory to tons of sample in industries.

7.0 APPLICATIONS OF SUPERCRITICAL FLUID EXTRACTION

Among the different extraction techniques used at analytical and preparative scale, supercritical fluid extraction (SFE) is one of the most used. From a simple literature search, it can be easily deduced the impact of SFE as sample preparation technique for the analysis of target compounds from natural products and foods. It is worth to mention that SFE has been also widely used in this field for process development, which is to extract target (bioactive or valuable) compounds from different matrices. Even though these processes usually offer clear advantages over traditional ones, the main drawback for industrial scale use is the lack of realistic economic studies. In this context, some papers have been published lately dealing with the assessment of the industrial economical feasibility of some developed processes, such as essential oil extraction from rosemary, fennel and anise and brewery spent grain management (C.G. Pereira, *et. Al.,* 2007). Therefore, SFE can be regarded as a possible tool not only from a laboratory point of view but also for the natural products and food industries.

Probably, the most extended use of SFE is in the food field. A high variety of samples, type of materials, target compounds and procedures have been published in the last years. A relatively new group of applications that have been recently developed which includes the extraction and fractionation of carbohydrates by SFE. The SFE technique is using supercritical CO_2 as the best option for this group of compounds due to the low polarity of carbon dioxide. SFE has been also used to remove unwanted compounds from other matrices. For example, supercritical CO_2 with water as co-solvent has been employed to selectively extract caffeine from green tea while avoiding the extraction of antioxidants from the matrix.

SFE has been widely used to value food industry by-products. These products are generated during food manufacturing and normally do not have any commercial value. By-products extraction allows the removal of valuable or interesting compounds that otherwise cannot be utilized. Industrial activities generate a large variety of byproducts and wastes ranging from manure to packing residuals. Strong research is focused in the development of new technologies and new uses for these materials in order to reduce their environmental impact. New processes are being developed to recover components producing high added value products. Mainly SFE has been tested for the extraction of lipids and lipophilic components (Herrero *et al.,* 2010).

Proprietary pharmaceutical product development is driven by continuous innovations in drug discovery, drug polymorph preparation, dosage form design and process engineering while meeting rigorous regulatory standards. Innovations in all these areas are feasible with the application of technologies utilizing supercritical fluids. Pharmaceutical companies are more and more urged to develop production processes with very low environmental impact, in particular to reduce the use of volatile organic compounds in medicine manufacturing as well as to avoid residues in the finished product. Other benefits of supercritical fluid technologies, apart from their mass transfer related properties, are linked to the reduced complexity of the process which stems from a diminution of the number of steps as well as to the improved process understanding and control. In general terms, the main use of supercritical fluids in pharmaceutics deals with the extraction of bioactive compounds from a mixture (purification from reactions, quantification of active enantiomer, extraction from natural matrices) or with the extraction of the matrix. In this case, crystallization and particle formation have undergone an enormous development in recent years (Herrero *et al.,* 2010).

The interest of SFE not only as an analytical tool but also for process development is discussed. It seems clear that SFE has an enormous interests nowadays and the of applications of SFE, including not only its use as sample preparation technique but also new and recent advances in different areas such as food science, pharmaceutical and environmental science.

8.0 SUMMARY AND FUTURE WORKS

It is necessary for the scientist and chemist to establish a simpler and a more efficient extraction method for the extraction of chemical components from different parts of the plants since the advanced invention of medicinal drugs and its application in the treatment of various diseases is getting more developed. The supercritical fluids like CO_2 under elevated pressure is able to provide more well-organized, efficient and appropriate technique for extraction of natural sources for coming future without losing the capability and activity of a medical drugs. The increasing demand of extracts for bioactive compounds of plants promotes continuous study for convenient extraction methods. Nonetheless, most of these techniques rely on different mechanisms and extraction improvement is developed from various processes. Therefore, comprehension about every aspect of extraction process is very important. Incorporation of hybrid methods should also be examined in consideration of plant material properties and selection of compounds. However, there are still deficient of complete experimental data in certain existing extraction methods. Appropriate selection of methods also give significant effects on the analysis of extraction effectiveness.

From another point of view, the increasing commercial significance of bioactive compounds and commodities full in these bioactive compounds may contribute to discover more advanced extraction techniques in future. Proper calculation of production costs which includes both capital and operating cost, should be accomplished in order to exploit supercritical fluid extraction technique at the scale of industrial level. In addition, the respective effects of the various process parameters which can influence the efficiency of SFE and also amount of extraction yield of targeted analytes is discussed in this project. SFE efficiencies are not only affected by experimental process parameters such as temperature, pressure (density), extraction time and modifier used but also by sample variables, extraction vessel variables and collection variables. However, a single standardized procedure is not applicable for all types of bioactive compounds from plant. Therefore, the procedures must be optimized case by case depending on the property of target analytes and nature of solid sample.

In conclusion, supercritical fluid technology has provides important advances for the extraction of bioactive compounds from natural sources compared to conventional extraction methods. Even if this technique is struggling with the limitation of cost–effectiveness factors in the event of low volume products, it is overcoming this drawback day-to-day and promising commercial benefits. Based on this study, it can be concluded that supercritical fluid extraction is recognized as one the favourable method for the extraction of the bioactive compounds from plant materials. ■

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ABBREVIATION

CO_2	Carbon dioxide
dl-THP	dl-Tetrahydropalmatine
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
GC	Gas chromatography
LC	Liquid chromatography
LLE	Liquid–liquid extraction
MAE	Microwave-assisted extraction
MAPs	Medicinal and aromatic plants
N_2O	Nitrous oxide
P	Pressure
PC	Critical pressure
Pr	Reduced pressure
PEF	Pulsed electric field assisted extraction
PLE	Pressurized liquid extraction
SC- CO_2	Supercritical carbon dioxide
SFE	Supercritical fluid extraction
SFs	Supercritical fluids
SLE	Solid–liquid extraction
SPE	Solid-phase extraction
T	Temperature
TC	Critical temperature
Tr	Reduced temperature
UAE	Ultrasound-assisted extraction
WHO	World Health Organization

PROFILES

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