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

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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.

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 interested 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 monograms 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