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Mini Review

Importance of HPLC in Analysis of Plants Extracts

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Abstract

When large numbers of investigating plants secondary metabolites from plants, it is important to have the means available to perform a characterization of the samples in order to provide a means of selecting species for study. This can be achieved by combining simple biological assays with High-Performance Liquid Chromatography (HPLC) analyses. HPLC is an extremely versatile technique; the reversed-phase method is able to handle compounds of a diverse polarity and molecular mass. Reversed phase chromatography has found both analytical and preparative applications in the area of phytochemical separation and purification. Once a candidate plant has been chosen, a suitable isolation procedure can be employed for the isolation of the active principles.

Keywords: Bioactive compound; Plant extraction; HPLC

Introduction

Plants are a valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeutically, natural products still remain as one of the best reservoirs of new structural types. The standardized extracts of plants, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [1]. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [2,3]. Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethnopharmacognosy [4]. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, anti-inflammatory, analgesic and wound healing activity were reported. In many cases the people claim the good benefit of certain natural or herbal products [5-7]. However, clinical trials are necessary to demonstrate the effectiveness of a bioactive compound to verify this traditional claim [8,9].

Although there are several hundred thousand plant species around the globe, only a small proportion has been investigated both phytochemically and pharmacologically. When one considers that a single plant may contain up to thousands of constituents, the possibilities of making new discoveries become evident [10]. The crucial factor for the ultimate success of an investigation into bioactive plant constituents is thus the selection of plant material. In view of the large number of plant species potentially available for study, it is essential to have efficient systems available for the rapid chemical and biological screening of the plant extracts selected for investigation.

Identification and characterization

Due to the fact that plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities, their separation still remains as big challenge for the process of identification and characterization of them. It is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as Thin-Layer Chromatography (TLC), column chromatography, flash chromatography, Sephardim chromatography and High-Performance Liquid Chromatography (HPLC), should be used to obtain pure compounds [11].

High performance liquid chromatography

HPLC is a versatile, robust, and widely used technique for the isolation of natural products, HPLC is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture [12,13]. Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants [14]. Natural products are frequently isolated following the evaluation of a relatively crude extract in a biological assay in order to fully characterize its properties. The resolving power of HPLC is ideally suited to the rapid processing of such multi component samples on both an analytical and preparative scale [11]. Several authors describe the use of HPLC for characterization and quantification of secondary metabolites in plant extracts, mainly phenol compounds, steroids, flavonoids, alkaloids [2,5-8].

Reversed-phase chromatography is the most commonly used separation technique in HPLC due to its broad application range. It is estimated that over 65% of all HPLC separations are carried out in the reversed phase mode. The reasons for this include the simplicity, versatility, and scope of the reversed-phase method as it is able to handle compounds of a diverse polarity and molecular mass [15,16], for example, to identify secondary plant metabolites [3,6,7]. In addition, the colloquial term used for the mobile phases in reversed phase chromatography is "buffer". However, there is little buffering capacity in the mobile phase solutions since they usually contain strong acids at low pH with large concentrations of organic solvents. Adequate buffering capacity should be maintained when working closer to physiological conditions [16].

In order to identify any compound by HPLC, a detector must

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first be selected. Once the detector is selected and is set to optimal detection settings, a separation assay must be developed. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed. UV detectors are popular among all the detectors because they offer high sensitivity [17] and also because majority of naturally occurring compounds encountered have some UV absorbance [12], Phenolics are frequently identified using UV-VIS and Photodiode Array (PDA) detectors at wavelengths 190-380 nm [18]. The high sensitivity of UV detection is bonus if a compound of interest is only present in small amounts within the sample. Besides UV, other detection methods are also being employed to detect phytochemicals among which is the Diode Array Detector (DAD) coupled with Mass Spectrometer (MS) [19]. Liquid Chromatography coupled with Mass Spectrometry (LC/MS) is also a powerful technique for the analysis of complex botanical extracts. It provides abundant information for structural elucidation of the compounds when tandem Mass Spectrometry (MS) is applied. Therefore, the combination of HPLC and MS facilitates rapid and accurate identification of chemical compounds in medicinal herbs, especially when a pure standard is unavailable [20].

The processing of a crude source material to provide a sample suitable for HPLC analysis as well as the choice of solvent for sample reconstitution can have a significant bearing on the overall success of natural product isolation [5]. The source material, e.g., dried powdered plant, will initially need to be treated in such a way as to ensure that the compound of interest is efficiently liberated into solution. In the case of dried plant material, an organic solvent (e.g., methanol, chloroform) may be used as the initial extracting and following a period of maceration, solid material is then removed by decanting off the extract by filtration [10,17]. The filtrate is then concentrated and injected into HPLC for separation. The usage of guard columns is necessary in the analysis of crude extract. Many natural product materials contain significant level of strongly binding components, such as chlorophyll and other endogenous materials that may in the long term compromise the performance of analytical columns. Therefore, the guard columns will significantly protect the lifespan of the analytical columns [21,22].

Conclusion

This review article showed some important parameters to analyze bioactive compounds occurring in plant material, since they consist of multi-component mixtures, and their separation determination still creates problems. HPLC is a versatile, reproducible chromatographic technique for the estimation of secondary metabolites in the plants. It has wide applications in different fields in term of isolation, quantitative and qualitative estimation of active molecules. In addition, this review has presented an overview of advanced extraction techniques to isolate and purify of compounds from plant-based sources, primarily by HPLC technique.

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