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ANALYTICAL TECHNIQUES IN QUALITY EVALUATION OF HERBAL DRUGS

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ABSTRACT

Different chromatographic and electrophoretic techniques commonly used for the quality evaluation of herbal medicines (HM) are comprehensively reviewed. Chemical fingerprints obtained by chromatographic technique and especially by hyphenated chromatographies, are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the "chemical integrities" of the herbal medicines and therefore be used for authentication and identification of the herbal products. Based on the conception of phyto equivalence, the chromatographic fingerprints of herbal medicines could be utilized for addressing the problem of quality control of herbal medicines. Several novel chemo metric methods for evaluating the fingerprints of herbal products, such as the method based on information theory, similarity estimation, chemical pattern recognition, spectral correlative chromatogram (SCC), multivariate resolution, etc.

Key words: Hyphenated chromatography, Thin layer chromatography, Chemical fingerprints, Herbal drugs.

INTRODUCTION

Plant derived products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics and are available in health food shops and pharmacies over the counter as or also as drugs prescribed in the non-allopathic systems. Herbal medicines widely used in health-care in both developed and developing countries are complex chemical mixtures prepared from plants and are limited in their effectiveness because they are poorly absorbed when taken orally. According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs. Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough remedies, memory enhancers and adopt gens. As per WHO definition, there are three kinds of herbal medicines: raw plant material, processed plant material and medicinal herbal products. Herbal drugs are finished labeled products that contain active ingredients such as aerial or underground parts of plant or other plant material or combination thereof, whether in the crude state or as plant preparations. The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies. Herbal medicine products are dietary supplements that people take to improve their health and are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants. Herbals are traditionally considered harmless and increasingly being consumed by people without prescription. However, some can cause health problems, not effective and may interact with other drugs.

Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. Quality evaluation of herbal preparation is a fundamental requirement of industry and other organization dealing with Ayurvedic and herbal products [1].

Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important.

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Scientifically validated and technologically standardized herbal medicines may be derived using a safe path of reverse pharmacology approach based on traditional knowledge database. This may play a vital role in drug discovery, development and therapeutics.

According to regulatory guidelines and pharmacopoeias macroscopic and microscopic evaluation and chemical profiling of the botanical materials is used for quality control and standardization. Thin layer chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) are valuable tools for qualitative determination of small amounts of impurities. Also many analytical techniques such as Volumetric Analysis, Gravimetric Determinations, and Gas Chromatography (GC), Column Chromatography (CC), High Performance Liquid Chromatography (HPLC) and Spectrophotometric methods are also frequently used for quality control and standardization [2]

Hence every single herb needs to be quality checked to ascertain that it confirms to quality requirement and delivers the properties consistently. Standardization assures that products are reliable in terms of quality, efficacy, performance and safety [3].

Analytical evaluation technique in Herbal Drugs

In general, quality control is based on three important Pharmacopoeias definitions:

Identity: Is the herb the one it should be?

Purity: Are there contaminants, e.g., in the form of other herbs which should not be there?

Content or assay: Is the content of active constituents within the defined limits.

It is obvious that the content is the most difficult one to assess, since in most herbal drugs the active constituents are unknown. Sometimes markers can be used which are, by definition, chemically defined constituents that are of interest for control purposes, independent of whether they have any therapeutic activity or not. To prove identity and purity, criteria such as type of preparation sensory properties, physical constants, adulteration, contaminants, moisture, ash content and solvent residues have to be checked. The correct identity of the crude herbal material, or the botanical quality, is of prime importance in establishing the quality control of herbal drugs [4]

Identity can be achieved by macro- and microscopically examinations. Voucher specimens are reliable reference sources. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification.

Purity is closely linked with the safe use of drugs and deals with factors such as ash values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation includes microbial contamination, aflatoxins, radioactivity, and pesticide residues. Analytical methods such as photometric analysis (UV, IR, MS, and NMR), thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations [5].

Chromatography and chemical fingerprints of herbal medicines

Chromatography is defined as technique of isolation and identification of components or compounds or mixture of it's into individual components by using stationary phase and mobile phase.

Strict guidelines have to be followed for the successful production of a quality herbal drug. Among them are proper botanical identification, photochemical screening, and standardization. Quality control and the standardization of herbal medicines involve several steps. The source and quality of raw materials, good agricultural practices and manufacturing processes are certainly essential steps for the quality control of herbal medicines and play a pivotal role in guaranteeing the quality and stability of herbal preparations [6-12].

The chemical constituents herbs in the herbal products may vary depending on stage of collection, parts of the plant collected, harvest seasons, plant origins (regional status), drying processes and other factors. Thus, it seems to be necessary to determine most of the phytochemical constituents of herbal products in order to ensure the reliability and repeatability of pharmacological and clinical research, to understand their bioactivities and possible side effects of active compounds and to enhance product quality control. Thus, several chromatographic techniques, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) and thin layer chromatography (TLC), can be applied as quality assessment parameters. The concept of phyto equivalence was developed in Germany in order to ensure consistency of herbal products. According to this concept, a chemical profile, such as a chromatographic fingerprint, for an herbal product should be constructed and compared with the profile of a clinically proven reference product.

By definition, a chromatographic fingerprint of an herbal drug is, in practice, a chromatographic pattern of the extract of some common chemical components of pharmacologically active and/or chemically characteristics. This chromatographic profile should be featured by the fundamental attributions of "integrity" and "fuzziness" or "sameness" and "differences" so as to chemically represent the herbal drug investigated. It is suggested that with the help of chromatographic fingerprints obtained, the authentication and identification of herbal medicines can be accurately conducted ("integrity") even if the amount and/or concentration of the chemically characteristic constituents are not exactly the same for different samples of drug (hence, "fuzziness") or, the chromatographic fingerprints could demonstrate both the "sameness" and "differences" between various samples successfully. Thus,

we should globally consider multiple constituents in the herbal drug extracts, and not individually consider only one and/or two marker components for evaluating the quality of the herbal products. However, in any herbal drug and its extract, there are hundreds of unknown components and many of them are in low amount. Moreover, there usually exists variability within the same herbal materials. Consequently, to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components is not an easy or trivial work. Fortunately, chromatography offers very powerful separation ability, such that the complex chemical components in herbal extracts can be separated into many relatively simple sub-fractions [13-16].

Thin layer chromatogram

TLC was the most common, versatile method of choice for herbal analysis before instrumental chromatography methods like GC and HPLC were established. Even nowadays, TLC is still frequently used for the analysis of herbal medicines since various pharmacopoeias such as Indian pharmacopoeia, Indian Herbal Pharmacopoeia, Ayurvedic pharmacopoeia; American Herbal Pharmacopoeia (AHP), British Pharmacopoeia, United State Pharmacopoeia, European Pharmacopoeia, Chinese drug monographs and analysis, Pharmacopoeia of the People's Republic of China, etc. Rather, TLC is used as an easier method of initial screening with a semi quantitative evaluation together with other chromatographic techniques as there is relatively less change in the simple TLC separation of herbal medicines than with instrumental chromatography. Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent [17,18].

Identification can be effected by observation of spots of identical Rf value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

HPTLC is one of the sophisticated instrumental techniques based on the full capabilities of TLC. It is most flexible, reliable and cost efficient separation technique. The advantage of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hypenation, and so on enable it to be powerful analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples and food stuffs. The advantages of using TLC/HPTLC to construct the fingerprints of herbal medicines are its simplicity, versatility, high velocity, specific sensitivity and simple sample preparation. Thus, TLC is a convenient method of determining the quality and possible adulteration of herbal products. It is worth noting that the new techniques of TLC are also being updated like forced-flow planar chromatography (FFPC), rotation planar chromatography (RPC), over pressured-layer chromatography (OPLC), and electro planar chromatography (EPC). A simple, but powerful preparative forced-flow technique was also reported; in this technique hydrostatic pressure is used to increase mobile-phase velocity. Parallel and serially-coupled layers open up new vistas for the analysis of a large number of samples (up to 216) for high throughput screening and for the analysis of very complex matrices [19-22].

Gas chromatography and volatile components in herbal medicines and GC–MS and herbal medicines

It is well-known that many pharmacologically active components in herbal medicines are volatile chemical compounds. Thus, the analysis of volatile compounds by gas chromatography is very important in the analysis of herbal medicines. The GC analysis of the volatile oils has a number of advantages. Firstly, the GC of the volatile oil gives a reasonable "fingerprint" which can be used to identify the plant. The composition and relative concentration of the organic compounds in the volatile oil are characteristic of the particular plant and the presence of impurities in the volatile oil can be readily detected. Secondly, the extraction of the volatile oil is relatively straightforward and can be standardized and the components can be readily identified using GC–MS analysis. The relative quantities of the components can be used to monitor or assess certain characteristics of the herbal medicines. Changes in composition of the volatile oil may also be used as indicators of oxidation, enzymatic changes or microbial fermentation.

The advantages of GC clearly lie in its high sensitivity of detection for almost all the volatile chemical compounds. This is especially true for the usual FID detection and GC–MS. Furthermore, the high selectivity of capillary columns enables separation of many volatile compounds simultaneously within comparatively short times. Thus, over the past decades, GC is a popular and useful analytical tool in the research field of herbal medicines [23-31]. Especially, with the use of hyphenated GC–MS instrument, reliable information on the identity of the compounds is available as well. However, the most serious disadvantage of GC is that it is not convenient for its analysis of the samples of polar and non-volatile compounds. For this, it is necessary to use tedious sample work-up which may include derivatization. Therefore, the liquid chromatography becomes another necessary tool for us to apply the comprehensive analysis of the herbal medicines.

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Advantages for GC–MS, that is: (1) with the capillary column, GC–MS has in general very good separation ability, which can produce a chemical fingerprint of high quality; (2) with the coupled mass spectroscopy and the corresponding mass spectral database, the qualitative and relatively quantitative composition information of the herb investigated could be provided by GC–MS, which will be extremely useful for the further research for elucidating the relationship between chemical constituents in herbal medicine and its pharmacology in further research. Thus, in our opinion, GC–MS should be the most preferable tool for the analysis of the volatile chemical compounds in herbal medicines [32-46].

High-performance liquid chromatography and HPLC–DAD, HPLC–MS and others

High performance liquid chromatography (HPLC), also known as high pressure liquid chromatography, is essentially a form of column chromatography in which the stationary phase consists of small particle (3-50μm) packing contained in a column with a small bore (2-5mm), one end of which is attached to a source of pressurized liquid eluent (mobile phase). The three forms of high performance liquid chromatography most often used are ion exchange, partition and adsorption. HPLC is a popular method for the analysis of herbal medicines because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. In general, HPLC can be used to analyze almost all the compounds in the herbal medicines. Thus, over the past decades, HPLC has received the most extensive application in the analysis of herbal medicines. Reversed-phase (RP) columns may be the most popular columns used in the analytical separation of herbal medicines.

It is necessary to notice that the optimal separation condition for the HPLC involves many factors, such as the different compositions of the mobile phases, their pH adjustment, pump pressures, etc. Thus, a good experimental design for the optimal separation seems in general necessary.

In order to obtain better separation, some new techniques have been recently developed in research field of liquid chromatography. These are micellar electrokinetic capillary chromatography (MECC), high-speed counter-current chromatography (HSCCC), low-pressure size-exclusion chromatography (SEC), reversed-phase ion-pairing HPLC (RPIPC-HPLC), and strong anion-exchange HPLC (SAX-HPLC). They will provide new opportunities for good separation for some specific extracts of some herbal medicines. On the other hand, the advantages of HPLC lie in its versatility for the analysis of the chemical compounds in herbal medicines, however, the commonly used detector in HPLC, say single wavelength UV detector, seems to be unable to fulfill the task, since lots of chemical compounds in herbal medicines are non-chromophoric compounds. Consequently, a marked increase in the use of HPLC analysis coupled with evaporative light scattering detection (ELSD) in a recent decade demonstrated that ELSD is an excellent detection method for the analysis of non-chromophoric compounds. This new detector provides a possibility for the direct HPLC analysis of many pharmacologically active components in herbal medicines, since the response of ELSD depends only on the size, shape, and number of eluate particles rather than the analysis structure and/or chromospheres of analytes as UV detector do. Especially, this technique is quite suitable for the construction of the fingerprints of the herbal medicines. Moreover, the qualitative analysis or structure elucidation of the chemical components in herbal drug by simple HPLC is not possible, as they rely on the application of techniques using hyphenated HPLC, such as HPLC-IR, HPLC–MS, HPLC-NMR, for the analysis of herbal medicines [47-50].

HPLC–DAD has become a common technique in most analytical laboratories in the world now. With the additional UV spectral information, the qualitative analysis of complex samples in herbal medicines turns out to be much easier than before. For instance, checking peak purity and comparing with the available standard spectrum of the known compound to the one in the investigated sample. Especially, with the introduction of electrospray mass spectrometry, the coupling of liquid chromatography and mass spectrometry has opened the new way to widely and routinely applied to the analysis of herbal medicines. HPLC chromatographic fingerprints can be then applied for documentation of complete herbal extracts with more information and on-line qualitative analysis becomes possible.

In last decades, the increasing usage of LC–MS and HPLC–DAD in the analysis of herbal medicines is quite obvious. Several good reviews have been published for the analysis of the bioactive chemical compounds in plants and herbal medicines, in which the technique used most is HPLC, especially the hyphenated HPLC techniques. Moreover, combined HPLC–DAD–MS techniques take advantage of chromatography as a separation method and both DAD and MS as an identification method. DAD and MS can provide on-line UV and MS information for each individual peak in a chromatogram. With the help of this hyphenation, in most cases, one could identify the chromatographic peaks directly on-line by comparison with literature data or with standard compounds, which made the LC–DAD–MS becomes a powerful approach for the rapid identification of phytochemical constituents in botanical extracts, and it can be used to avoid the time-consuming isolation of all compounds to be identified. Recently, the hyphenation between HPLC and NMR are also available, which might become a vital and an attractive analytical tool for the analysis of herbal medicines. In fact the tendency of the hyphenation or multi-hyphenation of the chromatography with the common used four spectroscopic detectors, says

UV, Fourier transformation infrared spectrum, MS and NMR, for structure elucidation of chemical compounds, is in progress.

DISCUSSION AND CONCLUSION

Western and traditional Chinese medical practices represent totally different philosophies. Thus, this is not a simple exercise of applying modern technologies to quality control of the products that have been in constant use for centuries. The progress on quality control of herbal medicines discussed in this review is just at its beginning stage of a long journey. Of course, the proposal of the use of chromatographic fingerprints of herbal medicines for quality control of herbal medicines is definitely a progress. However, using the chemical fingerprints for the purpose of quality control of herbal medicines can only address to the problem of comparing the integrated sameness and/or difference and controlling their stability of the available herbal products. The complex relationship between the chromatographic fingerprints and efficacy of the herbal medicines (QRFE) is not taken into account yet, which seems to be the most important aspect for the quality control of herbal medicines. As it is well known that the efficacy of traditional herbal medicines has a characteristic of a complex mixture of chemical compounds present in the herbs, thus how to evaluate reasonably their relationship is obviously not a trivial task. THMs represent a much more daunting challenge due to the natural variability of the individual herbs and the chemical complexity of the formulations. Moreover, the chemical profile by itself is insufficient in determining the efficacy of Herbal Medicines. This is where biochemistry, molecular biology, and cell biology are invaluable in establishing quantifiable and reproducible assays. Chemical fingerprints might be linked to these biological assays to provide assurance of efficacy and consistency. But the research work on this aspect, to our best knowledge, is far from sufficient to meet the criteria needed. Thus, the researches concerning the relationship between the chromatographic fingerprints and efficacy of the herbal medicines are urgent requirements for the quality control of herbal medicines. On the other hand, the works on possible contaminations in herbal products, such as excessive or banned pesticides, microbial contaminants, heavy metals, chemical toxins, should be also conducted concurrently. In fact, the research field of quality control of herbal medicines is really an interdisciplinary research. It needs crossover of chemistry, pharmacology, medicine and even statistics to provide a platform for the quality control of traditional herbal medicines and further to discover the novel therapeutics composed of multiple chemical compounds.

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