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Emerging green technologies for the chemical standardization of botanicals and herbal preparations

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ABSTRACT

As botanicals and many medicinal plants can be processed to become a food or a health supplement, a drug or cosmetics, chemical standardization is important for their quality control. Hence, the selection of appropriate extraction technologies and analytical techniques is required to provide a solvent-free solution for the chemical standardization of botanicals and herbal preparations.

Contents

1. Introduction

Botanicals or medicinal plants are known to contain one or many chemical constituents that may have therapeutic purposes. The classes of bioactive compounds present in medicinal plants include alkaloids, flavonoids, terpenes and saponins. Botanicals or medicinal plants can be processed to become a food or health supplement, a drug or cosmetics. Aromatic plants and spices are commonly used as food flavorings or food supplements, or as a source of essential oil. Currently, monographs of medicinal plants can be found in the United States Pharmacopeia (USP) [1], Chinese Pharmacopeia [2], WHO monographs for medicinal plants [3,4], Japanese Pharmacopeia (JP) [5] and others. Approaches for the chemical standardization of botanicals have been covered in a number of previous review papers [6–10]. For certain botanicals,

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a combination of chemical standardization with biological assay has been proposed to characterize the synergistic effect of the different constituents present [6,9].

Due to the complexity of medicinal plants and herbal medicines, holistic approaches with a combination of quality control (QC), elucidation of the properties of absorption, distribution, metabolism and excretion, and metabonomics evaluation of medicinal plants have been proposed [7]. The screening strategies of bioactive compounds by biochromatographic methods have been introduced for certain herbal medicines [10]. Also, there is a need to approach scientific proof and clinical validation with chemical standardization, biological assays, animal models and clinical trials for botanicals. We note that quality assurance of medicinal plants is the prerequisite of credible clinical trials. From a safety point of view, the misidentification or the mislabeling of plant material can lead to significant toxic effects in humans. The use of a wrong plant may result in unintended intoxication. Hence, standardization and effective control measures are required to monitor the quality of the medicinal plants and to exclude any possible contaminants arising from the misidentification of plants that would badly affect consumers of herbal medicine [8,11].

The different steps used for the chemical standardization of botanicals will include:

(1) pretreatment that will involve drying and grinding;
(2) selection of a suitable method of extraction;
(3) analysis of compounds using suitable chromatographic or spectroscopic methods; and,
(4) analysis of data based on bioactive or marker compounds or pattern-recognition tools.

The current methods in various pharmacopeia and other reports may require extensive use of organic solvents and can be time consuming. Moreover, organic solvents are expensive and their disposal is very costly. Hence, the use of green technologies to reduce and/or to eliminate the use or production of hazardous materials is highly desirable. The approaches to adapt the principles of green chemistry for the chemical standardization of botanicals are:

(1) to reduce the use of harsh organic solvents;
(2) to encourage the use of emerging extraction technologies; and,
(3) to use high-efficiency separation techniques with low usage of organic solvents.

Most important of all, the selection of extraction technologies and analytical techniques will largely be based on the inherent properties of compounds present in botanicals (Fig. 1). We note that different methods of extraction may affect the medicinal-plant profile and levels of markers or bioactive compounds obtained. Hence, depending on the physical and chemical properties of the target compounds present in the plant materials, a suitable extraction technology in combination with an analytical technique will be required. Also, a complex matrix may be encountered in herbal extracts for which tedious sample clean-up steps that involve multiple liquid-liquid extraction (LLE) steps may be required. Hence, approaches that can provide a simple solution for the extraction and analysis of target compounds in botanical extracts will be highly desirable.

Currently, we can find extensive discussion on the sample-preparation and extraction technologies for medicinal plants. However, there has been a limited number of review papers on green approaches that eliminate or reduce the use of organic solvents for the chemical standardization of botanicals (solid samples), which combine extraction technologies with analytical techniques. Hence, the focus of the current review paper is to identify emerging green approaches for the appropriate extraction technologies for solid samples and analytical techniques to form an energy-efficient and solvent-free solution for the chemical standardization of botanicals. Also, we discuss comparison of the advantages and the disadvantages of the various extraction technologies for solid samples and analytical techniques.

2. Extraction technologies

The traditional extraction techniques that are commonly used for the chemical standardization of botanicals include Soxhlet extraction, sonication, heating under reflux, blending and solid-liquid extraction. These techniques generally require long extraction times, large amounts of samples and organic solvents that may have potential negative effects on the environment and human health. At the same time, these characteristics mean that sample treatment may become an error-prone part of the method. Hence, there have been proposals to adopt emerging extraction technologies with various potential advantages {e.g., supercritical-fluid extraction (SFE), microwave-assisted extraction (MAE) and pressurized-liquid extraction (PLE) [12,13]}. The theory, the principles and the applications of each of the emerging extraction technologies have been covered in the various review papers cited in this present review.

From Fig. 2, the extraction mechanism in the various methods involves four sequential steps:

(1) first is the desorption of solutes from the active sites in the sample matrix under the operating conditions of the different methods of extraction;
(2) the second may involve the diffusion of extraction fluid into the matrix;
(3) next, depending on the sample matrix, the solutes may partition themselves from the sample matrix into the extraction fluid; and,
(4) finally, the analytes obtained can be analyzed *via* an appropriate analytical technique.

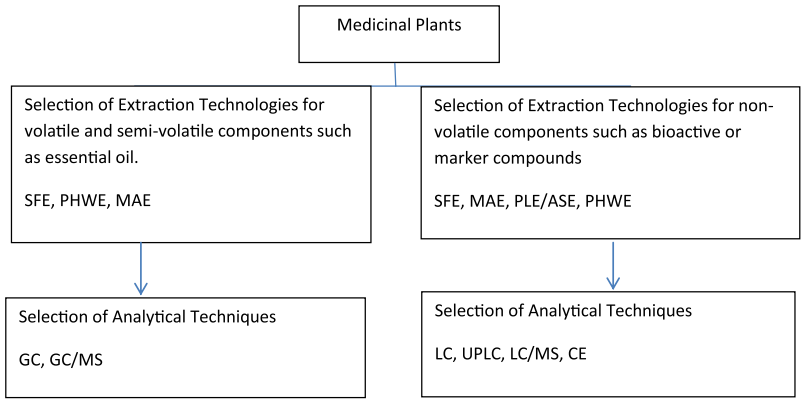

Fig. 1. Method-selection criteria for analysis of botanicals.

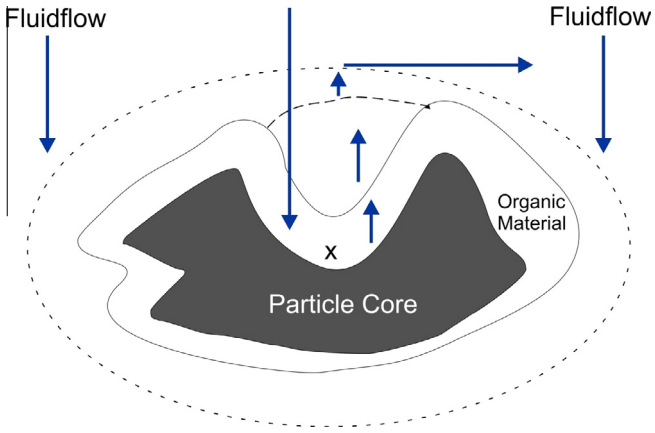

Fig. 2. The extraction mechanism in the methods described. The first step is the desorption of solutes from the various active sites in the sample matrix under the operating conditions of the different methods of extraction. The second step may involve the diffusion of extraction fluid into the matrix. Next, depending on the sample matrix, the solutes may partition themselves from the sample matrix into the extraction fluid.

Table 1 compares the various extraction technologies and their energy consumption. We note that emerging extraction tools have lower energy consumption than traditional methods of extraction (e.g., Soxhlet and heating under reflux).

For the extraction of bioactive or marker compounds in botanicals, one key challenge is that the target compounds are present naturally where significant analyte-matrix interaction is present. Hence, the spiking of target analytes into the plant matrix will not mimic the analyte-matrix interaction present naturally. Depending on the sample matrix, high recovery from spiking experiments may not imply that the method is accurate.

2.1. Ultrasound-assisted pretreatment of solid samples

Ultrasound-assisted pretreatment of solid samples is an emerging technique that is clean and energy efficient. Extraction using ultrasound can be performed on solid samples together with a suitable solvent on a bath sonicator or an ultrasonic probe. The unique conditions provided by acoustic cavitation can enhance solid-sample treatment. The method proposed can be performed at atmospheric pressure and room temperature. In most cases, a certain amount of organic solvent will be required to dissolve the target compounds from the medicinal plant. For ultrasonic extraction with an ultrasonic probe, a smaller volume (1–15 ml) of organic solvent will be needed [14]. Currently, works toward solvent-free approaches with ultrasound-assisted extraction (UAE) are limited. The energy consumption of ultrasound-assisted pretreatment of solid samples is similar to other methods of extraction stated in Table 1. UAE has been used to characterize polyphenolic compounds present in several *Salvia* species. Comparison with other methods of extraction with UAE showed that good method recovery was observed for certain target compounds in several *Salvia* species [15]. However, for the analysis of selected polyhalogenated pollutants in plants, it was observed that the extraction efficiency of UAE was lower than that of other emerging methods [16]. Depending on the medicinal plant-sample matrix, the method recovery or extraction efficiency may be lower than other methods of extraction. Hence, as a result of the complexity of botanical extracts, the extraction efficiency of methods using UAE will need to be thoroughly investigated.

2.2. Supercritical-fluid extraction (SFE)

Supercritical fluids (e.g., carbon dioxide) are substances above the critical pressure and temperature with properties ranging between liquid and gas. The extreme variability of their solvent power with pressure and temperature, and their low viscosity, enabling much faster mass transfer than in liquid, are the most important advantages of SFE [17–19]. One of the key features of SFE with CO_2 is that it is non-flammable, cost effective, easily accessible in its high purity and has no negative impact on the environment and human health. The critical point (Tc = 31.1 °C, Pc = 74 bar) allows for the extraction of thermally-labile solutes at a lower temperatures than conventional methods of extraction. SFE with CO_2 is usually carried out continuously or semi-continu-

Table 1
Comparison of technologies for the extraction of target compounds from botanicals and medicinal plants

Extraction technologies	Advantages	Disadvantages	Energy consumption
Ultrasound-assisted extraction with a bath sonicator Ultrasound-assisted extraction with an ultrasonic probe	Extraction can be performed at atmospheric pressure and room temperature Use of small volume of organic solvent (1–15 mL) for ultrasound-assisted extraction with an ultrasonic probe Very safe to use	Depending on the plant matrix, the extraction efficiency may need to be investigated thoroughly	Moderate
SFE	Green extraction technology with CO_2 as extractant Process can be scaled up for industrial production Suitable for thermally-labile substances Very safe to use	High cost for the high-pressure equipment needed May be difficult to extract polar components	Moderate
MAE	High throughput with the commercially-available system Suitable for thermally-labile substances Organic solvents and water can be used as an extractant	Amount of sample is to the volume of extractant used will be important A challenge to scale up MAE Potential explosion risks as a result of pressurization with closed vessel	Moderate
PLE/ASE	High throughput with the commercially-available system for ASE (laboratory scale) Suitable for thermally-labile substances Reduction in usage of organic solvent Very safe to use	High cost for the high-pressure equipment needed No commercial or high-throughput system for PLE in the dynamic mode	Moderate Moderately lower for PLE at room temperature
PHWE	Green extraction technology using water as extractant Suitable for thermally-labile substances Process can be scaled up for industrial production Ability to perform extraction at lower operating pressure Very safe to use	High cost for the high-pressure equipment needed for operation at higher pressure No commercial or high-throughput system for PHWE	Moderate

ously. SFE can be operated at an analytical scale or a plant scale. Botanicals will be placed into an extraction vessel and the supercritical fluid will be fed to the extractor by a high-pressure pump at a fixed flow rate. Finally, the substances extracted by SFE can be precipitated by temperature and/or pressure changes or by applying a mass-separating agent. Also, SFE equipment has been proposed to allow solvent regeneration and recirculation. For the fractionation of the extracts, SFE equipment can be operated with several separators in series at different pressures and temperatures. The main drawback of SFE, compared with traditional methods of extraction, is the high cost of the high-pressure equipment needed [17].

The various conditions that may affect the extraction efficiency of SFE are the fluid (e.g., CO_2), operating temperature (40–90 °C) and pressure (100–400 bar). The other important factors in SFE include particle size and shape of the plant material, moisture of the solid materials and solvent flow-rate. To increase the extraction efficiency of more polar substances from plant materials, a small amount of modifiers (e.g., methanol, ethanol and water) may be added.

Currently, a number of botanicals have been used as sources of bioactive compounds using SFE. Based on the operating conditions used, we note that the extracts or the active fractions are obtained with specific characteristics. The extracts obtained by SFE maintain the bioactivity of extracts obtained by traditional extraction methods, because SFE promotes a selective extraction and results in an extract enriched in the desirable compounds. The final result is that the SFE extract may be free of organic solvents and without loss of compounds due to degradation. SFE is very suitable for non-polar components. However, for certain classes of compounds that include the more polar constituents in medicinal plants, a lower yield or lower extraction efficiency than traditional methods may be observed.

Currently, SFE has been applied for the extraction of essential oils, phenolic compounds, carotenoids, tocopherols, and tocotrienols [17–22]. Response-surface methodology was applied to optimize the supercritical carbon-dioxide extraction of essential oil from *Cyperus rotundus* Linn. It was noted that the yield of *Cyperus rotundus* Linn by SFE was significantly higher than Soxhlet extraction with n-hexane [20]. Similarly, SFE was used to extract non-polar components (e.g., fatty acids from *Borago officinalis* L. flower) and it was observed that SFE was more effective than the conventional hydrodistillation method in extracting fatty acids and preserving its quality [21]. Despite the weaknesses stated, to eliminate the usage of organic solvents, SFE without any additives will remain the method of choice for the extraction of non-polar components in medicinal plants.

2.3. Microwave-assisted extraction (MAE)

MAE is one of the emerging techniques that have been widely employed for the extraction of bioactive and marker compounds from medicinal plants. Microwaves are non-ionizing electromagnetic waves that comprise an electric field and a magnetic field oscillating perpendicularly to each other in a frequency range (0.3–300 GHz). In addition, microwaves penetrate into certain materials and interact with the polar components to generate the heat needed for extraction. The heating of microwave energy acts directly on the compounds by ionic conduction and dipole rotation. These result in only selective, targeted materials that can be heated based on their dielectric constant. The efficiency of the microwave heating depends on the dissipation factor of the material, which measures the ability of the sample to absorb microwave energy and dissipate heat to the surrounding molecules. MAE has attracted significant attention in the analysis of medicinal plants due to its special heating mechanism, moderate capital cost, high-throughput capability and good performance under atmospheric conditions [12,23,24].

In general, MAE can be classified as closed or open vessel. For MAE with closed vessel, the extractions are carried out in a sealed vessel with different modes of microwave radiation. The uniform microwave heating with the high working pressure and temperature of the system allows fast, efficient extraction of bioactive compounds in botanicals. The pressure inside the closed extraction vessel is controlled in such a way that it would not exceed the working pressure of the vessel and the temperature can be regulated above the normal boiling point of the extraction solvent.

To counter the shortcomings of closed systems (e.g., safety issues) and to extract thermally-labile compounds, open vessel MAE was developed. For MAE with open vessel, more solvent can be added at a suitable point during the extraction process and the system has higher throughput. In addition, the upper part of the vessel is connected to a reflux unit to condense any volatile solvent [23–25].

The factors that may affect the efficiency of MAE are power and frequency of the microwaves, duration of the microwave radiation, moisture content of the botanical sample, concentration of solvent, ratio of solid to liquid, extraction temperature, extraction pressure and maybe the number of extraction cycles. Solvent and temperature are the most important parameters for MAE that affect the solubility of the bioactive or marker compounds. MAE can be applied to extract thermally-labile components (e.g., gastrodin in *Gastrodia elata* and stevioside and rebaudioside A from *Stevia rebaudiana* Bertoni) through optimization of the applied temperature [26,27]. On the whole, the choice of solvent takes into account not only its affinity with the target compound but also its ability to absorb microwave energy [22–24].

MAE has been widely reported as a good, reliable method in preparing samples for medicinal plants. We note that the extraction yield of MAE is higher and the extraction time needed is shorter than traditional methods of extraction [23]. With the availability of commercial MAE equipment, MAE has been widely used to extract a wide variety of compounds (e.g., flavonoids, and saponins from botanicals) [23–27]. Although organic solvents (e.g., methanol or ethanol, with or without the addition of water) are commonly used, we have reported that MAE with water shows higher extraction efficiency than heating under reflux with water for gastrodin in *Gastrodia elata* Blume [26] and stevioside and rebaudioside A from *Stevia rebaudiana* Bertoni [27].

Also, ionic liquids (ILs) are gaining wide recognition as novel, environment-friendly solvents in chemistry. Due to their excellent solvent properties (e.g., negligible vapor pressure, wide liquid range, good thermal stability, tunable viscosity, miscibility with water and organic solvents, good solubility and extractability for various organic compounds, room-temperature ILs are gaining attention as extracting solvents. MAE with ILs has been proposed as an alternative to conventional organic-solvent extraction for the extraction of components from medicinal plants [23,25,28]. Compared with water and common organic solvents (e.g., methanol and ethanol), the availability of selected ILs for the extraction of target compounds in medicinal plants may present a challenge. Also, the environmental impact of the selected ILs may need to be thoroughly evaluated.

Despite of the newer instrumental design of MAE (e.g., nitrogen-protected MAE and dynamic MAE), MAE with closed or open vessel systems will remain the preferred mode for the extraction of target compounds in botanicals. Lastly, to reduce reliance on organic solvents, it is clear that MAE with an environment-friendly extractant (e.g., water, aqueous surfactants and ILs) is an emerging trend for the extraction of botanicals.

Table 2
Comparison of static and dynamic PLE (for which the plant material is typically dispersed in a drying or inert sorbent (e.g., sodium sulfate diatomaceous earth, sand or others). The mixture of inert sorbent and plant sample is packed in a stainless-steel cell and inserted in a closed flow-through system.

Static PLE	Dynamic PLE
The extraction is performed in a static mode in the extraction cell for a predetermined time	The extraction solvent is continuously pumped through the extraction cell
The extraction process consists of one or several extraction cycles with replacement of the solvent between cycles in the static mode	The operation involves the flow rate set during the static time and the pump delivers the solvent at a constant flow rate for a certain time (e.g., 1.0–1.5 mL/min for 20–30 min)
The sample cell is purged with an inert gas to wash off the solvent from the cell and the tubing into the collection vial at the end of the last extraction cycle to avoid any loss or memory effects	No inert gas is needed
35–2008 bar can be applied	A lower pressure (10–50 bar) may be applied

2.4. Pressurized-liquid extraction(PLE)/accelerated-solvent extraction (ASE)

PLE/ASE was first introduced in 1995 at the Pittcon Conference by Dionex Corporation and it is also known as pressurized solvent extraction and enhanced solvent extraction. The technique is referred to as pressurized hot-water extraction (PHWE), subcritical water extraction (SWE) or superheated water extraction when water is used as the extractant.

PLE involves extraction of target compounds from medicinal plants using solvents at elevated temperature and pressure. The elevated temperature with pressure enhances the method performance compared to traditional methods of extraction carried out near to room temperature and atmospheric pressure. The advantages of using organic solvents at temperatures above their atmospheric boiling point are enhanced solubility and mass transfer. At the same time, methods using PLE significantly reduce the usage of organic solvent during the extraction process. For PLE, the pressure applied will increase the boiling point of the solvent used and allows the extraction to be carried out at temperature above the boiling point of the solvent [21,29].

For extraction by PLE, depending on the water content, the plant material is typically dispersed in a drying or inert sorbent (e.g., sodium sulfate, diatomaceous earth or others). The mixture of inert sorbent and plant sample is packed in a stainless-steel cell and inserted in a closed flow-through system. There are two main set-ups for PLE, namely static and dynamic instruments. For PLE in the dynamic mode, the extraction solvent is continuously pumped through the extraction cell. The operation involves the flow rate set during the static time and the pump delivers the solvent at a constant flow rate for a certain time (e.g., 1.0–1.5 mL/min for 20–30 min). Currently, there is no commercial dynamic PLE system available in the market.

By contrast, for PLE in static mode, once the set parameters of the extraction temperature and pressure are reached, the extraction is performed for a predetermined time. A common range is 5–15 min that is done in different cycles. Compared to PLE in dynamic mode, the extraction process comprises one or several extraction cycles with replacement of the solvent between cycles in the static mode. The sample cell is purged with an inert gas to wash off the solvent from the cell and the tubing into the collection vial at the end of the last extraction cycle to avoid any loss or memory effects. A wide range of extraction temperatures from room temperature to 200 °C and the applied pressure range of 35–200 bar can be applied for PLE.

One drawback of using pressurized-fluid technologies is that the higher applied pressure requires expensive equipment. However, we demonstrated that a laboratory-made dynamic PLE system at a lower applied pressure of 10–20 bar could be successfully applied to extract bioactive compounds in medicinal plants [26,27]. It was observed that the effect of pressure on the recovery or extraction efficiency of most substances in medicinal plants is usually negligible. Depending on the configuration of the extraction cell used, the volume of solvent required for dynamic PLE is comparable with PLE using static mode. Lastly, Table 2 compares static and dynamic PLE.

The main factors that will affect the extraction efficiency of PLE include the nature of the solvent used, applied temperature and number of cycles for static mode or time of extraction for the dynamic mode [29]. Optimization of the applied temperature for PLE is of key importance for the extraction of thermally labile compounds from botanicals. Based on various reports, PLE has been successfully applied for the extraction of phenolic compounds, alkaloids, lignans, carotenoids and others from botanicals [29,30]. The extraction efficiency of bioactive compounds by PLE is comparable with traditional methods of extraction [29,30]. For the extraction of thermally labile compounds, such as gastrodin and vanillyl alcohol in *Gastrodia elata* Blume, PLE at room temperature with a laboratory-assembled system was applied and the extraction efficiencies of the target compounds were found to be comparable with heating under reflux. For certain medicinal plants, PLE at room temperature was found to be rapid and highly energy efficient as heating was not required [31]. For the extraction of target compounds in certain botanicals where organic solvents are required, the significant reductions in time for sample preparation and organic solvents make it an attractive option.

2.5. Pressurized hot-water extraction (PHWE)

To eliminate the use of organic solvents, PHWE is a feasible option for the extraction of target compounds in food and herbal plants. The same equipment as PLE in the dynamic mode can be used for PHWE. However, a commercially-available ASE system was also applied for the extraction of ginsenosides in ginseng using water as the extractant [32]. Although a higher applied pressure at 50 bar was proposed in the earlier works for SWE [33], we observed that applied pressure of 10–20 bar for PHWE will give a method recovery comparable with traditional methods of extraction [26,27]. Hence, PHWE can be carried out using a simpler instrumental set-up for operation at lower applied pressure.

For PHWE, the plant sample needs to be dispersed with a certain quantity of sand or other inert material. This additional step is required as plant materials have a higher tendency to adsorb water during the course of extraction. Hence, the ground plant materials must be dispensed evenly with sand to prevent any potential blockage of the system.

By contrast, this step will not be required for PLE using an organic solvent (e.g., methanol) [26,27,33–35]. Hence, the development of methods where plant samples need not be dispensed by sand or other inert materials will be very desirable.

The parameters that may affect the extraction efficiency in PHWE include the applied temperature, extraction time and addition of a small percentage of organic solvents or surfactants [34,35]. Similar to PLE, optimization of the applied temperature will be critical for the extraction of thermally-labile compounds

in botanicals. Also, the higher applied temperature in PHWE can result in the degradation of the bioactive or marker compounds in medicinal plants [26,27]. We noted that PHWE without the addition of any additives showed extraction efficiency higher than heating under reflux with water for gastrodin in *Gastrodia elata* Blume [26] and stevioside and rebaudioside A from *Stevia rebaudiana* Bertoni [27]. For medicinal-plant extracts obtained with PHWE, where evaporation of water was needed, we noted that, by removing a small volume of the water required with a rotary evaporator and quantitatively transferring it to a suitable volumetric flask (50 mL), the drying process was found to be rapid and energy efficient [26,27].

For certain active ingredients (e.g., ginsenosides) that are hydrophobic in the respective medicinal plants, it was observed that the addition of additives [e.g., non-ionic surfactants (Triton X-100)] in water would enhance the extraction efficiency of PHWE [32]. In addition, the extraction efficiency of the PLE method at room temperature using surfactants (e.g., sodium dodecyl sulfate (SDS) and Triton X-100) in water is comparable with sonication using organic solvent for glycyrrhizin in *Radix glycyrrhizae* and ephedrine in *Ephedra sinica* [36]. One main disadvantage of using surfactant-assisted PLE arises from the difficulties encountered in the evaporation of medicinal plant extracts obtained with certain surfactants (e.g., SDS). However, by adjusting the sample size and time required for extraction in PHWE, the evaporation step using a rotary evaporator for medicinal plant extracts can be eliminated [36]. Surfactants from natural raw materials that possess good biodegradability and low toxicity are in increasing demand. Major classes of biosurfactants include glycolipids, lipoproteins, phospholipids and fatty acids and other complex biopolymers [37]. Hence, biosurfactants may provide a solvent-free solution and enhance the solubility of target compounds from medicinal plants using PLE and PHWE at a lower applied temperature.

At the same time, certain compounds (e.g., cytisine, sophocarpine, matrine, sophoridine and oxymatrine in *Sophora flavescens Ait.*) were extracted using SWE based on a commercially-available ASE system [38]. For certain compounds (e.g., curcumin in turmeric rhizomes, which has with limited solubility in water), the pH of the water was adjusted to enhance the extraction efficiency using PHWE [39].

Finally, the design and the scale-up of a pressurized fluid extractor for use with SFE, PLE and PHWE in food and bio-products were proposed [40]. Based on our earlier works [26,27], we note that a simpler version of equipment that operates at a lower pressure can be used for PLE and PHWE on the laboratory and industrial scales. Most important of all, the introduction of surfactants in the aqueous medium, adjustment of the pH of the extractant, lower applied pressure and extraction at a lower temperature for thermally-labile compounds will be the method of choice when using PHWE. Also, there is growing trend to use water as the extractant with the commercially-available ASE system [32,38].

3. Analytical techniques for the chemical standardization of botanicals

Analytical techniques [e.g., gas chromatography (GC), GC mass spectrometry (GC/MS), liquid chromatography (LC), LC/MS, and capillary electrophoresis (CE)] are commonly used to characterize the bioactive or marker compounds present in single medicinal plant and herbal preparations [6,7,41,42]. Comparison of the key characteristics of the various analytical techniques stated and energy consumption is presented in Table 3. It is critical that the botanical extracts obtained from various extraction technologies need to be compatible with the analytical techniques selected. Hence, the high salt content from aqueous medium with surfactants added for PHWE will not be suitable for LC/MS without a desalting step. Also, any additional drying steps for the medicinal plant extracts will be tedious and lead to higher energy consumption.

For the identification of medicinal plants, thin-layer chromatography (TLC) is considered a low-cost, simple, versatile and specific method. However, certain constituents present at a low level in the botanical extracts may present a challenge for methods using TLC. LC with UV detection is the most common method used for the analysis of target compounds in medicinal plants found in the various pharmacopeia, where the determination of marker or bioactive compounds is required [1,2,5]. In addition to various chromatographic methods, spectroscopic methods [e.g., Fourier-transform infrared (FTIR), near-infrared (NIR) and nuclear magnetic resonance (NMR) spectroscopy] are proposed for the QC of medicinal plants [7].

3.1. GC and GC/MS

GC and GC/MS are commonly-accepted methods for the analysis of volatile and semi-volatile components (e.g., essential oil) present in botanical extracts. GC using high-resolution capillary columns is noted for its high efficiency, selectivity and stability. Methods using GC and GC/MS are known to give good limit of detection, linear range and repeatability. Also, the chromatographic profile obtained provides a unique chemical fingerprint of the medicinal plant. Coupling with MS provides reliable information for the qualitative analysis of the multiple components present in botanical extracts. Recently, new sample-preparation methods for GC and GC/MS [e.g., solid-phase micro-extraction (SPME)] coupled with PHWE and MAE have been developed. Essential oil from *Fructus Amomi* was extracted using PHWE followed by concentration by SPME with final analysis by GC/MS [43]. Compared to traditional methods (e.g., steam distillation), these newer approaches (e.g., combination of PHWE with SPME) are simple, rapid, require lower sample size and may eliminate the use of organic solvent [7,10,41,42]. As the target compounds (e.g., alkaloids, flavonoids, terpenes, and saponins) often encountered in botanical extracts can be polar and thermally labile, it remains a challenge to obtain the required information using GC and GC/MS, even with any form of derivatization.

For the qualitative analysis of target compounds in botanical extracts, it is proposed that retention time and mass spectra of reference standards are compared with those obtained from samples. In addition, searches in mass spectral libraries available can provide tentative identification of unknown peaks in the chromatograms obtained. Although single or multiple internal standards are recommended for the quantitative analysis of target compounds in botanical extracts by GC or GC/MS, it was observed that acceptable repeatability of a method can be achieved without including internal standards [43]. Other than the analysis of volatile components (e.g., essential oil), methods using GC and GC/MS are very suitable for the detection of semi-volatile adulterants or undeclared compounds (e.g., paracetamol, and chlorpheniramine) that may be added to herbal medicine. Finally, to obtain further information on volatile, semi-volatile components in complex mixtures and primary metabolites in medicinal plants, comprehensive two-dimensional GC (GC $\times$ GC) is an important emerging technique [7,10].

3.2. LC and LC/MS

Among the analytical techniques for the analysis of thermally-labile bioactive or marker compounds in medicinal plants, reversed-phased chromatography (RP-C18), based on hydrophobic interaction of target compounds with a non-polar stationary phase, remains the most popular, due to its ease of operation and wide suitability for qualitative and quantitative analysis of herbal plants.

Table 3
Comparison of analytical techniques for the chemical standardization of botanicals and medicinal plants

Analytical techniques	Advantages	Disadvantages	Energy consumption
GC, GC/MS	Ability to analyze target compounds in complex matrix Confirmation of identity of unknown compounds with mass spectrometry library Minimal organic solvents required	Not suitable for polar and thermal labile components such as alkaloids, flavonoids, terpenes, saponins and others with or without derivatization	Moderately higher as a result of heating required for temperature programming
LC, LC/MS	Most common method with high method repeatability Convenient to be connected with MS for the analysis of target compounds in complex matrix Reduction of the usage of organic solvent with columns of smaller internal diameter Small amount of analytes are needed Ability to analyze target compounds in complex matrix such as herbal preparation using LC/MS with minimal sample preparation	Higher consumption of organic solvent when operated with LC columns of larger internal diameter Overlapping peaks observed for the analysis of target compounds in complex matrix using LC with UV detection	Moderate Energy consumption will be lower for methods using short column packed with smaller particle size
UPLC, UPLC/MS	Large decrease in the time for analysis and organic solvent usage Possibility of obtaining high efficiency separation Easy method transfer from LC	Back pressure increased observed Dedicated instrumentation may be required	Moderate Energy consumption will be lower for methods using short gradient runs or run time
CE, CE/MS (CZE and ERC)	Simplest and most versatile mode Possibility of obtaining high efficiency separation Easy to optimize, cheap and robust Highly suitable for the analysis of chiral components Total elimination of organic solvent for certain methods Ability to analyze target compounds in complex matrix such as herbal preparation with minimal sample preparation	Not suitable for neutral compounds Injection repeatability is not as precise as compared to LC with UV detection Lower sensitivity as compared to LC	Moderate

Also, many detectors (e.g., UV, DAD and evaporative light scattering detection (ELSD)] can be coupled with LC [7,9–11,41]. The coupling of LC with DAD will provide the UV spectra other than the retention time for the respective peaks that will be useful for the identification of unknown peaks in the botanical extracts. Similar to GC and GC/MS, most methods using LC are also known to give good limit of detection, linear range and repeatability. However, for the analysis of target compounds in herbal preparations that contain multiple medicinal plants, methods using LC without an additional sample-pretreatment step (e.g., tedious LLE) may present a challenge for qualitative and quantitative analysis of the target compounds.

The presence of constituents from other medicinal plants in herbal preparations will often give rise to overlapping peaks for the target compounds in the chromatograms obtained [44]. Depending on the medicinal plants, the problems may pose certain difficulties for the positive identification of unknown peaks in the botanical extracts. At the same time, LLE will require a large volume of hazardous organic solvents that are immiscible with water.

One disadvantage for methods using LC with conventional columns at 150 × 4.6 mm id packed with 5 μm particles (flow-rate 1.0–1.5 mL/min) is that a large volume of organic solvent will be required for high-throughput analysis. Hence, it is very desirable that green chromatography seeks to reduce and to eliminate organic solvents that are possible pollutants at source to protect human health.

For LC, certain approaches (e.g., reducing the internal diameter of LC columns, reducing particle size, operating at elevated temperature and switching to benign solvents and additives) are proposed [45]. To reduce the usage of organic solvents for the LC run, LC columns of 2.1 mm id and 3.9 mm id with a lower flow-rate of 0.2–0.6 mL/min have been proposed. In addition, the introduction of ultra-performance (UPLC) has shortened the analysis time, which will result in better separation. The use of relatively short columns (50 × 4.6 mm id), packed with sub-2 μm particles will provide high-speed separations while maintaining or increasing resolution. However, dedicated instruments (e.g., ultrahigh-pressure pump systems) have been proposed to overcome the high-pressure drop generated by such a sub-2 μm packing [46]. The other main concern for UPLC is a high back pressure, which can result in system blockage. However, we note that LC systems with binary pumps can be adapted quickly to perform separation of natural products using shorter LC columns with sub-2 μm and 3.5 μm packings. Through proper optimization of the gradient program, run time and oven temperature for the LC column, high-efficiency separation of natural products can be achieved for columns with smaller particle size on selected conventional LC systems or systems for UPLC. However, depending on the mobile phase and LC columns selected, we have noted that operating at elevated oven temperature will shorten the lifetime of the silica-based LC column. Also, methods using multi-dimensional liquid-separation systems (LC × LC) have shown powerful separation ability, high peak capacity and excellent detectability compared to single-dimension HPLC for the analysis of various components in medicinal plants [7,10].

The coupling of LC with MS has opened up new approaches for the qualitative and the quantitative analysis of bioactive and marker compounds in medicinal plants and herbal preparations. The modes of ionization in LC/MS that are most commonly used include electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). The choice on the mode of ionization will depend on the chemical properties of the target compounds in the botanical extracts. The other important component for MS instrumentation is the mass analyzer used {e.g., single quadruple, triple quadruple, ion-trap, time-of-flight, quadrupole time-of-flight (Q-TOF), and Orbitrap} [47]. With tandem MS, additional structural information can be obtained for the target compounds. These in-

clude using ion-trap to obtain structural information with MS^2 and MS^3 for senkirkine and senecionine in *Tussilago farfara* [44]. However, methods using LC/MS are still limited to conditions suitable for MS operations. There are restrictions on pH, solvent choice, choice of volatile salts that can be added into the mobile phase and flow-rate for LC in order to achieve optimal separation and sensitivity for MS detection [6,47].

The main advantage of using LC/MS in full-scan or selected reaction monitoring (SRM) mode is the ability to detect target compounds in complex mixtures with high sensitivity and the method developed is often without the need of an additional sample clean-up step (e.g., LLE). We noted that overlapping peaks for the target compounds are often observed in herbal preparations that contain multiple medicinal plants by LC with UV detection. However, using LC/MS, aristolochic acids I and II in herbal preparations can be detected with minimal sample pre-treatment [48]. For the analysis of senkirkine and senecionine in *Tussilago farfara* [44], it was demonstrated with standard-addition experiments that a significant matrix effect was not observed in the absence of an additional sample clean-up procedure for complex mixtures. The presence of overlapping peak, as detected by LC/UV for the target compound in *Tussilago farfara*, did not result in any significant matrix-induced interference in LC/MS. Although more expensive equipment (e.g., LC/MS) is needed for the methods proposed, we note that LC/MS will reduce the time needed for sample preparation and eliminate the use of organic solvent for sample pre-treatment. It has been noted that LC/MS^2 or LC/MS^3 can detect and determine the presence of the target compounds in the presence of overlapping peaks and complex matrices without the need for multi-dimensional liquid-separation systems [43,48].

The other main advantage for methods using LC/MS is that acceptable method repeatability was observed for the determination of target compounds present at low levels in botanical extracts using external standard calibration [44]. However, it has been observed that LC coupled with UV detection gives better injection repeatability than LC/MS. A wider linear range, compared to LC/MS, was observed for methods using LC coupled with UV or DAD.

Currently, the sort of comprehensive mass spectral library for GC/MS has not been found in LC/MS. Hence, identification of unknown compounds through library searches remains a challenge for methods using LC/MS. In addition, accurate-mass measurement using LC/TOF/MS and LC-Orbitrap/MS can provide tentative identification of unknown peaks [49,50]. However, a decision will be needed if the retention time, mass spectra of reference standard and other experiments are required for confirmation of the identity of the unknown peaks.

Direct analysis in real time (DART)-MS is a solvent-free method that relies upon desorption of condensed-phase analytes using a stream of hot gas (e.g., helium or nitrogen). The gas carrying active species derives from a plasma discharge flowing from the ion source onto a sample surface and is responsible for desorption and ionization of analyte molecules from the sample surface. For the rapid quality assessment of *Radix Aconiti*, DART/MS with multivariate data analysis was proposed [51]. However, the quantitative analysis of bioactive and marker compounds in medicinal plants with DART/MS will depend on the matrix and the usage of stable isotopes [52].

Based on the pharmacopeia and other works, LC and LC/MS remain techniques of choice for the analysis of target compounds present in medicinal-plant extracts. Depending on the analytes in the botanical extracts, the complete elimination of organic solvents for methods using LC and LC/MS will remain a major challenge. However, the adaptation of LC columns with smaller internal diameter and short columns (50×2.0 mm id) packed with sub-2 μm and 3.5 μm particles will significantly reduce the analysis time and the usage of organic solvents [45]. At the same time, concern will remain about the cost of the equipment and technical challenges in the development of methods for usage of the columns stated above. We note that adaption of smaller internal diameter LC columns stated above and coupling of LC with MS will propel the move towards green technology for the analysis of bioactive and marker compounds in medicinal plants.

3.3. Capillary electrophoresis (CE)

CE is a class of electro-migration techniques that use small-diameter capillaries to achieve high-efficiency separation for both large and small ions and molecules. For the separation of naturally-occurring charged and neutral species, different separation modes are applied {e.g., capillary-zone electrophoresis (CZE), electrokinetic chromatography (EKC) and capillary electro-chromatography (CEC) [53–55]}. The majority of commercially-available CE instruments use UV or DAD because of their simplicity, convenience and availability. The simplest mode of CE techniques is CZE and the target compounds are separated inside a narrow-bore capillary containing a buffer solution. In response to an applied voltage that creates an electric field across the capillary, the separation of the natural products will be based on the differences in electrophoretic mobility of ionic species in the buffer contained in the capillary. The separation mechanism is mainly based on differences in solute size and charge at a given pH [53–55]. For an uncoated fused-silica capillary, the electroosmotic flow is usually significant with most commonly-used buffers. Hence, with proper operating conditions, it is possible to separate both cations and anions in the same run for CZE [53]. The main factors that are known to affect separation in CZE include pH of running buffer, ionic strength, and applied voltage [55,56].

To address the separation of uncharged analytes in CE, EKC was introduced. EKC is commonly performed using charged surfactants [e.g., sodium dodecyl sulfate (SDS)], which are added to the buffer to form dynamic micelles to affect transport and separation of target compounds. The micelles added function as the stationary phase, termed pseudo-stationary phase (PSP) as it is not stationary but migrating. At the same time, the aqueous buffer solution serves as the mobile phase. Concurrently, the uncharged target compounds can be taken into the hydrophobic core of the micelles. Hence, separation is based on the differences in partitioning of the target compounds in the micellar PSP and aqueous buffer. For EKC, the selectivity of charged species can also be modified as the charged micelles added also provide ionic interaction [53,56]. Hence, optimization of the amount of additives (e.g., SDS) is required for EKC in addition to what is stated for CZE.

Permanently-charged molecules (e.g., anthocyanins, alkaloids and flavonoids) in botanicals are ideal candidates for CZE. However, crude plant extracts that contain acidic, basic and neutral compounds (e.g., alkaloids, catechins and terpenes) are suitable for analysis by EKC [53–58]. For traditional Chinese medicines (e.g., *Portulaca oleracea L*), certain techniques [e.g., field enhancement sample stacking (FESS) and CE separation] have been developed to analyze organic acids [56]. The key advantage of the CE method is its high-efficiency separation, which can be achieved for most target compounds with optimized operating conditions. Due to the unique separation mechanism in CZE, we note that it is possible to analyze target compounds (e.g., aristolochic acids) in complex mixtures (e.g., herbal preparations) without the need for an additional clean-up step (e.g., LLE). This would be impossible if the analysis was carried out using reversed-phase LC, where overlapping peaks for the target compounds are observed [58].

It has been demonstrated that methods with CE provide a simplified approach for the determination of marker compounds in complex mixtures. However, run failures for CZE could be observed for botanical extracts obtained from 100% organic solvent in our laboratory. Also, CZE and EKC can be carried out with aqueous buf-

fer solutions. Depending on the separation required, a small amount of organic solvent may be added. Hence, methods with CE will eliminate or significantly reduce the use of organic solvents. However, even with the inclusion of an internal standard, it remains a challenge to achieve sensitivity and injection repeatability for CE methods that are comparable to analysis by LC. Although FESS and field-amplified sample stacking can enhance the sensitivity of the method [57], injection repeatability will continue to remain the weakness compared to LC.

By controlling and overcoming the various problems stated above, CE with aqueous running buffer presents an attractive future trend for green analytical chemistry for the QC of target compounds in medicinal plants.

Lastly, there is an emerging trend of using CE with contactless conductivity detection, which is universal for CE in that all charged species based of electrophoretic separation can be quantified. At the same time, it is particularly attractive for those inorganic and organic ions that are not directly accessible by optical means [59]. Despite these advantages, there are limited applications of contactless conductivity detection with CE for the analysis of target compounds in medicinal-plant extracts.

4. Conclusions

Green approaches for the chemical standardization of botanicals are required to make therapeutic compounds and materials available to mankind, while minimizing harm to the environment. Based on environmental assessment tools (EATs) for the assessment of the impact on health, safety and environmental impact of LC methods, it was noted that reductions in the usage of organic solvents for preparing samples and analysis run time of the LC would lower the environmental impact of the method [60]. At the same time, life-cycle assessment (LCA), which involves evaluating the impact of raw materials acquisition, materials manufacture, production, use, reuse and maintenance, and waste management, can be used to assess the analytical methods proposed [61].

For the chemical standardization of botanicals, total elimination of organic solvent can be achieved with SFE, MAE and PHWE. However, the chemical standardization of botanicals will not be complete if a suitable analytical technique is not selected. Also, it is critical that the botanical extracts be compatible with the analytical techniques used. Any additional steps for the drying of extracts and sample pre-treatment to remove potential interfering components before analysis by the selected analytical technique are not desirable. Currently, conventional methods using LC with columns of smaller internal diameters and smaller particle size will reduce the usage of organic solvent and propel the move towards green chemistry. Furthermore, LC/MS and CE can provide an excellent solution for the analysis of target compounds in the presence of overlapping peaks in botanical extracts and herbal preparation without the need for additional sample clean-up. To move ahead, analytical techniques (e.g., DART with MS) and CE have the potential to be solvent-free approaches. Hence, even without the use of EAT and LCA, it is clear that solvent-free approaches for the chemical standardization of botanicals will reduce the impact of the manufacturing process and waste management compared to methods that have a significant requirement for organic solvents [45,59]. Nevertheless, there will always remain great scope of further research on green approaches for the chemical standardization of medicinal plants.

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