

Recent analytical techniques in quality control of indigenous system of medicine

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Abstract

Indigenous System of Medicine is gaining more and more attention towards its use all over the world, due to fewer side effects and less cost. Quality control of these medicines is the prominent part of this system of medicine for modernization and globalization. There are various analytical techniques available presently and screening of bioactive markers and compounds having therapeutic effects. Many hyphenated techniques are used for the quantitative determination of active principle in the drugs and also evaluating the therapeutic efficacy of the compounds, used against various diseases which prone to every layman. The comprehensive methods, such as fingerprint and multicomponent quantification are emphasized; generating the fingerprint profile is very much essential now-a-days. Hyphenated techniques like HPLC-MS, LC-MS-MS, GC-MS, CE-MS, LC-NMR, chemometric methods, and combination of chemical and biological methods such as biofingerprint, metabolic fingerprint are now more and more widely used. Therefore, the analysis and quality control of herbal medicines towards an integrative and comprehensive direction is needed, in order to better address the inherent holistic nature of drugs in traditional medicines.

Key words: Indigenous System of Medicine, Herbal drugs, Quality control, Hyphenated techniques.

Introduction

India has a rich heritage of traditional medicine and the traditional healthcare system have been flourishing for many centuries. Traditional medicine, defined by the WHO as “medical knowledge systems that developed over generations within various societies before the era of modern medicine, including the health practices, approaches, knowledge and beliefs, incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being” is used globally and has rapidly growing economic importance (Yadav *et al.*, 2012).

The knowledge of medicinal plants has been accumulated in the course of many centuries, based on different Indian Systems of Medicines (ISM) such as Ayurveda, Unani and Siddha. In India, it is reported that traditional healers use 2500 plant species and medicine. Herbal medicines which formed the basis of healthcare throughout the world, since the earliest days of mankind, are still widely used, and have considerable importance in international trade. Recognition of their clinical, pharmaceutical and economic value is still growing, although this varies widely between countries (Jayasuriya, 1990).

Medicinal plants constitute a major part in Traditional Systems of Medicine (TSM). Several regulations and controls on the use of medicinal plants in traditional medicine have evolved. On one hand, such regulations will help to cure different ailments through Indian indigenous resources and on the other hand, they will help in the screening and evaluation of natural resources through the development of potential lead components in order to provide better

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healthcare through ISM. Several lead molecules have been developed from ISM.

Herbal medicines have two special characteristics which distinguish them from chemical drugs; use of crude herbs and prolonged usage. A single herb may contain many natural constituents and a combination of herbs even more. Experience has shown that there are real benefits in the long-term use of whole medicinal plants and their extracts, since the constituents in them work in conjunction with each other. However, there is very little research on whole plants because the drug approval process does not accommodate undifferentiated mixtures of natural chemicals, the collective function of which is uncertain. To isolate each active ingredient from each herb, would be immensely time-consuming at unsupportable cost, and is almost impossible in the case of preparations.

Consequently, herbal medicinal products should be controlled by quality control systems, including standardization with marker compounds based upon legitimate science to ensure consistent high quality as well as valid efficacy and safety. There is still a great deal either unknown or misunderstood, regarding the actual active compounds of herbs. Bioavailability is one of the issues often ignored for standardization of herbs, due to the complexity of chemicals, even though it is essential for the utility of active ingredient(s) and is critical for assuring product quality. It is also beneficial for confirming the compliance of the subjects in clinical trials.

This may be the main reason why quality control of oriental herbal drugs is more difficult than that of western drug. As pointed in "General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines (World Health Organization, 2000).

Characteristics of traditional medicines are systematism, multitarget and multichannel and also due to the complex chemical constituents. If only few constituents are emphasized, the holistic nature of traditional medicines will be neglected, which needs to be studied and scientifically understood (Xie and Leung, 2009).

Challenges in traditional system of medicines

International diversity

Traditional medicine practices have been adopted in different cultures and regions, without the parallel advance of international standards and methods for evaluation. Many countries do not have national policies for traditional medicine. Regulating traditional medicine products, practices and practitioners is difficult due to variations in definitions and categorizations of traditional medicine therapies.

Safety, effectiveness and quality

Scientific evidence from laboratory findings to evaluate the safety and effectiveness of traditional medicine products and practices is lacking. While evidence shows some herbal medicines and some manual therapies (*e.g.*, massage) are effective for specific conditions, further study of products and practices is needed. Requirements and methods for research and evaluation are complex.

Knowledge and sustainability

Herbal materials for products are collected from wild plant populations and cultivated medicinal plants. The expanding herbal product market could drive overharvesting of plants and threaten biodiversity. Poorly managed collection and cultivation practices could lead to the extinction of endangered plant species and the destruction of natural resources. Efforts to preserve both plant populations and knowledge on how to use them for medicinal purposes is needed to sustain traditional medicine.

Patient safety and use

Many people believe that herbal medicines are natural or traditionally and they are safe (or carry no risk). However, traditional medicines and practices can cause harmful or adverse reactions if the product or therapy is of poor quality, or it is taken inappropriately or in conjunction with other medicines. Increased patient awareness about safe usage is important, as well as more training, collaboration and communication among providers of traditional and other medicines.

For modernization and global acceptance of herbal medicines, a key issue is the consistency and quality. Especially after the toxicological cases of aristolochic acids (AA) and pyrrolizidine alkaloids (PA), the quality and safety of herbal medicine attract more attention (Xiao and Liu, 2004). In China, traditionally along with identification of TCMs is performed according to its morphology, one or two markers (Rasheed *et al.*, 2012 b) TLC identification and/or content determination. The characteristics of multitarget and synergistic action of TCMs come from their multiple constituents. Thus, a comprehensive method which could reflect the variation of most constituents in the crude drugs is necessary, especially the variation, correlating with pharmacological and clinical efficacy. Thus, in recent years, the analysis of TCMs has begun to emphasize more on the integrative and holistic properties of TCMs (Li *et al.*, 2008).

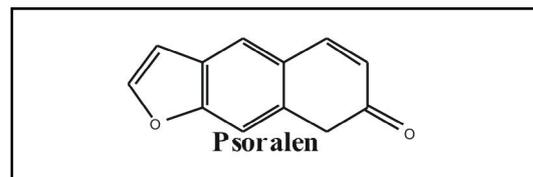
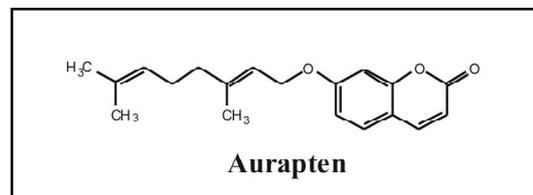
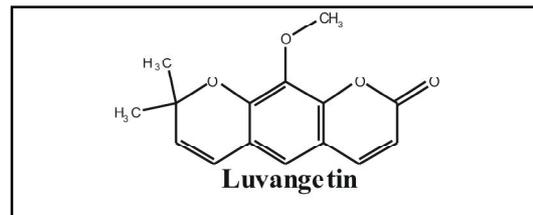
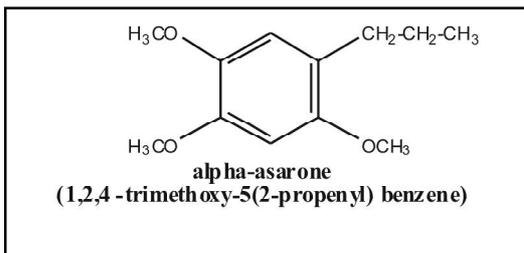
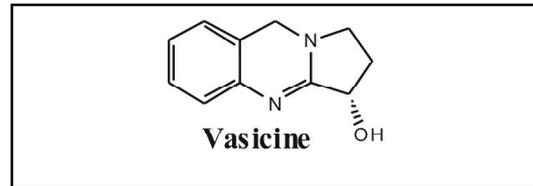
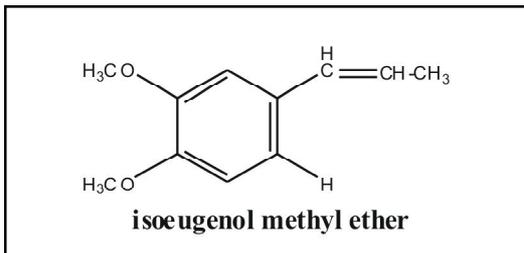
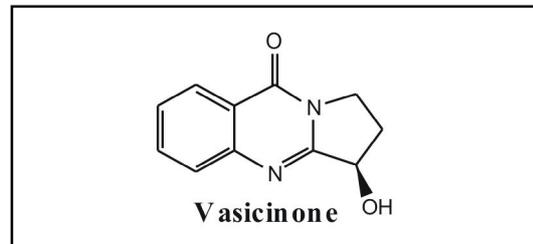
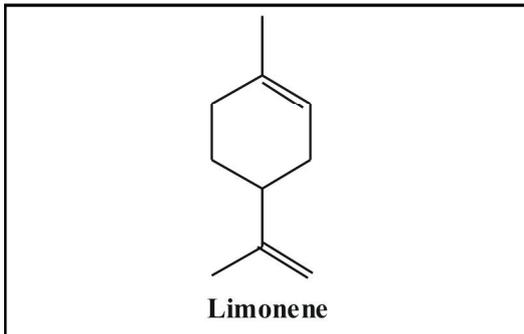
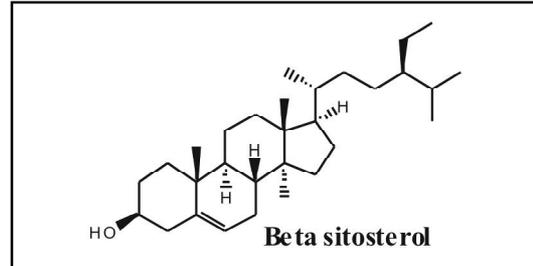
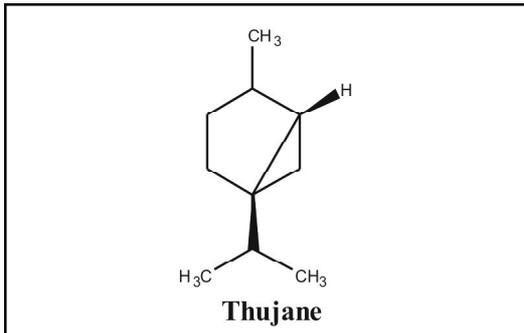
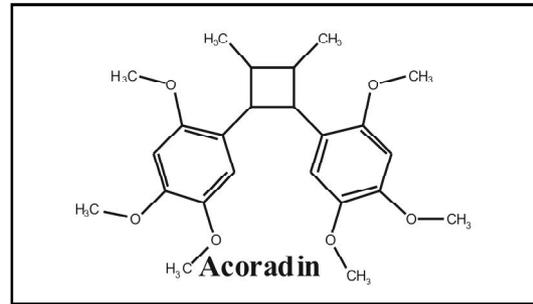
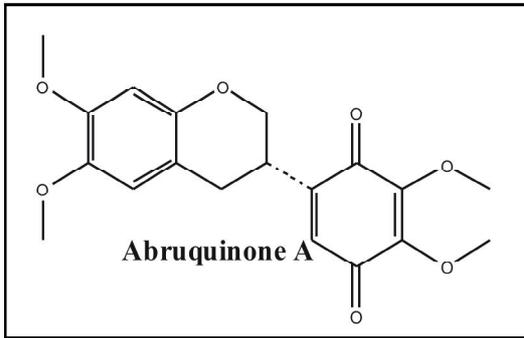
Importance and significance of traditional medicine

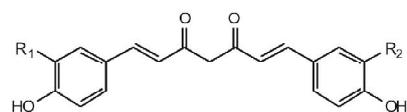
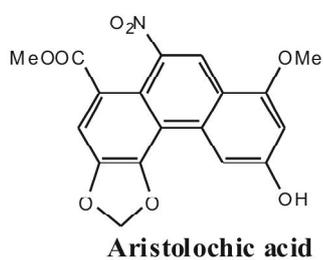
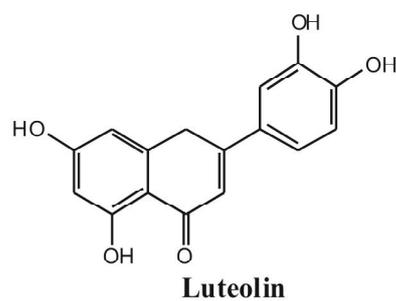
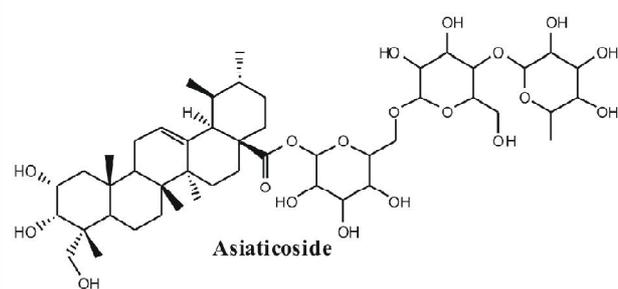
Traditional medicine uses several plant species for treatment particularly in case of skin diseases (Rasheed *et al.*, 2012 c), caused by microbial pathogens (Bacteria, Fungi and Viruses) (Sivaperumal *et al.*, 2009). Skin diseases are a common ailment and affect all ages from the neonate to the elderly and cause harm in number of ways. The physical examination of the skin and its appendages, as well as the mucous membranes, forms the cornerstone of an accurate diagnosis of cutaneous conditions. Skin diseases include several conditions like eczema, leucoderma, ringworm, scabies and many others without distinct symptoms. Modern medicines used in the treatment of skin diseases have side effects. Alternatively, herbal or plant based drugs are considered to be safe for the treatment of skin diseases. Few medicinal plants along with part used and chemical constituents present in them, have been depicted in the table, given below.

Table : Few medicinal plants used in the treatment of skin disorder

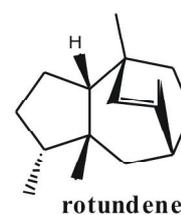
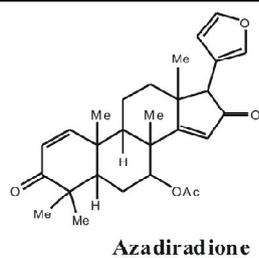
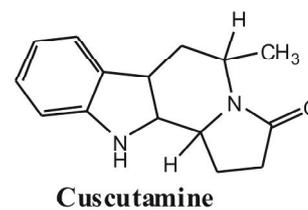
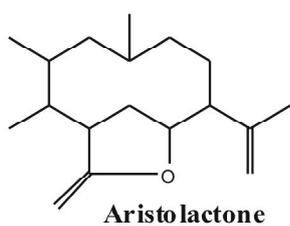
S.No	Botanical name	Part used	Chemical constituents	Method of preparation or uses
1.	<i>Abrus precatorius</i> L . (Fabaceae)	Leaf, seed, root	Abrin A - D	Fresh leaf paste applied over boils. Seeds and roots used in leucoderma
2.	<i>Acorus calamus</i> L. (Araceae)	Rhizome	Isoeugenol methyl ether; Alpha-asarone (1,2,4-trimethoxy-5 (2-propenyl) benzene); Thujane; Limonene; Acoradin; Beta-sitosterol	Rhizome paste is applied externally to cure scabies. Rhizome powder used to eliminate dandruff.
3.	<i>Adhatoda zeylanica</i> Medic(Acanthaceae)	Leaf	Vasicinone; 6-hydroxy peganine; Vasicine(Ahmad <i>et al.</i> , 2009)	Leaves are used to control acne and skin infections.
4.	<i>Aegle marmelos</i> L. Correa (Rutaceae)	Fruit	Luvangetin; Auraptin; Psoralen; Marmelide; Fagarine; Marmin; Lupeol; Eugenol; Citral; 1,8-Cineole; 4-isopropyl benzaldehyde; Citronellal (Maity <i>et al.</i> , 2009)	Young fruit is crushed with a piece of turmeric which is applied to cure ulcers.
5.	<i>Aristolochia indica</i> L. (Aristolochiaceae)	Leaf	Aristolochic acid; Aristolactone; Aristolochene; Ishwarol; 5 β -H, 7 β , 10 α -Selina-4(14), 11-diene (Sati <i>et al.</i> , 2011)	Leaf paste with coconut oil is applied on skin infected area.
6.	<i>Azadirachta indica</i> A. Juss.(Meliaceae)	Leaf, stem, bark	Azadirone; Epoxyazadiradione; Nimbin; Gedunin; Cazadiradione; Deacetylnimbin; 17-hydroxyaza- diradione	Leaf extract is applied externally on boils and blisters. The mixture of leaves, stem bark and coconut oil applied for all skin diseases.
7.	<i>Bacopa monnieri</i> L. (Scrophulariaceae)	Whole plant	Bacopaside A; Bacopasatin A; Bacopin	Plant extract on skin itching.
8.	<i>Cassia tora</i> L. (Cesalpinaceae)	Seed	Kaempferol; Leucopelargonidin; Rubrofusarin, Uridine; Beta sitosterol; Quercitrin, Stigmasterol; Chryso-obtusin; Phycion, Emodin; Chrysophanol; (Jain and Patil, 2010)	Seeds paste is applied on itching areas till cure. Leaf paste is applied in case of ringworm.
9.	<i>Centella asiatica</i> L. (Apiaceae)	Root	Centellin; Asiaticin; Centellicin; Asiaticoside (Zheng and Qin, 2007)	Leaf and root extract is applied on wounds
10.	<i>Curcuma longa</i> L. (Zingiberaceae)	Rhizome	Curcumin; Demethoxycurcumin; 1-(4-hydroxy-3-methoxyphenyl) -7-(3,4-dihydroxyphenyl)-1,6 -heptadiene-3,5-dione; 1-(4- hydroxyphenyl)-7-(3,4-dihy- droxyphenyl)- 1,6- heptadiene-3, 5-dione; Bisdemethoxycurcumin, ferulic acid, vanilic acid, vanillin. (Li <i>et al.</i> , 2011)	Paste of the rhizome is applied on the skin against psoriasis

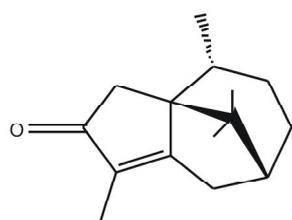
11.	<i>Cuscuta reflexa</i> Roxb. (Convolvulaceae)	Whole plant	Kaempferol; Quercetin; Luteolin; Caffeic acid; Cuscutamine; (Patel <i>et al.</i> , 2012)	Plant is crushed to paste and applied externally on white spots on face.
12.	<i>Cyperus rotundus</i> L. (Cyperaceae)	Root	Cyprotene; Cypera-2, 4-diene; a-copaene; Cyperene; rotundene; Valencene; Cyperotundone; Mustakone; Cyperol (Meena <i>et al.</i> , 2010)	Root paste is applied to treat blisters.
13.	<i>Jatropha curcas</i> L. (Euphorbiaceae)	Latex	5 α -stigmastane-3, 6-dione; Nobiletin; β -sitosterol; Taraxerol; 2S-tetracosanoic Acid glyceride -1;5-hydroxy-6,7-dimethoxy coumarin; Jatropholone A; Jatropholone B; 6-methoxy-7-hydroxycoumarin; Caniojane; 3-hydroxy-4-methoxybenzaldehyde; 3-methoxy-4-hydroxy benzoic acid; daucosterol (Ling-Yi <i>et al.</i> , 1996)	Latex of stem and leaves with mustard oil is applied to cure scabies.
14.	<i>Lawsonia inermis</i> L. (Lythraceae)	Stem bark	Lawsone; 2-hydroxy-1:4 naphthoquinone; Gallic acid; Glucose; Mannitol; Laxanthone I (Chaudhary <i>et al.</i> , 2010)	Stem bark paste is applied externally till cure skin eruptions.
15.	<i>Melia azedarach</i> L. (Meliaceae)	Stem bark	Limonene; Carvacrol; Phytol; Quercetin; Kampherol; Beta-Sitosterol; Stigmasterol; (Sen and Batra, 2012)	Stem bark and fruit paste is applied to cure leucoderma and wound.
16.	<i>Ocimum basilicum</i> L. (Lamiaceae)	Leaf	methyl cinnamate; Linalool; β -elemene; Camphor; Methyl eugenol, Eugenol, estragole: Safrole (Kathirvel and Ravi, 2012)	Leaf paste is applied to cure ulcers.
17.	<i>Phyllanthus emblica</i> L. (Euphorbiaceae)	Leaf	Gallic acid; Ellagic acid; 1-O-galloyl-beta-D-glucose; 3,6-di-O-galloyl-1-D-glucose; Chebulinic acid; Quercetin; Chebulagic acid; Corilagin; 3-ethylgallic acid (3-ethoxy-4, 5-dihydroxy-benzoic acid; isostrictiniin; 1,6-di-O-galloyl-beta-D-glucose (Zhang <i>et al.</i> , 2003)	Leaf powder and oil mixed together and the paste is applied to cure burn wound.
18.	<i>Plumbago zeylanica</i> L. (Plumbaginaceae)	Leaf, Root, Root bark	Plumbagin; Chitranone; Zeylanone; Maritinone; Dihydrosterone; Maritinone; Lupeol; α - and β -amyrin; and γ -taraxesterol; Daucosterol (Min <i>et al.</i> , 2011)	A paste of leaf with root bark and coconut oil applied on skin infected area. Root paste is applied externally in case of ringworm and psoriasis.
19.	<i>Tamarindus indica</i> L. (Ceasalpinaceae)	Stem bark, seed	Lupanone; Lupeol; <i>n</i> -hexacosane; Eicosanoic acid; Beta-sitosterol; Apigenin; Catechin; Procyanidin B2 (Bhadoriya <i>et al.</i> , 2011)	Dry stem bark powder mixed with oil is applied on burn wound, seed paste is applied externally to cure scabies.



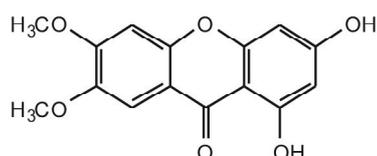


- 1 $R_1 = \text{OCH}_3$, $R_2 = \text{OCH}_3$ (1) Curcumin;
 2 $R_1 = \text{OCH}_3$, $R_2 = \text{H}$ (2) Demethoxycurcumin;
 3 $R_1 = \text{OH}$, $R_2 = \text{OCH}_3$ (3) 1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxyphenyl)-1,6-heptadiene-3,5-dione;
 4 $R_1 = \text{OH}$, $R_2 = \text{H}$ (4) 1-(4-hydroxyphenyl)-7-(3,4-dihydroxyphenyl)-1,6-heptadiene-3,5-dione;
 5 $R_1 = \text{H}$, $R_2 = \text{H}$ (5) Bisdemethoxycurcumin.

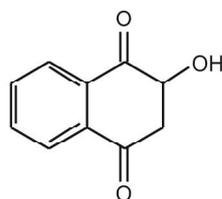




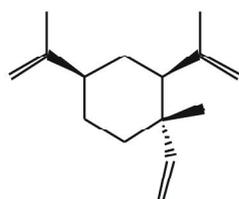
cyperotundone



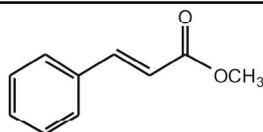
Laxanthone I



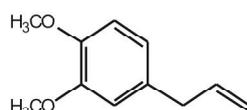
**Lawsonone
(2-hydroxy-1,4-Naphthoquinone)**



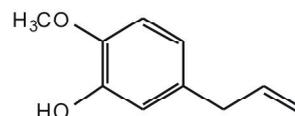
Beta elemene



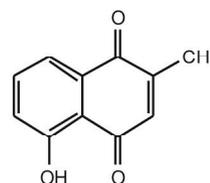
Methyl cinnamate



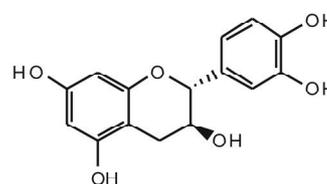
Methyl eugenol



Eugenol



Plumbagin



Catechin

The data related to quality and quantity along with the safety and efficacy on traditional medicine are far from the criteria needed to support its use worldwide. The reasons for the lack of research data are due to not only to healthcare policies, but also lack of adequate or accepted research methodology for evaluating traditional medicine.

New comprehensive method for biochromatographies based on studying the properties of absorption, distribution, metabolism and excretion (ADME) need to analyze traditional system of medicine and to screen the bioactive markers; also some other systems biology, including genomics and proteomics have been introduced to study these, more clearly and more scientifically which are described in detail. There has been a great scientific work, carried out by the Chinese people in respect of herbal medicine. Similarly we need to follow our research in the same directions.

Screening strategies of bioactive markers by biochromatography

Affinity chromatography is based on the biological interactions between biologically active compounds and immobilized proteins, enzymes and antibodies. It has been successfully applied to rapidly probe drug-protein binding and to study anticooperative, noncooperative and cooperative protein-ligand interactions (Evans *et al.*, 1989; Domenici *et al.*, 1991; Noctor *et al.*, 1992). Screening of traditional medicine for bioactive compounds, using *in vivo* models is time-consuming and complex and cannot be used

for the direct screening of bioactive components in traditional medicine. Organs, tissues, and cellular models have been studied *in vivo* and *in vitro* (Jiang *et al.*, 2010). Modern pharmacological studies have revealed that an important indication of drug action is its ability to bind with some receptors, channels, and/or enzymes on cell membranes or inside the cells. High-throughput screening methods, using membrane receptors or channels as targets, have been extensively developed and employed as promising approaches to the efficient screening of lead compounds as drug candidates from natural resources (Liang *et al.*, 2005; Su *et al.*, 2005; Qi *et al.*, 2006; Lei *et al.*, 2008; Li *et al.*, 2006). Furthermore, the ability of cell interactions is one of the key factors in the biological activities of a drug, thus, biochromatographic techniques, especially those involving immobilized artificial membranes and liposomes, have been developed for the analysis and screening of biologically active compounds in herbal medicines (Zhang *et al.*, 2007; Dong *et al.*, 2005; Yu *et al.*, 2007; Mateen *et al.*, 2010).

Biological fingerprinting analysis is a powerful and efficient method of screening and analyzing bioactive compounds and

uses proteins (Shang *et al.*, 2010; Wang *et al.*, 2007), DNA (Su *et al.*, 2005; Guo *et al.*, 2006), membranes (Su *et al.*, 2007), and even cells (Wang *et al.*, 2009) as the targets. The identification of biologically active compounds from complex TCM mixtures *via* biological fingerprinting and obtaining results with high sensitivity and specificity presents a great challenge for many researchers (Shang *et al.*, 2010; Wang *et al.*, 2007; Wang *et al.*, 2009; Su *et al.*, 2005).

A simple, rapid and high sensitive method for analysis of 13 components in *Angelica sinensis* root as ferulic acid, *Z*-ligustilide, *E*-ligustilide, *Z*-butylidenephthalide, *E*-butylidenephthalide, 3-butylphthalide, 3-butylidene-4-hydroxyphthalide, senkyunolide A, 6,7-epoxyligustilide, senkyunolide F, senkyunolide H, senkyunolide I, and 6,7-dihydroxylicustilide were quantified by Gas Chromatography-Mass Spectrometry (GC-MS), coupled with Pressurized Liquid Extraction (PLE). The analyzed results showed that interaction of multiple chemical compounds contributes to the therapeutic effects of medicines. However, evaluation of activities of different *Angelica* root is helpful to elucidate the mechanism of therapeutic effects (Lao *et al.*, 2004).

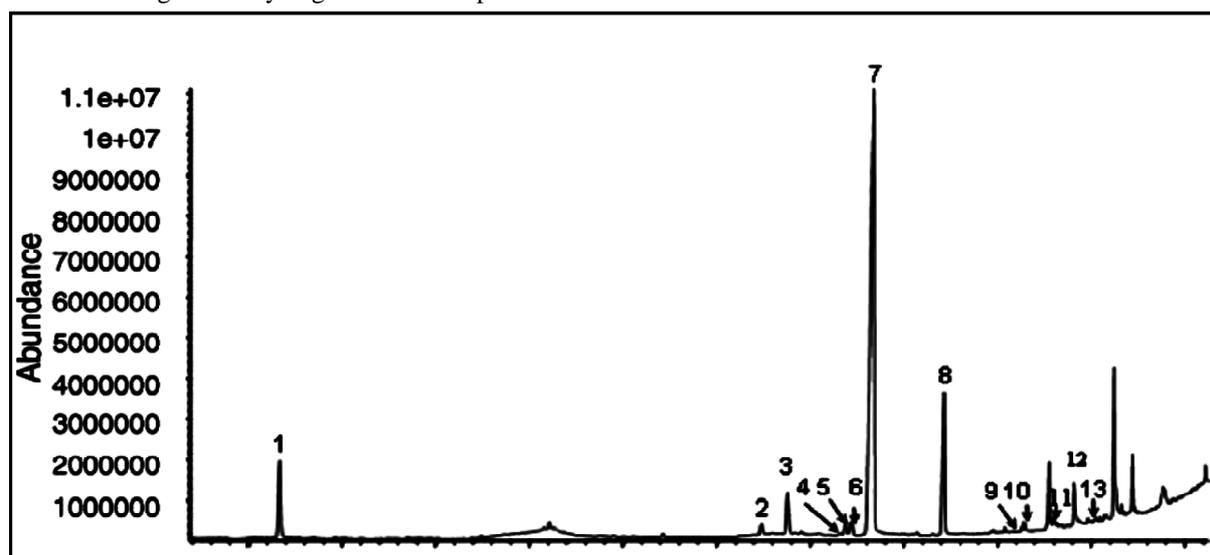


Figure: GC-MS total ion chromatograms of PLE extract from *Angelica sinensis*, *Angelica acutiloba* and *Angelica gigas*. (1) Ferulic acid; (2) *Z*-ligustilide; (3) *E*-ligustilide; (4) *Z*-butylidenephthalide; (5) *E*-butylidenephthalide; (6) 3-butylphthalide; (7) 3-butylidene-4-hydroxyphthalide; (8) senkyunolide A; (9) 6,7-epoxylicustilide; (10) senkyunolide F; (11) senkyunolide H; (12) senkyunolide I; (13) 6,7-dihydroxylicustilide (Lao *et al.*, 2004).

Screening strategies of bioactive markers by metabolic fingerprint

Microdialysis-HPLC techniques for evaluation of bioactive compounds with human serum

The possibility of the method for, further, studying binding behavior of the active components in TCMs to proteins in plasma where some preliminary results have been achieved. The chromatograms of microdialysis samples from the extracted solution of *Rhizoma chuanxiong* and the mixed

solution of *Rhizoma chuanxiong* with plasma, resulting in some peaks exhibited the affinity to proteins in plasma and the recovery conveniently indicated their affinity capacity to proteins in plasma. Two primary active components were identified as ferulic acid and 3-butylphthalide, and the 3-butylphthalide, showed the higher affinity capacity than ferulic acid. The method provides a simple way to study the binding behaviors of active components in TCMs with proteins in plasma with automatic operation (Huang *et al.*, 2004).

Qualitative and quantitative methods of TSM

Chromatographic methods such as TLC, SFC, HPLC, GLC, HPLC, CE and other hyphenated techniques, involving spectroscopic methods, such as LC-MS, LC-NMR, LC-IR are widely used in recent era for traditional medicines.

In TLC fingerprinting, the data that can be recorded, using a high-performance TLC (HPTLC) scanner, includes the chromatogram, retardation factor (R_f) values, the color of the separated bands, their absorption spectra, λ_{max} and shoulder inflection/s of all the resolved bands. All of these, together with the profiles on derivatization with different reagents, represent the TLC fingerprint profile of the sample (Rasheed *et al.*, 2010 a; 2011 a; 2012 a; Naikodi *et al.*, 2011 b). The information so generated has a potential application in the identification of an authentic drug, in excluding the adulterants and in maintaining the quality and consistency of the drug. HPLC fingerprinting includes recording of the chromatograms, retention time of individual peaks and the absorption spectra (recorded with a photodiode array detector) with different mobile phases (Makhija and Vavia, 2001; Wen *et al.*, 1992; Lee *et al.*, 1994; Lee *et al.*, 1995; Escarpa and Gonzalez, 2001; Tsao and Yang, 2003). Similarly, GLC is used for generating the fingerprint profiles of volatile oils and fixed oils of herbal drugs. Furthermore, the recent approaches of applying hyphenated chromatography and spectrometry such as High-Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD), Gas Chromatography-Mass Spectroscopy (GC-MS), Capillary Electrophoresis- Diode Array Detection (CE-DAD), High-Performance Liquid Chromatography-Mass Spectroscopy (HPLC-MS) and High-Performance Liquid Chromatography-Nuclear Magnetic Resonance Spectroscopy (HPLC-NMR) could provide the additional spectral information, which will be very helpful for the qualitative analysis and even for the online structural elucidation (Patil and Shettigar, 2010).

Supercritical fluid chromatography (SFC)

Supercritical fluid chromatography is a hybrid of gas and liquid chromatography that combines some of the best features of each. SFC permits the separation and determination of a group of compounds that are not conveniently handled by either gas or liquid chromatography. SFC has been applied to a wide variety of materials, including natural products, drugs, food and pesticide (Matthew and Henry, 2006). These compounds are either nonvolatile or thermally labile so that GC procedures are inapplicable or contain no functional group that makes possible detection by the spectroscopic or electrochemical technique employed in LC (Patil and Shettigar, 2010).

Types of hyphenated technique

GC-MS: With MS as the preferred detection method, and single- and triplequadrupole, ion trap and time-of-flight (TOF)

mass spectrometers as the instruments most frequently used, both LC-MS and GC-MS are the most popular hyphenated techniques, in use today. GC-MS, which is a hyphenated technique developed from the coupling of GC and MS, was the first of its kind to become useful for research and development purposes. Mass spectra obtained by this hyphenated technique, offer more structural information, based on the interpretation of fragmentations. Sometimes, polar compounds, especially those with a number of hydroxyl groups, need to be derivatized for GC-MS analysis. The most common derivatization technique is the conversion of the analyte to its trimethylsilyl derivative (Wilson and Brinkman, 2003).

It is a valuable analytical tool for the analysis of mainly nonpolar components and volatile natural products (Nozal *et al.*, 2002; Siano *et al.*, 2003; Myinff *et al.*, 1996; Mediros and Simoneit, 2007), *e.g.*, mono- and sesquiterpenes. They described a method, using direct vaporization GC-MS to determine approximately 130 volatile constituents in several Chinese medicinal herbs. They reported an efficient GC-MS method for the separation and structure determination of the constituents in ether-extracted volatile oils of Chinese crude drugs: *Jilin ginseng*, *Radix Aucklandiae*, and *Citrus tangerina* peels. Saponins are steroidal or triterpenoidal glycosides that occur widely in plant species of nearly 100 families. As saponins are highly polar compounds and difficult to volatilize, the application of GC-MS is mainly restricted to the analysis of aglycones, known as sapogenins or saponins. Sometimes, precolumn derivatization of saponins can be used to attach a chromophore that facilitates UV detection at higher wavelengths.

LC-IR: The hyphenated technique developed from the coupling of an LC and the detection method infrared spectrometry IR or FTIR is known as LC-IR or HPLC-IR. While HPLC is one of the most powerful separation techniques available today. The IR or FTIR is a useful spectroscopic technique for the identification of organic compounds, because in the mid-IR region, the structures of organic compounds have many absorption bands that are characteristic of particular functionalities, *e.g.*, -OH, -COOH, and so on. However, combination of HPLC and IR is difficult and the progress in this hyphenated technique is extremely slow. In addition, as a detection technique, IR is much less sensitive compared to various other detection techniques, *e.g.*, UV and MS. The recent developments in HPLC-IR technology have incorporated two basic approaches based on interfaces applied in HPLC-IR or HPLC-FTIR.

The solvent-elimination approach is the preferred option in most of the LC-IR operations, the mobile phase solvent is eliminated, IR detection is carried out in some medium that has a transparency for IR light. Generally, KBr or KCl salts are used for the collection of sample components in the eluent,

and heating up the medium before IR detection eliminates the volatile mobile phase solvents (Jinno *et al.*, 1982; Bourne *et al.*, 1990).

LC-MS: This hyphenated techniques, LC-MS or HPLC-MS refers to the coupling of an LC (Liquid Chromatography) with a Mass Spectrometer (MS), the information obtained from a single LC-MS run, on the structure of the compound, is rather poor. However, this problem has now been tackled by the introduction of tandem Mass Spectrometry (MS-MS), which provides fragments through collision-induced dissociation of the molecular ions produced. The use of LC-MS-MS is increasing rapidly.

An LC-MS combines the chemical separating power of LC with the ability of an MS to selectively detect and confirm molecular identity. MS is one of the most sensitive and highly selective methods of molecular analysis, and provides information on the molecular weight as well as the fragmentation pattern of the analyte molecule. The information obtained from MS is invaluable for confirming the identities of the analyte molecules (Niessen and Tinke, 1995; Dugo *et al.*, 2000; Wolfender *et al.*, 1998). Hyphenated techniques such as; HPLC coupled to UV and Mass Spectrometry (LC-UV-MS) have proved to be extremely useful in combination with biological screening for a rapid survey of natural products.

LC-NMR: In this technique, technological developments have allowed the direct parallel coupling of HPLC systems to NMR, giving rise to the new practical technique HPLC-NMR or LC-NMR, which has been widely known for more than last 15 years. The first online HPLC-NMR experiment, using superconducting magnet, was reported in the early 1980s. However, the use of this hyphenated technique in the analytical laboratories started in the latter part of the 1990s only.

LC-NMR experiments can be performed in both continuous-flow and stop-flow modes. A wide range of bioanalytical problems can be addressed, using 500, 600, and 800 MHz systems with ^1H , ^{13}C , ^2H , ^{19}F , and ^{31}P probes. The main prerequisites for online LC-NMR, in addition to the NMR and HPLC instrumentation, are the continuous-flow probe and a valve installed before the probe for recording either continuous-flow or stopped-flow NMR spectra.

A UV-Vis detector is also used as a primary detector for LC operation. Magnetic field strengths higher than 9.4 T are recommended, *i.e.*, ^1H resonance frequency of 400 MHz for a standard HPLC-NMR coupling. The analytical flow cell was initially constructed for continuous-flow NMR acquisition. However, the need for full structural assignment of unknown compounds, especially novel natural products, has led to the application in the stopped-flow mode (Albert, 1995).

CE-MS: When an MS detector is linked to a CE system for acquiring online MS data of the separated compound, the resulting combination is termed as CE-MS. CE is an automated separation technique, introduced in the early 1990s. CE analysis is driven by an electric field, performed in narrow tubes, and can result in the rapid separation of many hundreds of different compounds. The versatility and the many ways that CE can be used, mean that almost all molecules can be separated, using this powerful method. It separates species by applying voltage across buffer-filled capillaries, and is generally used for separating ions that move at different speeds when voltage is applied, depending on their size and charge. The solutes are seen as peaks as they pass through the detector and the area of each peak is proportional to their concentration, which allows quantitative determinations. Analysis includes purity determination, assays, and trace level determinations (Dunayevskiy *et al.*, 1996).

Isolation and analysis of natural products

Crude natural product extracts, which represent extremely complex mixtures of numerous compounds, can be analyzed successfully by using appropriate hyphenated techniques. Among the various hyphenated techniques, LC-MS are the two most extensively used for natural product analysis. LC-NMR, as well as different multiple hyphenated techniques like LC-NMR-MS have also become popular most recently. LC-MS, if the ionization technique is chosen appropriately, can be an extremely powerful and informative tool for screening crude plant extracts. The currently available various types of LC-MS systems, allow the analysis of small nonpolar compounds to large polar constituents like oligosaccharides, proteins, and tannins present in natural product extracts. Alkaloids are a large group of nitrogen-containing secondary metabolites of plant, microbial, or animal origin. Various hyphenated techniques have been used in the analysis of several types of alkaloids today. GC-MS has become the method of choice for the analysis of various pyrrolizidine and quinolizidine types of alkaloids. The coumarins are the largest class of 1-benzopyran derivatives that are found mainly in higher plants. HPLC-PDA can be used successfully in the analysis of various phenolic compounds, including coumarins, because of the presence of significant amounts of chromophores in these molecules. The HPLC-PDA determination of coumarins, where absorption spectra are registered with a PDA detector, provides useful information about the identity of the molecule including oxidation pattern. Among the hyphenated techniques, LC-MS, LC-NMR, and CE-MS could be useful for the rapid initial screening of crude extracts.

Chemical fingerprinting and quality control of herbal medicine

Generally, in the context of drug analysis, fingerprinting method is used to highlight the profiles of the sample matrix,

which is often sufficient to provide indications of the source and method of preparation. In herbal medicines, the profile depends not only on the preparation processes but also on the quality of the crude herb source material. The use of hyphenated techniques, *e.g.*, LC-MS, CE-MS, LC-NMR, or LC-NMR-MS, in chemical fingerprinting analysis for quality control and standardization of medicinal herbs has attracted immense interest in recent years (Cai *et al.*, 2002; Schaneberg *et al.*, 2003).

Analytical chemistry: It is useful in determination of drug and identification of its degraded products. It is systematically applied to monitor impurity profiles during pharmaceutical

development and scaling up and supports the safety evaluation of batches used in clinical studies.

Chemotaxonomy: Chemical taxonomy or chemotaxonomy is based on the principle that the presence of certain secondary metabolites is dictated by various enzymes, involved in the biosynthesis of these compounds. Hence, chemical profiling of these secondary metabolites, either by complete isolation and identification, or by separation and online identification, using modern hyphenated techniques, could provide useful information with regard to the taxonomic or even phylogenetic relationships among various species.

Table: Tools that help in the search and identification of metabolites in drug metabolism

Stage	Tools	Uses
Presynthesis compound for drug discovery	In silico	Assistance with chemical synthesis efforts to select or eliminate compounds.
Available compounds for screening and early drugs discovery	In silico LC-MS-MS	Assistance with synthetic efforts to block or enhance metabolism. Identification of simple or major metabolites. For example, dealkylations and conjugations such as glucuronide. Prediction of metabolites that are likely to be formed <i>in vivo</i> .
Late drug discovery and candidate selection	LC-MS-MSQT of (high resolution and exact mass measurement) MS ³ H-D exchange	Determination of metabolic differences between species. Identification of potential pharmacologically active or toxic metabolites.
Preclinical and Clinical development	LC-MS-MSQT of (high resolution and exact mass measurement) MS ³ H-D exchange Radioactivity Detector LC-MS-NMR	Determination of the percentage of metabolite formed <i>in vitro</i> or <i>in vivo</i> synthesis of metabolites for toxicology testing comparison of human pathways drug-drug interactions.

H-D= Hydrogen-deuterium; LC= Liquid chromatography; MS=Mass spectrometry; MS-MS= Tandem mass spectrometry; MS³= ion trap; NMR= Nuclear magnetic resonance; QTof= Quadrupole time-of-flight. (Nassar and Talaat, 2004)

DNA methods and others

There are two general approaches, used for DNA-based authentication of medicinal plants: the first one covers determination of the nucleotide sequence of one or more genetic loci in the plants of interest, and identification of the nucleotide sequence that is characteristic of a given species; the second one, rather than focusing on specific genetic loci, makes use of species-specific variations (polymorphisms) of the nucleotide sequence that are spread randomly over the entire genome, resulting in characteristic “fingerprints” of genomic DNA.

Comprehensive methods

Fingerprint

The chromatographic fingerprinting is based on the chromatographic separation and identification of marker

compound from other constituents. For these purposes, TLC, HPTLC, HPLC, LC-MS, LC-NMR, GC-MS, GC-FID and SFC methods are used. The other method used is DNA fingerprinting. As the DNA fingerprint of genome remain the same, irrespective of the plant part used while the phytochemical content will vary with the plant part used, physiology and environment. Hence, this is well established and highly precious method for standardization of herbal drug (Rajkumar and Sinha, 2010; Teo *et al.*, 2008; Drasar and Moravcova, 2004; Yang *et al.*, 2010; Liang *et al.*, 2009; Zhou *et al.*, 2009; Zeng *et al.*, 2007).

The WHO estimates that about 80% of the population living in the developing countries, relies almost exclusively on Traditional Medicine (TM) for their primary healthcare needs. The wide spread use of TM among both rural and urban

population, could be attributed to cultural acceptability, physical accessibility and economic affordability, as well as efficacy against certain types of diseases, as compared to modern medicine (Mukherjee, 2002; Gedif and Hahn, 2002).

By definition, a chromatographic fingerprint of a Herbal Medicine (HM) 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 HM 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 this HM (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 HM extracts, and not individually consider only one and/or two marker components for evaluating the quality of the HM products.

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 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. TSMs represent a much more daunting challenge due to the natural variability of the individual herbs and the chemical complexity of the formulations. Many pharmacopoeias are constantly evolving, and those of current western origin may incorporate both traditional and novel uses of phytomedicines.

Clues for new drug discovery from traditional system

Pharmacokinetic studies: Pharmacokinetic studies are designed in such a way that humans and test animals should have similarities for that aspect. Selection of suitable animals is noteworthy for functional screening may precisely follow the human ailment *i.e.*, infectious disease or induce a

condition that mimics the affliction, as chemical injury to the liver or pancreas needed to understand hepatoprotective or hypoglycaemic agents, respectively.

Utilization of traditional knowledge as information to develop new therapeutic ingredients: When targeted to a specific disease that is clearly recognized by conventional and traditional diagnostic methods, where appropriate bioassays can elicit the therapeutic rationale for use, and where field studies are applicable to establish its value. Linking these ‘targeted’ medicinal uses to appropriate functional and mechanistic assays, frequently provides a drug of high therapeutic potential.

Chemical fingerprints obtained by chromatographic and electrophoretic techniques, 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. Several novel chemometric 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.* which shows that the combination of chromatographic fingerprints of herbal medicines and the chemometric evaluation might be a powerful tool for quality control of herbal products.

Combination of fingerprint with multicomponent quantification

Since fingerprint is mainly a qualitative method, used for the authentication, batch-to-batch consistency evaluation, and quality control of TCMs, combination of fingerprint with multicomponent quantification would be much powerful, since the qualitative and quantitative information of the products can be achieved at the same time. It has been proved to be a comprehensive method to evaluate the quality consistency, stability and reasonability of the manufacturing processes. By this method, *Psoralea corylifolia*, *Belamcanda chinensis* and some TCM preparations, such as Shuang- Huang-Lian oral liquid, Xuesetong injection, *etc.* were analyzed successfully (Qiao *et al.*, 2007; Cao *et al.*, 2006; Lai *et al.*, 2006; Pinelli *et al.*, 2007; Ding *et al.*, 2007).

Conclusion

In general, one or two markers or pharmacologically active components in herbs and or herbal mixtures were currently employed for evaluating the quality and authenticity of herbal medicines, in the identification of the single herb or HM preparations, and in assessing the quantitative herbal composition of an herbal product. This kind of the determination, however, does not give a complete picture of

a herbal product, because multiple constituents are usually responsible for its therapeutic effects. These multiple constituents may work 'synergistically' and could hardly be separated into active parts. Moreover, the chemical constituents in component herbs in the HM products may vary, depending on harvest seasons, plant origins, 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) with recent hyphenated techniques, can be applied for this kind of documentation. In this way, the full herbal product could be regarded as the active 'compound'. The concept of phytoequivalence 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 a herbal product should be constructed and compared with the profile of a clinically proven reference product.

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Conflict of interest

The authors declare no conflict of interest.

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