Available online at SciVarse ScienceDirect

Chinese Herbal Medicines (CHM)

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Review

A New Concept on Quality Marker for Quality Assessment and Process Control of Chinese Medicines

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ARTICLE INFO

Article history

Received: October 15, 2016

Revised: November 12, 2016

Accepted: November 18, 2016

Available online:

December 20, 2016

DOI:

10.1016/S1674-6384(17)60070-4

ABSTRACT

Chinese medicine (CM) is the most typical conventional therapy compared with any other traditional or alternative medicine systems. The active components of CMs are either primary or secondary metabolites generated by metabolic and biosynthetic enzymes in plants, protecting the plants from environmental stress. The characteristics of these metabolites are diverse, complicated and unique. In this paper, current approaches for quality assessment were extensively reviewed, a new concept of quality marker (Q-marker) was then proposed for CM quality assessment. Additionally, definition of the Q-marker, as well as the relevant methods, were discussed, on the basis of the biosynthetic pathways of secondary metabolites and source of biological active components. Study design of Q-marker is complex system for quality assessment and production process control of CM products with transitivity and traceability. Therefore, the system with characteristics of transmission and traceability is expected to be established for regulation of quality. Upon the concept which the transitivity and traceability in the quality assessment and production process control covered the entire process, such as raw materials, decoction slices, processing, extraction and production can be further enhanced. The transitivity and traceability will inevitably require close attention to "who, what, where, when, and why" details at each stage of Q-markers of CM production form raw materials to patent product. The establishing quality standards are enablers of many and various transitivity and traceability solutions, not a solution in them. It means that the transitivity and traceability system is readily link between products and across borders in quality. According to the thinking mode and methods of investigation on quality assessment of CM product, we focus on the entire process, in terms of safety and effectiveness and quality control. The standard preparation of CM or CM decoction is not only the basis for study of Q-marker, but also the basis for transmission and traceability of the quality of CM product.

Key words

Chinese medicine; formulation; medicinal resource; quality administration; quality marker; quality standard; quantitative analysis; secondary metabolites

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Fund: National Natural Science Fundantion of China (No. 81430096)

1. Introduction

Chinese medicine (CM) is the most typical conventional therapy comparing with any other traditional or alternative medicine systems. According to the origin, CMs cover traditional medicines, herbal medicines, folk medicines, and phytomedicines in China. CMs are well known for their pharmacological effects widely used for thousands of years. The widespread and global acceptance as well as the utilization of herbal medicines is suggestive of their safety and efficacy. Therefore, CM has become an important and indispensable part of public healthcare globally. Challenges, however, still remain, with respect to the new drug research and development (R&D) of CM. For instance, those issues related to finance, ethics, quality and standardization of product, study design and regulatory affairs need to be fully addressed.

It is well known that the difference can be attributed to the regulation of CMs in developed countries where CMs are produced and utilized in accordance with requirements of good agricultural practice (GAP), good manufacturing practice (GMP), good laboratory practice (GLP) and good clinical practice (GCP) for establishing appropriate specifications of their products, intermediates and starting substances in R&D of pharmaceuticals.

According to literature, the active components of CMs are either primary or secondary metabolites generated by metabolic and biosynthetic enzymes in plants with diverse, complicated and unique features. In order to promote the rapid development of pharmaceutical industry, improve the quality standard system, such as pharmacopoeia standards, and product quality standards of Chinese medical materials and their formulations, current approaches for quality assessment were analyzed, a new concept of quality marker (Q-marker) of CMs and their products was then proposed (Liu et al, 2016; Zhang et al, 2016a; 2016b; Xiong and Peng, 2016; Kang et al, 2014; Chen et al, 2016; Ding et al, 2016; 2017; Zhou et al, 2017). In the following sections, the factors affecting the quality of CMs and secondary metabolites, quality standards and regulatory affairs to define quality markers of CMs, as well as the research methods, in terms of evaluation of applications, were discussed.

2. Chinese medicine is a complex system

2.1 Chinese medicine is a multi-component system

CM product is a multiple-component complex system (Figure 1), in particular its chemical components remain unclear, which makes it rather difficult to define the functions of CM from material basis and chemical properties. In terms of chemistry, CM consists of a large number of constituents, which may undergo biotransformation or chemical changes. In addition, the contents of chemical substances are generally complicated and changes, in terms of quality and quantitative criteria of products, increasing difficulties for CM development, for instance, the origins, culture conditions, harvest, parts of the plants used as medicines, formulation production and clinical application. It is of great importance to strengthen the quality control and standard for ensuring the effectiveness and safety of CM products. Due to the complexity of traditional Chinese medicine (TCM), many issues, particularly quality and relevant standards still remain unsolved (Liu, 2013; 2001; Li et al, 2015).

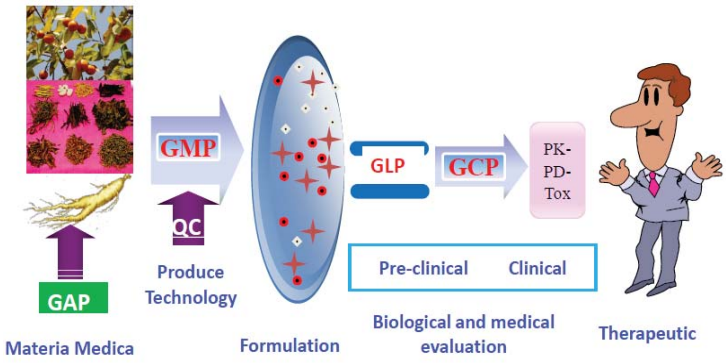

Figure 1 CM product is a complex system

2.2 Difficulties and challenges of CM quality control

The difficulties of quality assessment of CM products can be listed as following: (1) It is associated with Good Agricultural Practice, Good Collection Practice (GACP), and Good Supplement Practice (GSP); (2) Specific eco-geographic regions (EGRs) of botanical raw materials is of particular importance; (3) How to reduce the variability from the plant raw materials to production process; and (4) Based on well-known mechanism of action (MOA) and TCM theory, potency evaluation of bioactive response become flexible for control of the manufacturing process. Based on the above statement, it is suggested as follows: (1) CM products are complex with multiple chemical components (known and unknown active components) and natural variations; (2) Generally CM product is the mixture of the active pharmaceutical ingredients (API) and inactive ones from raw materials of herbs; (3) CM products are very difficult to satisfy the identical requirement of quality, safety and efficacy as that of chemical drugs; and (4) Based on TCM theory, the principle of multiple-flavor prescription is also far from that of chemical composition of western drugs.

3. Primary and secondary metabolites

A variety of compounds, in terms of their chemical structures, are produced by plants. These compounds can be classified as either primary or secondary metabolites. The metabolites are extensively distributed in different tissues and organs. These naturally occurring products are synthesized in plants through diverse pathways of biotransformation, which are an integral part of the entire developmental program of plants. Primary metabolites are ubiquitous in plants and fulfill essential metabolic roles, while natural products refer to the compounds that are differentially distributed in the plant kingdom and fulfill a very broad range of physiological roles, which are considered essential for their adaptive significance in protection against environmental constraints (Caretto et al, 2015).

The secondary metabolites in Chinese medicinal materials (medicinal plants) and formulations are the substantial basis of study of CMs and product quality of CM under the guidance of TCM theory. These compounds are not only the bases for investigating CM products, product quality control and establishing quality standards, but also the key resources for new drug discovery. Recently, there has been increasing interest in natural product research, due to the failure of other drug discovery approaches to discover lead compounds in key therapeutic areas.

3.1 Primary metabolites

Primary metabolites are the type of metabolites that is directly involved in normal growth, development, and reproduction. Generally, it performs a physiological function in the organism (i.e. an intrinsic function). The primary metabolites are typically existed in many organisms or cells of plant, including ethanol, lactic acid, and certain amino acids. While the secondary metabolites are not directly involved in those processes, but usually have an important relational function. A secondary metabolite is typically present in a taxonomically restricted set of organisms or cells (plants, fungi, and bacteria), for instance ergot alkaloids, naphthalenes, nucleosides, phenazines, quinolines, terpenoids, peptides and growth factors. Interestingly, plant growth regulators may be recognized as both primary and secondary metabolites, due to their role in plant growth and development (Prins et al, 2010; Buchanan et al, 2015; Buchanan, 2012). Some of them are intermediates between primary and secondary metabolism (Seigler, 2012).

3.2 Secondary metabolites

Unlike primary metabolites, harmful consequence may not occur, for instance death, in the absence of secondary metabolites, but long-term impairment of the organism's survivability, fecundity, or aesthetics, or no significant change at all. Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group. Secondary metabolites often play a key role in defense mechanism of plant against herbivory and other interspecies defenses. Secondary metabolites acting as medicines, flavorings, and recreational drugs are used by human beings.

The secondary metabolites were initially reviewed by Ahmad in 1979 (Ahmad, 1979). Over the past decades, efforts were made on study of secondary metabolites, of which 1242 review articles discussing this issue were published (Figure 2).

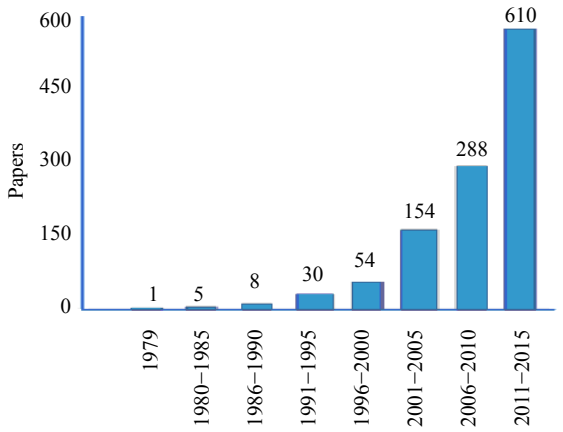

Figure 2 Review articles published on secondary metabolites

In the early years of studying secondary metabolites of the plant species, biological testing was less considered, but increasingly there has been a focus on the biological properties of these compounds. Systematic secondary metabolites-biosynthesis genes relationship might give insight into the molecular level (Zhai et al, 2016).

Most of the metabolites of interest were classified as secondary metabolites, based on their biosynthetic pathway. At present, many secondary metabolites, for example, terpenes, phenols, alkaloids, steroids, glycosides, from traditional Chinese medicines and pharmaceutical products have been used in treatment of diseases, for quality control and/or quality standards, and as leading compounds for drug innovation (Table 1). Berenbaum analyzed the interactions between secondary metabolites of plant and ecological and evolutionary processes in his book (Berenbaum, 2012), covering molecular biology and microbiology, ecology, and evolutionary theory, which is valuable for investigating the relationship between specific eco-geographic regions (EGRs) and quality of botanical raw materials.

Plant phenolics are the most widely distributed natural products. Several types of phenolic compounds such as esters, amides, and glycosides of hydroxycinnamic acids, flavonoids, proanthocyanidins, and their relatives have been identified. Polymeric phenolics such as lignin, suberin, and melanins have also been found in plants. Polyphenolic compounds are produced in plants by different biosynthetic pathways. The glycolytic and pentose phosphate pathways provide precursors to the shikimate pathway. Phenylalanine, produced by the shikimate route, is the precursor of phenylpropanoid metabolism to diverse specific flavonoid pathways (Sofia et al, 2015).

Table 1 Major types of secondary metabolites

Compound types	Secondary metabolites	Origins
Flavonoids	rutin, quercetin, ginkwanin sciadopitysin, gingetin, isogingetin	*Sophora japonica* L., *Ginkgo biloba* L.
Alkaloids	hyoscyamine, atropine, cocaine, scopolamine, tetrodotoxin, codeine, morphine	*Datura stramonium* L., *Atropa belladonna* L., *Erythroxylon coca* L., *Solanaceae*, Fugu, Salamanders, *Papaver somniferum* L.
Terpenoids	azadirachtin, artemisinin, tetrahydrocannabinol,	Neem tree, *Artemisia annua* L., cannabis
Saponins	saponins	*Panax ginseng* C. A. Mey , *Panax notoginseng* (Burk) F.H.Chen
Steroids	steroids	*Chionographic japonica* (Willd.) Maxim., *Hibiscus tiliaceus* L.
Glycosides	modified sugar molecules	*Ophiopogon japonicus* (Linn. f.) Ker-Gawl., *Cynanchum atratum* Bunge, *Ginkgo biloba* L.
phenols	stevia phenols, allylpyrocatechol, resveratrol	*Stevia rebaudiana* Bertoni, *Suaeda glauca* Bunge，*Polyg*, *Polygonum cuspidatum* Sieb. et Zucc
Biphenyls	phytoalexins	*Polygonum cuspidatum* Sieb. et Zucc, *Vaccinium* spp., *Reynoutria japonica* Houtt.

3.3 Biosynthetic pathway of secondary metabolites

Many secondary metabolites are always at low levels in plants. Since different enzymatic systems are involved in the biosynthetic pathways, secondary metabolites are produced in different ways.

3.3.1 Biosynthetic pathway of flavonoids

Flavonoids are widely presented in medicinal plants. These compounds normally exist in the form of glycosides. Flavonoids were demonstrated to be able to regulate lipid metabolism, expand coronary artery, reduce vascular fragility and exert other pharmacological functions.

Great progress has been made in study of flavonoid-biosynthetic pathway and enzymatic reactions. (Koes et al, 1994; Jung, 2000; Schijlen et al, 2004). Generally, flavonoid compounds include 2-phenylchromans and 3-phenylchromans. 2-phenylchromans (flavonoids) include flavanones, flavones, flavonols, flavanol and anthocyanidins, 3-phenylchromans (isoflavonoids) include isoflavones, isoflavans, and pterocarpans.

Structurally, flavonoid biosynthesis related genes can be divided into either the structural gene coding for the enzyme or the genes for regulations. Multiple flavonoids metabolic biosynthetic pathways is the fundamental for the diversity of these compounds.

Chalcone synthase (CHS) catalyzes the initial step of this pathway; chalcone isomerase(CHI) catalyzes intramolecular cyclization reaction; isoflavone synthase (IFS) catalyzes Synthesis of (2*S*)-flavanone (5,7,4′-trihydroxy chalcone) or (2*S*)-5-deoxy flavanones (7,4′-hydroxy chalcone) and transfers from the C-2 position to the C-3 position of the B ring, and then through the isoflavones dehydratase heterocycle C-2. Flavanone 3-β-hydroxyalse (F3H）promotes CHI catalytic synthesis of (2*S*)-flavanone or (2*S*)-5-deoxy flavanones C-3-hydroxylation, for generating flavanonols. Flavonol synthase (FLS) catalyzes *C*-3-hydroxylation, forming various flavonols, while dihydroflavonol 4-reductase (DFR）is a key enzyme in the biosynthesis of anthocyanins and phlobaphenes (Schijlen et al, 2004).

Up to date, discovery of new drugs from natural resources is of particular interest to the pharmaceutical industry. Natural products have been acting as the source of new drugs since ancient times. Plants are the good sources of secondary metabolites with beneficial properties. Natural products will definitely become important sources of new pharmaceutical compounds (Favela-Hernández et al, 2010). Recently, there has been a renewed interest in natural product research, due to the failure of alternative drug discovery methods to deliver many lead compounds in key therapeutic areas.

Salvia miltiorrhiza Bunge is a traditional Chinese medicine. Over 40 tanshinones, such as tanshinone I, tanshinone IIA, cryptotanshinone, and dihydrotanshinone I, have been isolated from *S. miltiorrhiza*. *S. miltiorrhiza* is therefore considered as a model for analyzing plant secondary metabolism pathways, due to its two typical secondary metabolites (Xu et al, 2016; Liu, 2016).

3.3.2 Biosynthesis pathway of alkaloids

In the biosynthesis process of berberine, *L*-tyrosine exists in plants as the starting material. Berberine is then produced in two steps (Sato et al, 2001). The first stage of berberine synthesis occurs in the cytoplasm, in the presence of norcoclaurine synthase, dopamine and acetaldehyde formed by condensation of 4-hydroxyphenyl-benzyl isoquinoline alkaloid of norcoclaurine. It accepts methyl adenosine methionine, after several methylation into reticuline. In the second step, reticuline is transported into the endoplasmic reticulum vesicles, and forms a ring under the action of berberine-bridge, further generating the alkaloid skeleton, scoulerine. Scoulerine gets methyl adenosylmethionine to form tetrahydrocolumbamine, which is catalyzed by canadine

synthase, and then formed methylene dioxymethamphetamine ring structure into canadine. The last step of the synthesis is through enzymatic oxidation of tetrahydro-berberine, and then uses hydrogenation as substrate of oxidative dehydrogenation, and ultimately forming berberine.

The biosynthetic pathways of *Corydalis yanhusuo* were studied by Beaudoin and Facchini (Beaudoin and Facchini, 2014). The intermediates/end-products in the biosynthesis pathways were already known, but only a few have been identified in *C. yanhusuo* bulbs. However, all the known enzymes were identified in *C. yanhusuo* bulb transcriptome, indicating that the biosynthesis in different plant species shared most of common steps, especially those in the upstream pathways. For example, (*S*)-reticuline is the key intermediary branch-point in biosynthesis of different types of benzylisoquinoline alkaloids and exists in a varied abundance in many medicinal plants (Farrow et al, 2012). The biosynthesis pathway includes reticuline 7-*O*-methyl-transferase, norreticuline 7-*O*-methyltransferase, berbamunine synthase, corytuberine synthase, salutaridine synthase, and columbamine *O*-methyltransferase. *Corydalis yanhusuo* is widely used in traditional Chinese medicines. So far, about 60 alkaloids have been identified from dried *C. yanhusuo* bulbs (He et al, 2007; Sun et al, 2014; Zhan, 2014). The active alkaloids include tetrahydropalmatine, corydaline, protopine, columbamine, berberine, dehydrocorydaline, tetrahydro-columbamine and palmatine. All of these compounds are biosynthesized from tyrosine and have a common basic benzylisoquinoline subunit (Hagel and Facchini, 2013; Qing et al, 2015; Ahmad, 1979; Zhai et al, 2016).

3.3.3 Biosynthetic pathways of phenolics

There are more than 20 phenolic acids in *S. miltiorrhiza*. Two biosynthetic pathways are involved in the biosynthesis of the processor of phenolic acid: (1) the phenylpropanoid pathway and (2) the tyrosine-derived pathway (Figure 3) (Di et al, 2013). Through the phenyl-propanoid pathway, phenylalanine is transformed into 4-coumaroyl-CoA by phenylalanine ammonia-lyase (PAL), cinnamic acid 4-hydroxylase (C4H) and 4-coumarate: CoA ligase (4CL). In the tyrosine-derived pathway, tyrosine is metabolized to 4-hydroxyphenyllactate through tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate reductase (HPPR), finally forming 3,4-dihydroxyphenyl-lactic acid (DHPL).

Tanshinones are a group of abietane-type norditerpenoid quinone that is mainly accumulated in the roots of *S. miltiorrhiza*. The biosynthetic pathway of tanshinones could be divided into three steps: (1) the formation of precursors for all terpenoids; (2) the construction of skeletons of tanshinones; and (3) the post-modification of the skeleton, such as oxidation, methylation, decarboxylation or cyclization to produce the various tanshinones. This pathway has been explored for more than two decades, and most genes involved in tanshinone biosynthesis have been cloned (Figure 4)(Dong et al, 2011; Gao et al, 2014; Guo et al, 2013).

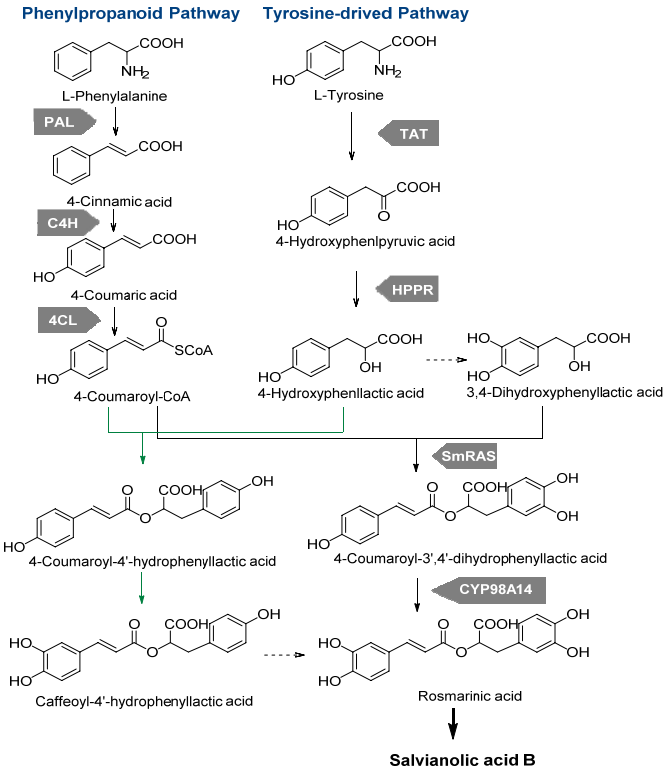

Figure 3 Biosynthesis of phenolic acids in *S. miltiorrhiza*

RA is mainly biosynthesized from the pathway presented in black arrows in *S. miltiorrhiza*, and parially from the pathway presented in green arrows. PAL: phenylalanine ammonialyase; C4H: cinnamic acid 4-hydroxylase; 4CL: 4-coumaroyl CoA ligase; TAT: tyrosine aminotransferase, HPPR: 4-hydroxyphenylpyruvate reductase; RAS: rosmarinic acid synthase

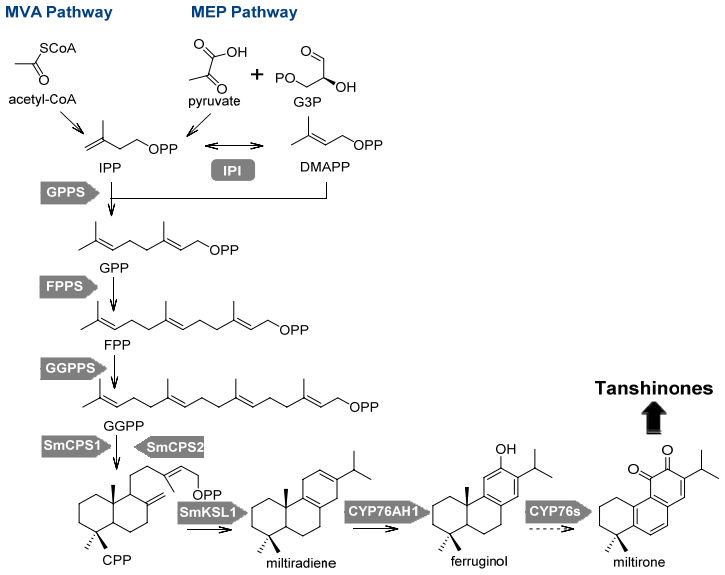

Figure 4 Biosynthesis of tanshinones in *S. miltiorrhiza*

G3P: glyceraldehyde-3-phosphate; IPP: isopentenyl pyrophosphate; DMAPP: dimethylallyl pyrophosphate; IPI: IPP isomerase; GPP: geranyl diphosphate; GPPS: GPP synthase; FPP: farnesyl diphosphate; FPPS: FPP synthase; GGPP: geranylgeranyl diphosphate; GGPPS: GGPP synthase; CPP: copalyl diphosphate; SmCPS: CPP synthase; SmKSL: kaurene synthase-like cyclase

In *S. miltiorrhiza*, tanshinones are mainly synthesized by the MEP pathway, while the MVA pathway could be explained as being involved in cell growth. In the MVA pathway, IPP is synthesized from two acetyl-CoA molecules.

The 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) converts 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to MVA with two NADPH as the rate-limiting step of the MVA pathway (Dai et al, 2011).

3.4 Quantitative and qualitative analysis of secondary metabolites

A great number of secondary metabolites are produced by plants as natural antioxidants from *Moringa oleifera* Lam. *Ocimum tenuiflorum* L. is known for its wide applications in food and pharmaceutical industry. As to compare the levels of phenolic and flavonoid in *M. oleifera* and *O. tenuiflorum* Phenolic and flavonoid contents were studied by spectrophotometry and paper chromatography in *M. oleifera* and *O. tenuiflorum* Higher phenolic and flavonoid levels were observed in *M. oleifera* leaf and flower. *O. tenuiflorum* flower presented higher phenolic content and low flavonoid, by comparison of that in *M. oleifera*. Flavonoids such as biflavonyl, flavones, glycosylflavones, and kaempferol were identified by chromatography. Phytochemical analysis for flavonoid, tannins, saponins, alkaloids, reducing sugars, and anthraquinones were tested positive for *M. oleifera* and *O. tenuiflorum* leaf as well as flower. In the present study, higher phenolic and flavonoid content indicated the natural antioxidant nature of *M. oleifera* and *O. tenuiflorum*, signifying their medicinal importance (Sankhalkar et al, 2016).

Plants are rich in secondary metabolites with medicinal and aromatic features. Secondary metabolites such as alkaloids, iridoids, and phenolics generally produced by plants for defenses have been implicated in the therapeutic properties of most medicinal plants. Hence, quantification of these metabolites will increase the possibility to discover new and effective drugs from plant sources and also to scientifically validate the existing traditional practices. Quantification of a large group of phytochemicals is described by Thangaraj (Thangaraj, 2015).

4. Fundamental of quality markers from secondary metabolites of CMs

Medicinal plants are the major source of Chinese medicines. Plant secondary metabolites, which are formed by biological and non-biological factors, are the substantial basis of the active components and mechanism of action, particularly an important source of drug discovery. (1) Many secondary metabolites are derived from the primary metabolites. The structures are diverse. In general, secondary metabolites include alkaloids, flavonoids, terpenoids, anthraquinone, coumarins, lignans compounds. Different types of compounds have their own origins (e.g. family, genus, species, subspecies, varieties, etc.), also they have significant value in biological and medical applications. (2) Biosynthetic pathway of chemicals of Chinese medicines (biosynthesis pathway) is based on the chemical and the biological basis of genetic studies. The secondary metabolites such as alkaloids, flavonoids, terpenoids, anthraquinone, coumarins, lignans are different, in terms of their chemical structures. The compounds with various sub-structures laid under many factors (genetic, growth differentiation and environmental factors) formed the diversity and specificity of different secondary metabolites in plants. (3) Based on their diversity and specificity, differences of Chinese medicine ingredients can be identified.

Differences from certain active chemical ingredients are reflected as markers for quality control of CMs.

5. Quality markers

5.1 What is CM quality markers

CM quality marker (Q-marker) is a chemical constituent and can be defined, according to the following four basic conditions: (1) quality marker exists in herbs, pieces, extracts, unilateral or compound formulations; (2) quality marker should be analyzed through either qualitative and quantitative approach; (3) based on principle of multiple-flavor prescription of traditional Chinese medicine (TCM) (such as the King (*Jun*), minister (*Chen*), assistant (zuo) and guide (*Shi*), as well as the compatibility of TCM and modern pharmacological study, the drug effect (such as effectiveness and safety) should be demonstrated to be associated with the identified quality marker, (4) the quality marker is a chemical substance that are transferable and traceable in the process of production and preparation.

5.2 Quality marker determination and quality standard of Corydalis Rhizoma

Corydalis Rhizoma is taken as an example here for demonstrating standards and research model proposed, based on the concept of Q-markers. Through specific biosynthesis pathway analysis of chemical composition, as well as the potency, efficacy, pharmacodynamics, pharmacokinetics and network pharmacological analysis, the biological activity of chemical components were confirmed. The chemical constituents of *Corydalis Rhizoma* were identified using high performance liquid chromatography/electrospray ionization quadruple time-of-flight mass spectrometry (HPLC/ESI-Q-TOFMS). The source and specificity of chemical constituents were confirmed by analyzing biosynthetic pathway and component specificity. Through qualitative identification and quantitative analysis of 11 batches of samples of *Corydalis Rhizoma*, similarity analysis and principal component analysis, fingerprint control methods were established. Eventually, seven alkaloidal compounds including tetrahydropalmatine, corydaline, coptisine, palmatine, dehydrocorydaline, tetrahydro-jatrorrhizine, and protopine have been selected as Q-markers, in the meanwhile quality control methods of multi-component assay and fingerprint have been established.

5.3 Determination of quality markers according to biosynthesis of different plant sources

For example, analysis of quality markers of *Coptis chinensis* Franch and *Phellodendron amurense* Rupr suggested that benzylisoquinoline alkaloids are important secondary metabolites in medicinal plants. As shown in Figure 3, the biosynthetic process formed several relevant alkaloids: (1) norcoclaurine presents in *Aconitum japonicum* Tbunb, *Nelumbo uncifera* Gaertn, and *Gnetum parvifolium* (Warb.) C.Y. Cheng ex Chun; (2) reticuline in *Annona reticulate* L., *Annona squamosa* L., *Nelumbo nucifera* Gaertn and *Magnolia officinalis* Rehd. et Wils; (3) scoulerine in *Gueldenstaedtia multiflora* Bunge; (4) tetrahydro-columbamine in *Jatrorrhiza palmate* (DC.) Miers and roots of *Coptis chinensis* Franch; (5) canadine presents in *C. chinensis*, *P. amurense* Rupr., and *Berberis pruinosa* Franch.; (6) berberine and coptisine presents in *C. chinensis*; and (7) sanguinarine in *Chelidonium majus* L., *Corydalis mucronifera* Maxim., *Macleaya cordata* (Willd. R. Br.) and *Eomecon chionantha* Hance. While *C. chinensis* and *P. amurense* both contain berberine, jatrorrhizine, and palmatine. Berberine are presented in *C. chinensis* and *P. amurense*. Coptisine is a special type of alkaloid in *C. chinensis* and phellodendrine in *P. amurense* only. Therefore, these differences can be distinguished from the source of alkaloids (Table 2).

Table 2 Diversity of benzylisoquinoline alkaloids in different medicinal plants

Compounds	Berb	Cand	Copt	Jatr	Norc	Palm	Phel	Retc	Sang	Scol	Tetc
Aconitum japonicum					+						
Annona reticulate								+			
Annona squamosa								+			
Berberis pruinosa		+									
Chelidonium majus									+		
Coptis chinensis	+	+	+	+		+					+
Corydalis mucronifera									+		
Eomecon chionantha				+		+			+		
Gnetum parvifolium					+						
Gueldenstaedtiamultiflora										+	
Jatrorrhiza palmate											+
Macleaya cordata				+					+		
Magnolia officinalis								+			
Nelumbo uncifera					+			+			
Phellodendron amurense	+	+					+				

Berb: berberine; Cand: canadine; Copt: coptisine; Jatr: jatrorrhizine; Norc: norcoclaurine; Palm: palmatine; Phel: phellodendrine; Retc: reticuline: Sang: sanguinarine: Scol: scoulerine: Terc: tetrahydrocolumbamine

5.4 Quality markers of CM compound formulas

In TCM, four elements, "king-minister-assistant-guide", are the basic prescription principles for the individual therapy in CM. TCM physicians diagnose diseases and analyze herb-herb interaction to prescribe medicines by either reducing or adding herbs. With respect to complex formulations of CM, chemistry study in combination with pharmacological, toxicological study, as well as emerging pharmacokinetic study of CM plays a key role in CM modernization, offering new approaches to explain scientific fundamental of compound formulations and further promote international recognition of CMs. A new concept of pharmacokinetic marker (PK-marker) to study pharmacokinetics of CM complex formulations was proposed in 2008. The PK-marker is defined as follows: (1) PK-marker must be associated with efficacy, (2) PK-marker exists in biological sample and can be determined by analytic method, and (3) PK-marker should reflect the relationship between concentration and time (Liu, 2013; Xiao et al, 2008; Lu et al, 2008).

Pharmacokinetic study of "Bi-qi capsule", for example was performed to illustrate the function of pharmacokinetics (PK) in understanding compatibility of components/ingredients. After rats were orally administrated with compounds of brucine and strychnine, King drug of Biqi capsule, as well as all components of Biqi capsule at same dose, pharmacokinetic patterns of "king", "minister", "assistant", and "guide" drugs were compared to study the PK interaction of the four drugs. The resulting data suggested that there was no significant difference between brucine and strychnine in either "king" drug or as single compound, in terms of pharmacokinetic parameters. Time to reach peak concentration of brucine and strychnine, however, were delayed. Peak concentrations were also lowered. Effective concentrations in circulation were remarkably prolonged, but the overall absorption was not altered, implying that non-"king" drugs may change the pharmacokinetic behavior of both strychnine and brucine in rats, in particular the absorption process (Xu et al, 2009) (Figures 5A and 5B).

Studies on pharmacological and toxicological interaction of Biqi capsule showed that brucine and strychnine in "king" drug are key bioactive components, while non-"king" drugs reduce the toxicity and enhance the efficacy. According to PK and PD studies, combined with the definition of quality markers, the brucine and strychnine is therefore considered as identification markers for quality indicators. The result of this study has now been adopted by Chinese Pharmacopoeia 2015 edition.

5.5 Determination process of quality marker

The four-stage process to determine the Q-markers, as well as the pathways of improving quality of CMs were illustrated in Figure 6, covering preparation, fingerprints/standards to establish standards of decoction extracts,

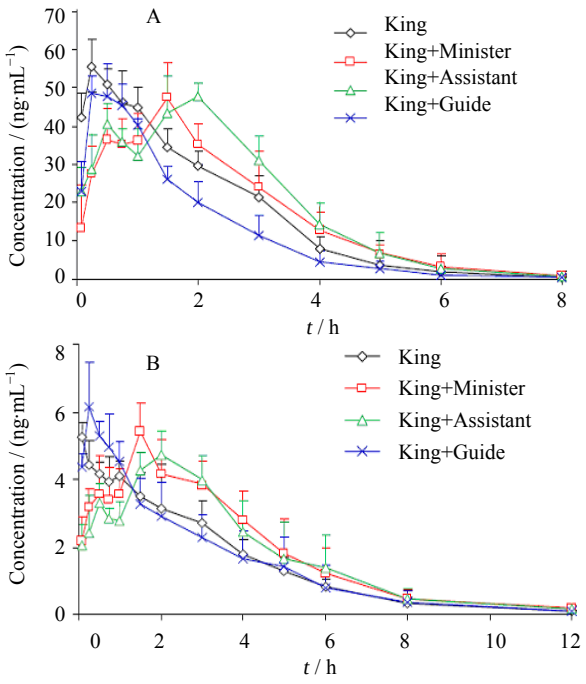

Figure 5 Effect on blood concentration of strychnine (A) and brucine (B) in minister, adjuvant, and guide drugs to strychnine (A) and brucine (B) in king drug

biological effects-oriented quality markers for quantification and qualification.

With respect to definition of quality markers, consideration should be given to the following basic principles: (1) Q-markers of CMs are chemical substances existing in raw materials, pieces, decoction, extracts, single herb formulation or compound formulation; (2) Q-markers should be associated with efficacy and/or to special bioactivity (efficacy or safety); (3) Q-markers should be determined by either qualitative or quantitative analysis methods for quality control of medicinal raw materials, pieces and processed products, decoction and compound preparations; (4) Based on the guidance of TCM theory, for instance compatibility of prescription (such as the King (*Jun*), minister (*Chen*), assistant (*Zuo*), and guide (*Shi*), the Q-markers should be related to traditional prescription; (5) Q-markers should be traceable in the quality control process from "Resource-Pieces-Processing-Extract" to product. Quality assessment system of traditional Chinese medicine could be therefore improved by Q-marker proposed through further investigations (Figure 7).

On March 4, 2016, the China State Council issued a legal document on promoting the healthy development of the pharmaceutical industry, indicating that the *Chinese Pharmacopoeia* must act as the core of the national drug standards and enhance the basic quality standards. In order to improve the scientific rationality and operability of the standard, as well as to strengthen the status of the standard, especially for specifications of quality control standards of traditional medicines, herbs and folk medicines and the productions, the new concept of quality marker was proposed.

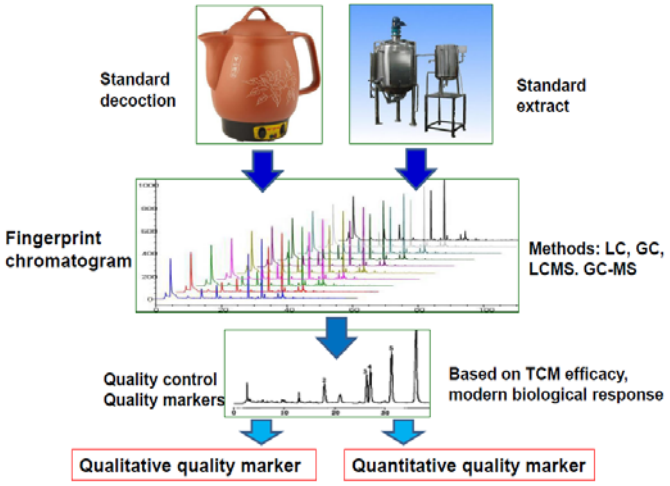

Figure 6 Key stages of study of Q-marker in Chinese medicines

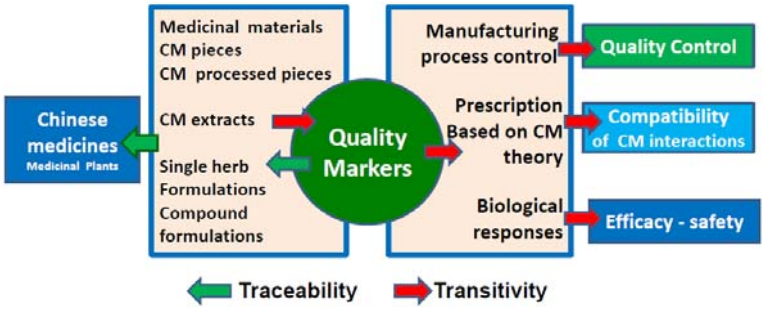

Figure 7 Quality management system on the basis of Q-marker in medical materials and products

Since Chinese pharmacopeia is the fundamental compendium in quality control of pharmaceuticals, the pharmaceutical industries should monitor the production process from field, manufacture process to market, in compliance with the requirement of pharmacopeia as to ensure the strategic security of the national drug products. It is a mandatory responsibility, especially to improve the sustainable development of CM products.

A new concept on the Q-marker of CM was discussed. Establishment of the quality control and tracing system in the whole process of CM production become essential. Q-marker is derived from the biosynthesis of traditional Chinese medicine-based organisms, and undergoes biotransformation and chemical change in processing and pharmaceutical processes. The Q-marker eventually exerts its clinical functions through drug delivery in the form of complex preparations.

6. Study design and significance of quality marker for quality assessment and production process control of CM products

6.1 Study design

Study design of Q-marker is a complex system, especially in complex materials, multiple-technical industries and long supply chains into the markets for quality assessment and production process control of CM products with transitivity and traceability.

The transitivity and traceability will inevitably require close attention to "what, where, when, and why" (4W) details at each stage of Q-markers of CM production from raw materials to the finished products. The established quality standards are enablers of many and various transitivity and traceability solutions, not a solution in them. It means that the transitivity and traceability system is readily to link between products and across borders in quality.

For designing the transitivity and traceability system of process control, the first step is to establish high-level objective and process scope. The scope should extend from procurement of all marker components to the depiction of final product units. Scope also identifies all the markers in transitivity and traceability process.

The second step is to assess the drug efficacy and safety and other risks associated with the CM products to be transitivity and traceability, and the quality management program already should be in relative place of the process control. There drive critical decisions on the levels of quality data to be captured and stored about production lots and finished products.

The third step is to report information on transitivity and traceability. The information includes identification of Q-markers of the raw materials, decoction-pieces, processed-pieces, extracts, intermediates and finished products in the production chain to answer "what, where,

when and why" questions.

6.2 Study significance

The quality assessment of CM products includes raw materials, pieces, decoction, preparation products, the basic quality elements, such as quality standards and quality control of the production process. The scientific research on determining the Q-marker plays an important role in quality standards and control of CM products. The important role in (1) to assess the authenticity of the clear source of raw materials; (2) to assess standard of processing procedure; (3) to assess the standard formulation process control based on reasonable reproduce; (4) to assess regulation from raw materials to finished products, and (5) to assess the Q-marker identified is associated with the safety and efficacy of the CM products.

According to our thinking mode and methods of investigation on quality assessment of CM products, we focus on the entire process, in terms of safety and effectiveness and quality control. The standard preparation of CM or CM decoction is not only the basis for studying Q-marker, but also the basis for transmission and traceability of the quality of CM products, covering herbs-pieces-processing-extract-intermediates. The new system with characteristics of transmission and traceability is expected to be established for regulation of CM quality.

Conflict of interest statement

The authors declare no conflict of interest.

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