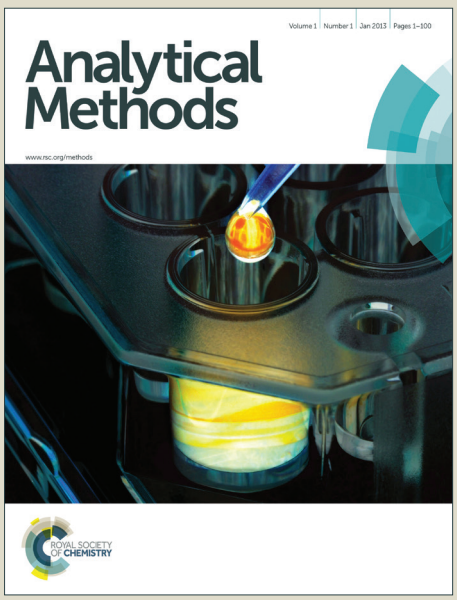
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Mass Spectrometry Based Molecular Profile Dissects the Complexity of Traditional Chinese Medicine

Short title: Molecular Profiling of TCM

Wenhu Liu[1#], Xiaojuan Guo[1#], Qiang Li[1#], Guang Xu[1#], Mengpei Feng[1#], Tianbing Guan[1], Leyu Yan[1], Wenna Nie[1], Tony Parker[2]*, Haitao Lv[1, 2]*,

[1]Chongqing University Innovative Drug Research Centre, School of Chemistry and Chemical Engineering, Chongqing 401331, P.R. China

[2]Tissue Repair and Regeneration Program, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, QLD 4059, Australia

[#]These authors are equally contributed to this manuscript.

Corresponding authors

Haitao Lv, Ph.D.

Email: haitao.lu@cqu.edu.cn

Phone: 86-2365678464

Fax: 86-2365678450

Abstract

Traditional Chinese Medicine (TCM) has yielded many medical benefits for the prevention and treatment of numerous diseases in China, Asian countries and western countries. Yet TCM is acting as an important complementation to modern western medicine for facilitating drug discovery and development. However, many questions have emerged with TCM development, highlighting the chemical complexity, the fact that TCM preparations contain undefined bioactive compounds that lead to unidentified mechanisms of action, toxicity and adverse effects. These questions concerning the therapeutic efficacy, molecular mechanisms of action and safety issues have prevented the broader applications of TCM in the biomedical niche related to the drug discovery and development. Herein, this review shall explore the applicability of metabolomics as a systems biology strategy for addressing above questions present in TCM field which is supposed to considerably promote modern drug discovery and development.

Key words: Molecular Profile, Metabolomics, Drug Discovery and Development, Traditional Chinese Medicine; Chemical Complexity

1 Introduction

Traditional Chinese Medicine (TCM) derives from a series of medical practices from ancient China that involves marked differences from modern medicine in regards to treatment tools and the philosophy for diagnosis and therapeutics. TCM continues to play an important role in health maintenance and improvement for many people in China, some other Asian countries [1] and even in some western worlds**.** Indeed, it has been reported that approximately 60% of the population of mainland China and Hong Kong, have consulted with traditional medicine practitioners at least once [1]. In addition, there is a growing population in some western countries that receive Traditional Chinese Medicine treatments [1]. Moreover, global statistics indicate that TCM is in fact an important complementary practice to western medicine with reference to disease diagnosis and treatments.

It is well known that refined mixtures of multiple Chinese medicinal plants or minerals, described as "TCM formula", are the primary format for the delivery of TCM in clinical practice by TCM doctors [2]. Indeed, the numbers of Chinese medicinal plants in a typical TCM formula vary from one to about a dozen. Importantly, the small-molecule compounds present in Chinese medicinal plant are chemically diverse and rather complex. Moreover, the number of distinct compounds varies from dozens to hundreds or even thousands in an undefined Chinese medicinal plant, extract or preparation and this complexity is what primarily accounts for the marked discrepancy in their physicochemical properties [3, 4]. It is understandable that the inherent chemical complexity of TCM results in many challenges for the modern scientific study of its purported therapeutic effects, the potential mechanisms of action of the many unidentified bioactive

compounds, and importantly their potential toxicity and adverse effects [5-8] (**Figure 1**). The identification of the chemical composition in TCM formulae is crucial in order to begin to address such challenges.

Conventionally, the chemical compounds in TCMs are often identified via the combined use of NMR, low-resolution mass spectrometry, UV, IR and X-ray technologies, following preparative steps using HPLC and various chromatography resin chemistries to isolate individual compounds for pharmacological screening [9-11]. While their efficiency is lower compared to high-throughput chromatography combined with mass spectrometry methods, these approaches typically embrace a number of shortcomings during the process of chemical isolation and identification. These include: cost effectiveness due to the time consuming nature of isolation of individual compounds for testing; loss of potentially important bioactive trace compounds; and loss of integrated and holistic effects of the original TCM contributed by multiple bioactive compounds, however they might be important complementation to the current advanced mass spectrometry assay.

Advances in analytical instrumentation such as mass spectrometry have markedly enhanced analytical efficiency, which have allowed researchers to profile and efficiently identify the compounds present in diverse TCMs [12-14]. Apart from being utilized to assess the global chemical profile of TCM formulae and medicaments, mass spectrometry approaches can be extended to explore the metabolic signatures of drug metabolism and pharmacokinetics to evaluate the processing of TCMs in the body. In addition, mass spectrometry based metabolomics studies can be used to investigate the biological mechanisms underpinning potential therapeutic actions and toxicity in the host [15-21]. This

may lead to the discovery of new molecular targets for pharmaceutical development into new classes of therapeutics for a range of important clinical ailments.

The capability of mass spectrometry is significantly underpinned by the prior separation of the molecular constituents of samples using chromatographic techniques (High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC)) [22-25]. Herein, the combined technologies of chromatography and mass spectrometry have been utilized for metabolomics assay as an emerged and novel systems biology driven omics method that is being broadly explored and exploited for addressing the complex questions raised from TCM niche, such as chemical profile, identification and characterization of chemical and biochemical compounds, dissection of therapeutic mechanisms and compatibility principle of TCM, etc [26-33].

During this review, we will emphasize the potential application of mass spectrometry-based chemical profiling to unveil the chemical composition of TCMs and their main mechanisms of action. In addition, we will focus on some selected cases that assist in better understanding the modern analytical approach being adopted to dissect the inherent chemical complexity of TCM. Finally, in this review we will also focus attention on the practicality of using mass spectrometry-based metabolomics to the study of TCM **(Figure 2).**

2 Mass spectrometry facilitates the identification of small-molecule compounds in TCM

Differences in the cultivation of particular species of plants used in TCM, such as location of production, results in different levels and ratios of molecular constituents in

individual plants. This variable chemical complexity basically leads to differences in the concentrations of bioactive compounds and toxic ingredients associated with specific TCMs [3, 4, 34]. This in turn partially accounts for the unpredictable uniformity and quality of TCM formulations, which markedly limits their clinical efficacy and broad acceptance in the international medical community [34-36]. However, the purported benefits of TCM provide an acceptable rationale for the comprehensive investigation of TCMs to identify the bioactive molecular constituents in order to strengthen the quality and reliability of TCM treatments.

In recent years, assorted analytical approaches have been applied for the identification of chemical compounds present in a diversity of TCM formulae [37-40]. These studies represent a growing body of evidence that suggest that biochemical profiling of TCM formulae may be a favorable strategy for application of scientific consistency and quality control. Currently, high-throughput chromatography / spectroscopy is critical for the establishment of chemical profiles of TCM with, for example, LC-UV, LC-ELSD to LC-MS and GC-MS commonly being used. Amongst these techniques, ultra-performance LC or GC -MS has the highest potential to identify large numbers of the diverse chemical compounds present in TCM preparations, rather than using conventional HPLC-UV [41-46].

For example, an Ultra-Performance Liquid Chromatography (UPLC-MS) based chemical profile was developed to identify the chemical composition of Yinchenhao Tang (YCHT), a typical TCM for treatments of diverse liver diseases [47, 48]. At the end of the study, 45 individual chemical species were provisionally identified [4] with holding promise in quality control of YCHT **(Figure 3).**

As mentioned above, LC-MS based chemical profiling is becoming widely accepted as a crucial technique for quality control of TCM preparations. LC-MS chemical profiles were established to evaluate the quality of the TCM *Radix scrophulariae*, which is found in various locations of production. From eight batches of *Radix scrophulariae* samples, six common chemical compounds were identified, including cetoside, angoroside C, cinnamic acid, harpagoside, sibirioside A and scrophuloside B [44]. In short, the quality of *Radix scrophulariae* from different origins can be controlled through selectively targeting and measuring these six common compounds [44].

In addition, simultaneous quantitation of key compounds using qualitative LC-MS and quantitative HPLC have been used for quality control of *Spirodela polyrrhiza*, a well-known TCM. A total of 18 flavonoids were identified, and 14 were described in this TCM plant for the first time. Furthermore, five common flavonoids were quantified from different sample batches of *Spirodela polyrrhiza*, including luteolin 8-C-glucoside, apigenin 8-C-glucoside, luteolin 7-O-glucoside, apigenin 7-O-glucoside and luteolin, [49].

The TCM plant *Fructus evodiae* (Wuzhuyu) is widely used in numerous preparations and its bioactive compounds include the indoloquinazoline alkaloids rutaecarpine, evodiamine and dehydroevodiamine [50]. The LC-MS method used to identify these compounds was initially developed for simultaneous determination of five indoloquinazoline alkaloids present in 12 commercial products from different pharmaceutical companies, and the method was designed as an overall quality control of those commercial products [50].

Taken together, the studies discussed above suggest that mass spectrometry coupled with liquid chromatography is currently one of the most valuable tools for

identification and quantification of the small-molecule constituency of TCM preparations. Moreover, it intrinsically implies that these techniques can be used for the simultaneous determination of key common compounds between batches of important TCMs and thereby can also be utilized as an approach for the implementation of TCM quality control.

3 Mass spectrometry characterizes metabolic signatures in TCM

Characterization of alterations to the metabolite profile of patients treated with TCM could typify the phenotype of biochemical processes after selected drugs are applied against a given disease. TCM treatment possesses similar therapeutic patterns as western medicine, via chemical compounds exerting biological activities; consequently, the characterization of metabolic signatures of bioactive compounds may improve our understanding of the therapeutic and toxic effects of TCM. This approach might permit optimization of doses and formulations used in clinical applications.

Generally, LC/MS based metabolite profiling and pharmacokinetic assays of bioactive compounds can be used to characterize primary metabolic signatures in TCM. [18, 19, 51, 52]. Scoparone is a bioactive compound derived from *Artemisia capillaris* Thunb, a plant that is broadly used to treat diverse liver diseases. Metabolite profiling using UPLC/MS allowed the identification four novel metabolites in scoparone treated rats, including scopoletin, isoscopoletin, isofraxidin and fraxidin [53] **(Figure 4).** The biochemical annotation performed in this study suggested that the generation of these metabolites largely accounted for the therapeutic action of the scoparone against broad liver disorders and jaundice[53].

Pharmacokinetics assays represent a potential tool for better understanding the compatibility principles of TCM formulae [54 55]; it can allow researchers to investigate the reasons why the therapeutic efficacies of most TCM formulae are better than the individual compounds contained in the TCM preparations alone. For example, the metabolic signatures arising from a pharmacokinetics assay of YCHT, composed of *Artemisia capillaris* Thunb, *Cape Jasmine* Fruit and *Radix ec Rhizoma Rhei* [55], revealed that scoparone, genposide and rhein were the major bioactive compounds, and that the combined use of the three TCMs resulted in far better pharmacokinetics parameters (e.g. *AUC*, $T_{1/2}$, and *Cmax*, etc.) compared to the individual extracts or dual combinations of two of the TCMs components [55] as the *AUC* was increased for each targeted compounds while combined use of tree TCMs was exploited, then *Cmax* approached a higher level accordingly, as well as $T_{1/2}$ come slowly that is relevant to utilization alone of each defined TCM, by which a sound therapeutic outcome might be captured at a holistic aspect.

Metabolite profiling of samples obtained from animals subjected to TCM treatments have also revealed differential patterns in the host metabolic response resulting from the administration of the TCM preparations. Of note, many of the metabolites observed in the *in vivo* studies were frequently identified as the constituents that accounted for the primary therapeutic efficacies of TCM medicaments. UPLC/MS-based metabolite profiling was explored to identify a total of 45 compounds present in YCHT [4]. Noticeably, that is in some degree partially different from 21 metabolites that were characterized in the study using rats [56]. However, the pharmacokinetics assay revealed

that only nine these compounds could be regarded as the potentially bioactive compounds in YCHT [56].

LC/MS-based metabolite profiling of *Fructus corni* (*Cornus officinalis)*, a well-known TCM herb, identified seven new compounds and three new metabolites for the first time [25] that markedly differed from *in vitro* chemical composition after dosing the medicine**.** PHY906 is a TCM preparation with therapeutic potential against metastatic colorectal cancer. Similarly, LC/MS-based metabolite profiling had identified 33 chemical compounds and new metabolites in patients with metastatic colorectal cancer, which is in contrast to a total 57 compounds and 27 metabolites found *in vitro* with the extract [52].

In short, characterization of metabolite signatures of TCM preparations is an effective method for the identification of potential bioactive compounds and their associated therapeutic actions. At this point, LC/MS-based metabolite profiling and pharmacokinetics assays are emerging as valuable tools for the study of TCM herbal preparations and their compatibility effects in treated patients and laboratory animals.

4 Mass spectrometry based metabolomics annotates systems toxicity of TCM

Good quality TCM is typically characterized by a high therapeutic efficacy against the targeted diseases with generally low toxicity for the host. However, the chemical complexity of some remedies means that those TCMs not only exert therapeutic actions on the targeted diseases, but also can cause toxicity for the host [57, 58]. In TCM preparations there are multiple bioactive compounds, and this is obviously different from western medicine, whereby medicines typically involve a single bioactive compound as

the active constituent. This means that the conventional approaches for assessment of toxicity as used in the western medicine is often not sufficient for the evaluation of toxicity of the TCM medicaments in the first instance.

In recent years, there has being growing evidence recognizing that mass spectrometry-based metabolomics is emerging as an advantageous tool for the assessment of toxicity in TCM by globally and simultaneously permitting profiling of metabolites from multiple metabolic pathways directly associated with cellular metabolism, tissue metabolism, energy metabolism, cell signaling, cell proliferation and development. The perturbed patterns in these metabolic pathways from toxicities caused by the use of TCM can be revealed by metabolomics [59-61].

Chuanwu, the mother root of *Aconitum carmichaelii* Debx., is a valuable ingredient utilized in numerous classic TCM preparations. But its use in a broader clinical context has been impeded due to the potential toxicity on the host [62]. Mass spectrometry-based metabolomics complemented by the use of ingenuity pathway bioinformatics analysis enabled phenotyping of multiple metabolic perturbations in rats treated with these root extracts and demonstrated that Chuanwu caused toxicity [62]. The perturbed metabolic pathways in rat were identified to be linked to the Chuanwu-induced toxicity, and several of the metabolites exhibiting alterations in their levels were identified as potential biomarkers that can allow the monitoring of the toxic process induced by the use of Chuanwu [62] **(Figure 5).**

Rational processes (denoted as "*Pao Zhi*" in TCM) prior to the clinical application of TCM medicaments could enhance therapeutic efficacy and attenuate possible toxic effects of the preparations [63]. Marked alterations in chemical composition of specific

TCMs resulting from a defined processing method has been shown to mostly account for the therapeutic advantages delivered by the processed TCM [64].

Ideally, mass spectrometry-based metabolomics can be utilized to differentiate the metabolic impact of pre- and post-processed Traditional Chinese medicinal plants on the host. For example, metabolomics analysis of rat urine was able to distinguish metabolic alterations induced by Chuanwu from metabolic effects induced by the processed product obtained from the same medicinal roots [61]. It was observed that the processed products induced significantly less metabolic perturbations when compared to the alterations caused by the Chuanwu [61]. Together these results suggest that in some cases the processed medicaments have markedly different chemical composition and that the toxic compounds may be reduced in the extract as a result of the processing.

Many of the bioactive compounds isolated from TCM medications have been observed to exert the preferred therapeutic actions against the targeted diseases. However, extensive purification leads to the generation of compound derived toxicity against the host, as a number of purified compounds isolated from the defined TCM plants have been observed to exert toxicity in multiple tissues while they were utilized alone [65], which can be efficiently characterized via mass spectrometry-based metabolomics method. GC/MS and LC/MS-based metabolomics approaches were utilized to unveil the pathological outcomes of aristolochic acid-induced nephrotoxicity, a compound isolated from *Caulis Aristolochiae manshuriensis*, a well-known medicinal plant commonly used in TCM [65]. Consistent with histopathological examinations, aristolochic acid-induced systemic metabolic disturbance was found to induce perturbations in free fatty acid generation,

energy and amino acid metabolism and deregulation in gut microbiota; consequences that evidently account for aristolochic acid-induced nephrotoxicity [65].

Similarly, alkaloids from the TCM plant *Aconitum* sp. were found to cause multiple metabolic perturbations in experiments using rats. The approach was carried out using a GC/MS-based metabolomics assay and preliminary data indicated that the toxic action of hypaconitine is different from that of aconitine and mesaconitine on the host [66].

In summary, mass spectrometry-based metabolomics is increasingly gaining importance as a tool for the characterization of systematic toxicity bought about by TCM. Importantly, this has significant consequences for the elucidation of the potential mechanisms of toxicity of many compounds that currently have unclear consequences as TCM remedies.

5 Mass spectrometry based metabolomics explores therapeutic discovery of TCM

TCM usually exerts therapeutic actions in the host via simultaneous interactions with multiple targets [67, 68]. Recently, there has been growing evidence to support mass spectrometry-based metabolomics as a promising strategy to explore the therapeutic actions of TCM [21, 69]. (**Figure 6**)

Alcohol liver disease is a significant health issue in modern societies [70, 71]. Yin Chen Hao Tang (YCHT) has been identified as a potential preventative treatment for ALD. An LC/MS-based metabolomics strategy was used to study metabolic perturbations caused by alcohol ingestion and the associated therapeutic actions of YCHT [20]. The results of this study revealed that YCHT treatment could markedly help to improve the recovery from metabolic perturbations caused by alcohol ingestion. The identification of

ceramide (d18: 1/25:0) provided further support for the involvement of the sphingomyelin signaling pathway in alcohol-induced hepatotoxicity, thus providing significant insights in the relevancy and therapeutic action of YCHT for the treatment of alcohol-induced hepatotoxicity [20] **(Figure 7).**

A LC/MS-based metabolomics strategy has also been applied for the screening and identification of biomarkers during the early period of acute myocardial infarction in wistar rats and to characterize the therapeutic actions of a TCM Shexiang Baoxin Pill [72]. This study identified obvious metabolic alterations related to inflammation, hypertrophy of cardiac muscle tissue and oxidative injury, potentially associated with the pathological changes during the early period of acute myocardial infarction [72]. Furthermore, the metabolomics data demonstrated that Shexiang Baoxin Pill pretreatment may have resulted in protective effects against the acute myocardial infarction (AMI) via positive regulation of steroid hormone biosynthesis [72].

TCM preparations are often used to treat diseases by exerting combinational effects of multiple medicinal plants; the basic prepared law is denoted as the 'compatibility principle' that was employed to guide the preparation of TCM formulae by TCM doctors. Mass spectrometry-based metabolomics is a useful strategy for annotating the compatibility principles of TCM. For example, a LC/MS -based metabolomics strategy was designed to study the synergetic effects of TCM Shuanglong Formula, composed of *Panax ginseng* and *Salvia miltiorrhiza*, against AMI in rats [73]. The resulting metabolomics data revealed that Shuanglong Formula might diminish cardiac injury due to myocardial infarction, by regulation of myocardial energy metabolism. Moreover, the study also indicated that Shuanglong Formula exerted holistic effects

effects related to the improvement of recovery from myocardial infarction when compared to *Panax ginseng* or *Salvia miltiorrhiza* treatments alone [73].

LC/MS-based metabolomics has also been exploited to develop a deeper understanding of the functional deficiencies in kidneys caused by thyroxine and reserpine, as well as the potential therapeutic actions of Liu Wei Di Huang Wan, and its associated compatibility advantages [74]. The obtained revealed metabolic perturbations of kidney yin deficiency caused by thyroxine and reserpine and the associated therapeutic efficacy of Liu Wei Di Huang Wan. Moreover, its compatibility advantage was also annotated at the metabolic level [74].

6 Future perspective

TCM has been of appreciable medical value for combating numerous diseases in China, Asian countries and even the western world in recent decades.

Recently progress in this regard has been achieved in the application of mass spectrometry-based molecular profile of chemicals derived from TCM preparations in order to answer questions about TCM. We believe that mass spectrometry-based molecular profile can be utilized to address the underlying scientific basis of some of the observed beneficial effects of TCM. Such effects are encompassed in the 'Yin-Yang' homeostasis theory of TCM; 'Qi'-blood theory; molecular basis of acupuncture; biochemical basis of TCM purse-diagnosis; and molecular signatures of TCM 'Syndrome' (**Figure 8**).

However, many barriers will remain following application of mass spectrometry-based based molecular profile strategies that we can apply to assist a better understanding

on the above questions of TCM. These barriers will typically include rapid and validated identification of small-molecule metabolites due to the limitation in the development of instrumental sciences; metabolic pathway mapping; functional annotation for the discovered small molecules; and chromatographic limitations for broader coverage of the vast diversity of small molecules present in a metabolome or TCM preparation. These shortcomings presented are narrowing our understanding to TCM questions we have and desire to answer.

Altogether, it is feasible that the explorative application of mass spectrometry-based molecular profile for chemical profiling may well promote progression in the scientific study of TCM. Particularly noticeable is the fact that mass spectrometry-based metabolomics strategies have the intrinsic potential to yield much broader investigational approaches to addressing the questions that underlie in TCM and to couple the biological effects of TCM to modern scientific understanding of human physiology.

Acknowledgements

This work was supported by National Natural Science Foundation of China Grant (81274175), the Startup Funding for "Hundred Young-Talent Scheme" Professorship provided by the Chongqing University in China (0236011104401), and the Queensland University of Technology Vice Chancellor's Research Fellowship Grant (150410-0070/08), Open Grants for Key Instrumental Platform Usage Provided by the Chongqing University in China (2013121564, 201406150008 and 201412150114), and Natural Science Foundation of Chongqing Grant (China) (CSTC2014JCYIA10109).

The authors declare no conflict of interest.

References

1. F. Cheung, *Nature*, 2011, 480, S82-S83.
2. X. Wang and B. Zhang, *Zhongguo Zhong Yao Za Zhi*, 2010, 35, 1346-1348.
3. G. Cao, C. Zhang, Y. Zhang, X. Cong, H. Cai, B. Cai, X. Li and J. Yao, *J Sep Sci*, 2011.
4. X. Wang, W. Sun, H. Sun, H. Lv, Z. Wu, P. Wang, L. Liu and H. Cao, *J Pharm Biomed Anal*, 2008, 46, 477-490.
5. C. Xiang, X. Qiao, Q. Wang, R. Li, W. Miao, D. Guo and M. Ye, *Drug Metab Dispos*, 2011, 39, 1597-1608.
6. X. L. Song, Y. Q. Gao, X. Niu and Y. C. Si, *Zhong Xi Yi Jie He Xue Bao*, 2003, 1, 177-179.
7. N. Gilbert, *Nature*, 2011, 480, S98-99.
8. Z. Xu, *Nature*, 2011, 480, S90-S92.
9. Q. H. Wang, H. X. Kuang, B. Y. Yang, Y. G. Xia, J. S. Wang and L. Y. Kong, *J Nat Prod*, 2011, 74, 16-20.
10. H. X. Kuang, B. Y. Yang, Y. G. Xia and Q. H. Wang, *Molecules*, 2011, 16, 5833-5839.
11. X. Wu, J. Ruan, V. C. Yang, Z. Wu, J. Lou, H. Duan, J. Zhang, Y. Zhang and D. Guo, *Fitoterapia*, 2012, 83, 166-169.
12. Y. Zhang, J. Pan, J. Zhong, Y. Wang, X. Fan and Y. Cheng, *J Chromatogr A*, 2012.
13. L. P. Kang, K. Yu, Y. Zhao, Y. X. Liu, H. S. Yu, X. Pang, C. Q. Xiong, D. W. Tan, Y. Gao, C. Liu and B. P. Ma, *J Pharm Biomed Anal*, 2012.
14. X. Dai, R. Hu, C. Sun and Y. Pan, *J Chromatogr A*, 2011.

15. G. Lu, Z. Dong, Q. Wang, G. Qian, W. Huang, Z. Jiang, K. S. Leung and Z. Zhao, *Planta Med*, 2010, 76, 825-830.

16. X. Wang, B. Yang, A. Zhang, H. Sun and G. Yan, *J Proteomics*, 2012, 75, 1411-1427.

17. P. Jiang, W. Dai, S. Yan, Z. Chen, R. Xu, J. Ding, L. Xiang, S. Wang, R. Liu and W. Zhang, *Mol Biosyst*, 2011, 7, 824-831.

18. L. Chen, L. Liu, W. Zhu, H. Zhang, Z. Yan and H. Liu, *Pharmazie*, 2011, 66, 684-689.

19. K. Duan, Z. Yuan, W. Guo, Y. Meng, Y. Cui, D. Kong, L. Zhang and N. Wang, *J Ethnopharmacol*, 2011, 135, 201-208.

20. X. Wang, H. Lv, H. Sun, L. Liu, B. Yang, W. Sun, P. Wang, D. Zhou, L. Zhao, S. Dou, G. Zhang and H. Cao, *J Pharm Biomed Anal*, 2008, 48, 1161-1168.

21. S. Yan, B. Wu, Z. Lin, H. Jin, J. Huang, Y. Yang, X. Zhang, Z. Shen and W. Zhang, *Mol Biosyst*, 2009, 5, 1204-1213.

22. Y. Qi, L. Qu, Y. Wu and G. Fan, *J Ethnopharmacol*, 2011, 137, 487-494.

23. M. Chen, M. Su, L. Zhao, J. Jiang, P. Liu, J. Cheng, Y. Lai, Y. Liu and W. Jia, *J Proteome Res*, 2006, 5, 995-1002.

24. Z. H. Su, S. Q. Li, G. A. Zou, C. Y. Yu, Y. G. Sun, H. W. Zhang, Y. Gu and Z. M. Zou, *J Pharm Biomed Anal*, 2011, 55, 533-539.

25. G. Cao, H. Cai, Y. Zhang, X. Cong, C. Zhang and B. Cai, *J Pharm Biomed Anal*, 2011, 56, 118-125.

26. Y. Chen, Z. Zhao, H. Chen, T. Yi, M. Qin and Z. Liang, *Phytochem Anal*, 2014.

27. M. Guo, L. Zhang, H. Liu, L. Qin, Z. Zhang, X. Bai and X. Gao, *J Sep Sci*, 2014, 37, 2844-2850.

28. J. S. Tian, G. J. Peng, X. X. Gao, Y. Z. Zhou, J. Xing, X. M. Qin and D. Guan-Hua, *J Ethnopharmacol*, 2014, 158PA, 1-10.

29. J. Li, L. Yang, Y. Li, Y. Tian, S. Li, S. Jiang, Y. Wang and X. Li, *Mol Med Rep*, 2015, 11, 1324-1333.

30. P. Ji, Y. Wei, H. Sun, W. Xue, Y. Hua, P. Li, W. Zhang, L. Zhang, H. Zhao and J. Li, *J Chromatogr B Analyt Technol Biomed Life Sci*, 2014, 973C, 45-54.

31. W. Yao, H. Gu, J. Zhu, G. Barding, H. Cheng, B. Bao, L. Zhang, A. Ding and W. Li, *Anal Bioanal Chem*, 2014, 406, 7367-7378.

32. G. Dong, J. Wang, P. Guo, D. Wei, M. Yang and L. Kong, *Mol Biosyst*, 2015, 11, 407-417.

33. S. N. Zhang, X. Z. Li, Y. wang, N. zhang, Z. M. Yang, S. M. Liu and F. Lu, *J Ethnopharmacol*, 2014, 156, 290-300.

34. K. Chan, *Trends Pharmacol Sci*, 1995, 16, 182-187.

35. D. Chen, S. S. Zhao and K. S. Leung, *J Sep Sci*, 2009, 32, 2892-2902.

36. L. Yi, L. W. Qi, P. Li, Y. H. Ma, Y. J. Luo and H. Y. Li, *Anal Bioanal Chem*, 2007, 389, 571-580.

37. L. Wang, G. Gao, Y. Bai, W. Luo, C. Lin and Q. Jia, *Pharm Biol*, 2011, 49, 595-601.

38. B. Avula, Y. H. Wang, C. S. Rumalla, Z. Ali, T. J. Smillie and I. A. Khan, *J Pharm Biomed Anal*, 2011, 56, 895-903.

39. Y. Zhang, G. Cao, J. Ji, X. Cong, S. Wang and B. Cai, *J Sep Sci*, 2011, 34, 1429-1436.

40. W. N. Lin, H. Y. Lu, M. S. Lee, S. Y. Yang, H. J. Chen, Y. S. Chang and W. T. Chang, *Am J Chin Med*, 2010, 38, 205-218.

41. X. Wang, H. Lv, H. Sun, X. Jiang, Z. Wu, W. Sun, P. Wang, L. Liu and K. Bi, *J Sep Sci*, 2008, 31, 9-15.

42. Y. Lu, W. Ma, R. Hu, A. Berthod and Y. Pan, *J Chromatogr A*, 2009, 1216, 4140-4146.

43. Y. Jiang, S. P. Li, Y. T. Wang, X. J. Chen and P. F. Tu, *J Chromatogr A*, 2009, 1216, 2156-2162.

44. J. Jing, C. O. Chan, L. Xu, D. Jin, X. Cao, D. K. Mok, H. S. Parekh and S. Chen, *J Pharm Biomed Anal*, 2011, 56, 830-835.

45. Y. Ni, M. Mei and S. Kokot, *Anal Chim Acta*, 2012, 712, 37-44.

46. R. Pan, F. Guo, H. Lu, W. W. Feng and Y. Z. Liang, *J Pharm Biomed Anal*, 2011, 55, 391-396.

47. H. Lv, H. Sun, X. Wang, W. Sun, G. Jiao, D. Zhou, L. Zhao, H. Cao and G. Zhang, *J Sep Sci*, 2008, 31, 659-666.

48. X. Wang, H. Lv, H. Sun, W. Sun, L. Liu, P. Wang and H. Cao, *Arzneimittelforschung*, 2008, 58, 336-341.

49. X. Qiao, W. N. He, C. Xiang, J. Han, L. J. Wu, D. A. Guo and M. Ye, *Phytochem Anal*, 2011, 22, 475-483.

50. Y. Zhou, S. H. Li, R. W. Jiang, M. Cai, X. Liu, L. S. Ding, H. X. Xu, P. P. But and P. C. Shaw, *Rapid Commun Mass Spectrom*, 2006, 20, 3111-3118.

51. Y. Ling, M. Chen, K. Wang, Z. Sun, Z. Li, B. Wu and C. Huang, *Biomed Chromatogr*, 2011.

52. W. Zhang, M. W. Saif, G. E. Dutschman, X. Li, W. Lam, S. Bussom, Z. Jiang, M. Ye, E. Chu and Y. C. Cheng, *J Chromatogr A*, 2010, 1217, 5785-5793.

53. X. Wang, H. Lv, H. Sun, L. Liu, W. Sun and H. Cao, *Rapid Commun Mass Spectrom*, 2007, 21, 3883-3890.

54. Q. Zheng, P. F. Yue, B. Wu, P. Y. Hu, Z. F. Wu and M. Yang, *J Ethnopharmacol*, 2011, 137, 221-225.

55. H. Lv, H. Sun, W. Sun, L. Liu, P. Wang, X. Wang and H. Cao, *Phytomedicine*, 2008.

56. X. Wang, H. Sun, A. Zhang, G. Jiao, W. Sun and Y. Yuan, *Analyst*, 2011, 136, 5068-5076.

57. W. Y. Jiang, *Discov Med*, 2005, 5, 455-461.

58. M. Wang, R. J. Lamers, H. A. Korthout, J. H. van Nesselrooij, R. F. Witkamp, R. van der Heijden, P. J. Voshol, L. M. Havekes, R. Verpoorte and J. van der Greef, *Phytother Res*, 2005, 19, 173-182.

59. C. Ma, K. Bi, M. Zhang, D. Su, X. Fan, W. Ji, C. Wang and X. Chen, *J Pharm Biomed Anal*, 2010, 53, 559-566.

60. B. Sun, L. Li, S. Wu, Q. Zhang, H. Li, H. Chen, F. Li, F. Dong and X. Yan, *Anal Biochem*, 2009, 395, 125-133.

61. X. Wang, H. Wang, A. Zhang, X. Lu, H. Sun, H. Dong and P. Wang, *J Proteome Res*, 2012, 11, 1284-1301.

62. H. Dong, A. Zhang, H. Sun, H. Wang, X. Lu, M. Wang, B. Ni and X. Wang, *Mol Biosyst*, 2012.

63. H. Sun, B. Ni, A. Zhang, M. Wang, H. Dong and X. Wang, *Analyst*, 2012, 137, 170-185.

64. H. S. Yu, B. P. Ma, L. P. Kang, T. Zhang, F. J. Jiang, J. Zhang, P. Zou, Y. Zhao, C. Q. Xiong, D. W. Tan, X. B. Song and K. Yu, *Chem Pharm Bull (Tokyo)*, 2009, 57, 1011-1014.

65. Y. Ni, M. Su, Y. Qiu, M. Chen, Y. Liu, A. Zhao and W. Jia, *FEBS Lett*, 2007, 581, 707-711.

66. B. Sun, S. Wu, L. Li, H. Li, Q. Zhang, H. Chen, F. Li, F. Dong and X. Yan, *Rapid Commun Mass Spectrom*, 2009, 23, 1221-1228.

67. H. Wei, C. Hu, M. Wang, A. M. van den Hoek, T. H. Reijmers, S. Wopereis, J. Bouwman, R. Ramaker, H. A. Korthout, M. Vennik, T. Hankemeier, L. M. Havekes, R. F. Witkamp, E. R. Verheij, G. Xu and J. van der Greef, *PLoS One*, 2012, 7, e30332.

68. X. Wang, B. Yang, H. Sun and A. Zhang, *Anal Chem*, 2012, 84, 428-439.

69. Y. M. Lao, J. G. Jiang and L. Yan, *Br J Pharmacol*, 2009, 157, 1128-1141.

70. W. Z. Mehal, *J Hepatol*, 2012, 56, 311-312.

71. T. Gunji, N. Matsuhashi, H. Sato, K. Iijima, K. Fujibayashi, M. Okumura, N. Sasabe and A. Urabe, *J Clin Gastroenterol*, 2011, 45, 808-813.

72. P. Jiang, W. Dai, S. Yan, Z. Chen, R. Xu, J. Ding, L. Xiang, S. Wang, R. Liu and W. Zhang, *J Ethnopharmacol*, 2011, 138, 530-536.

73. X. Liang, X. Chen, Q. Liang, H. Zhang, P. Hu, Y. Wang and G. Luo, *J Proteome Res*, 2011, 10, 790-799.

74. P. Wang, H. Sun, H. Lv, W. Sun, Y. Yuan, Y. Han, D. Wang, A. Zhang and X. Wang, *J Pharm Biomed Anal*, 2010, 53, 631-645.

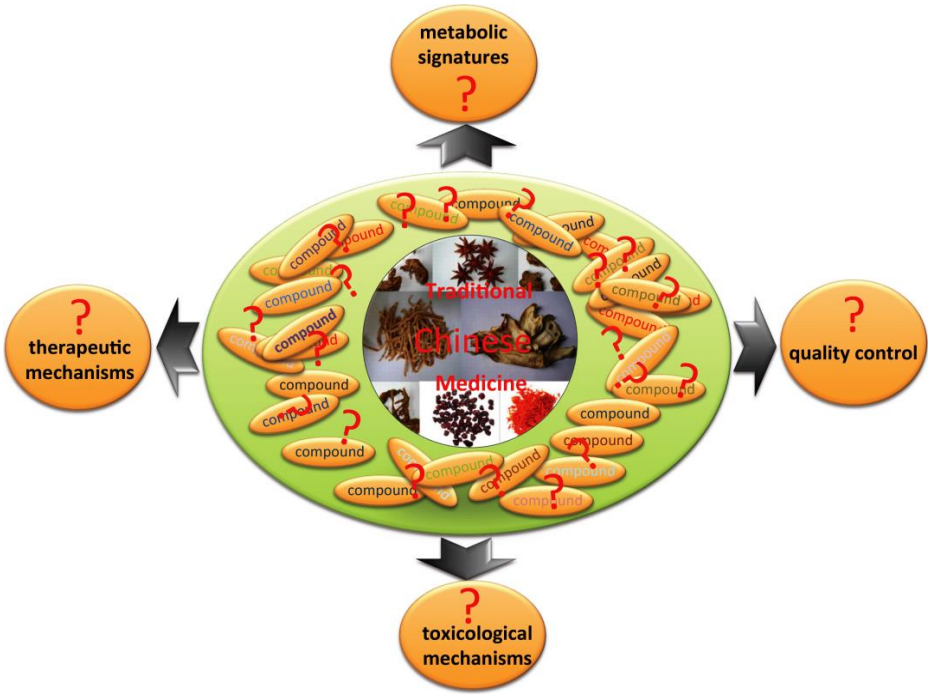


Figure 1. Schematic illustration that manifests the complexity of chemical compounds blends contained in Traditional Chinese Medicine formulae. The complexity and lack of definition of Chinese medicinal plants largely accounts as the major impediment for the development of TCM globally and acceptance by modern science. Furthermore, there are several important questions in the TCM field that cannot be adequately addressed due to the chemical complexity of TCM formulae and Chinese medicinal plants, which intrinsically involve uncertainties such as the identity of bioactive constituents and related metabolic features, undefined therapeutic mechanisms, and unstable quality of the basic products. Additionally, the toxicity of TCM formulae cannot be easily predicted.

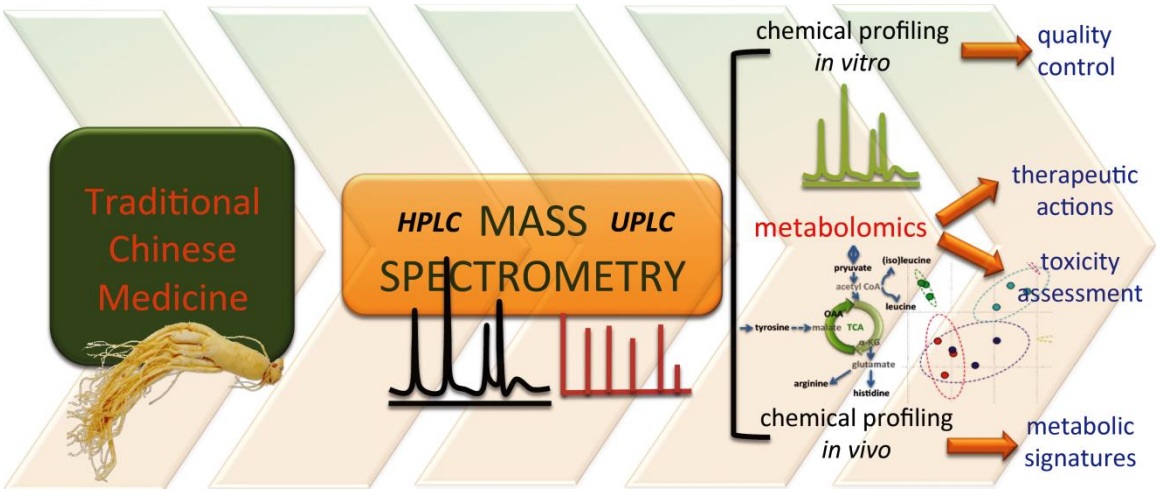


Figure 2. Mass spectrometry can help to identify and characterize *in vitro* and *in vivo* chemical compounds and to perform rational quality control and establish metabolic signatures of TCM formulae and Chinese medicinal plants. There is a significant potential application for metabolomic studies of the toxicity and therapeutic actions of TCM formulae and Chinese medicinal plants for the investigation of a large range of biological contexts.

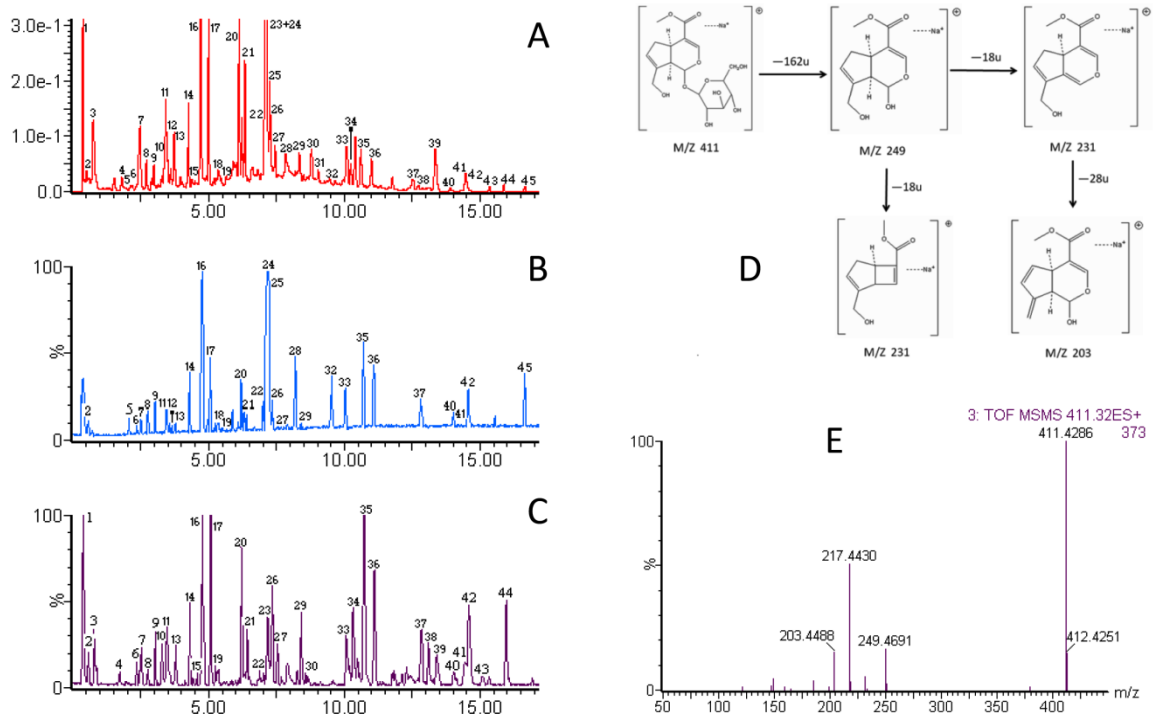


Figure 3. Chemical characterization of *in vitro* extract of *Artemisia capillaris* (Yin-Chen-Hao) by UPLC-UV-Q-TOF. (A) UPLC-UV chromatogram of an extract of *A. capillaris* at 254 nm. (B) TIC chromatogram of the same extract using Q-TOF detection in positive ESI mode. (C) TIC chromatogram of the extract using Q-TOF detection in negative ESI mode. (D) Fragmentation pathway of the geniposide identified in the *A. capillaris* extract by collision-induced dissociation. (E) Parent and products mass spectra of geniposide analyzed from the *A. capillaris* extract in positive ion mode. The figure was reproduced from Wang, X., et al. (2008), with permission Copyright © 2008, Elsevier.

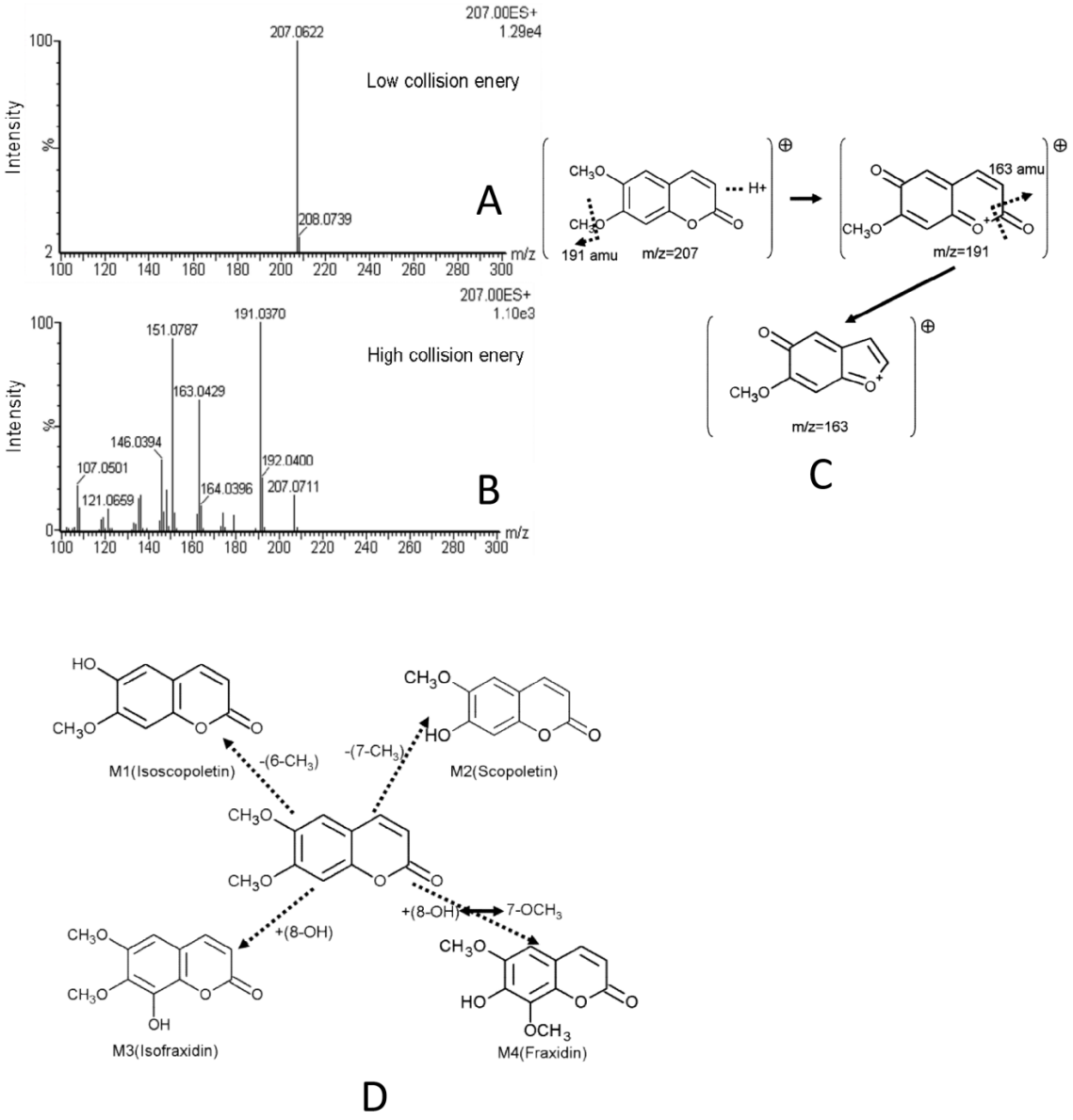


Figure 4. Metabolite characterization of the bioactive compound scoparone in the extract of *A. capillaris*. (A) and (B), mass spectra of full scan and product ion scan of scoparone; (C) The fragmentation pathway of the scoparone using collision-induced dissociation. (D) Mass spectrometry-based targeted screening of scoparone in rats allowed for the identification of several derivatives of this molecule as a consequence of the metabolism of this bioactive compound in the animal's blood. The figure was reproduced from Wang, X., et al. (2007), with permission Copyright © 2007, Willey.

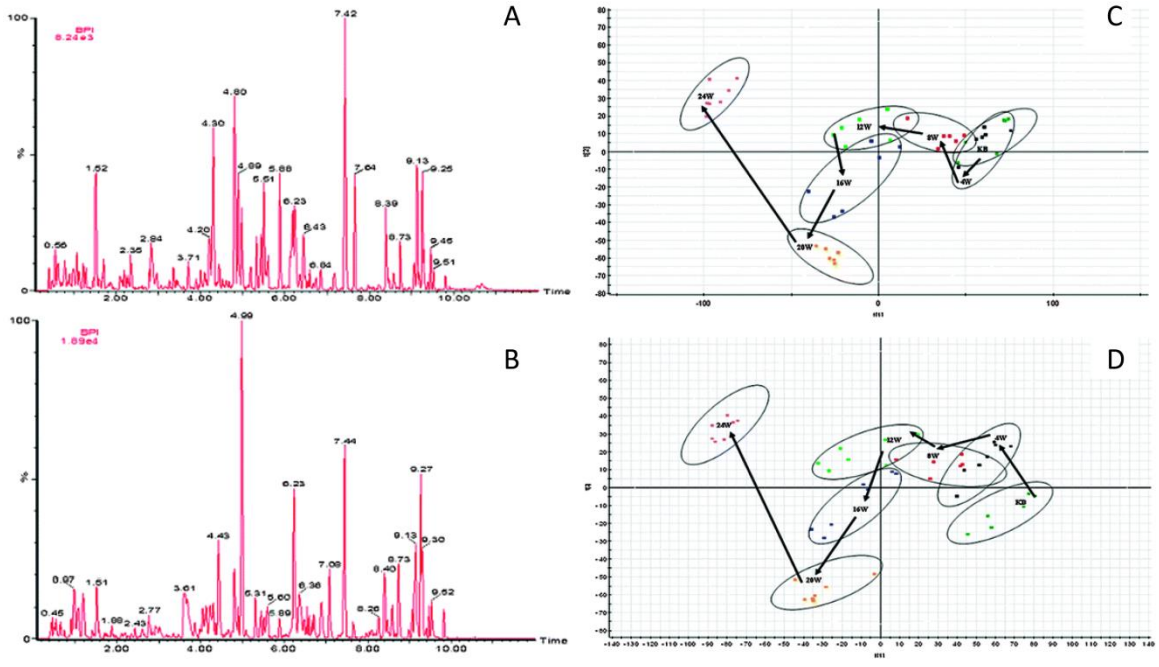


Figure 5. Base peak intensities (BPI) chromatograms of urine sample of quality control analyzed by UPLC-HDMS using ESI detection in positive mode. (A) and negative mode (B). The principal components score plots reflect time-dependent fluctuations of numerous metabolites after oral administration of high-doses of Chuanwu to rats. PCA for metabolites detected using ESI in positive mode (C) and negative mode (D). KB, control group; 4W, 1st month; 8W, 2nd month; 12W, 3rd month; 16W, 4th month; 20W, 5th month; 24W, 6th month. The figure was reproduced from Dong, H., et al. (2012), with permission Copyright © 2012, RSC.

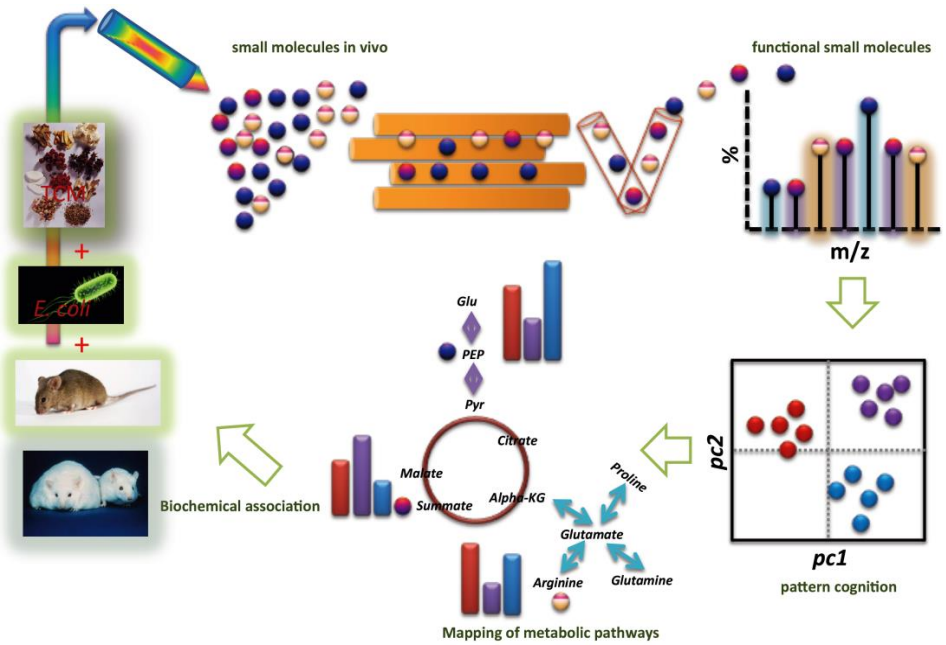


Figure 6. The workflow for a mass spectrometry-based metabolomics strategy to investigate Traditional Chinese Medicine formulae and Chinese medicinal plant extracts. The use of metabolomics permits annotation of the complexity of TCM systems by globally profiling small molecules from the TCM medications and the host. Likewise, the biochemical responses of a host, with a defined disease, to the TCM can be characterized by identifying differential metabolites and their associations to specific metabolic pathways. The results and their proof-of-knowledge associations to specific metabolic pathways may help to elucidate mechanisms of action of the TCM.

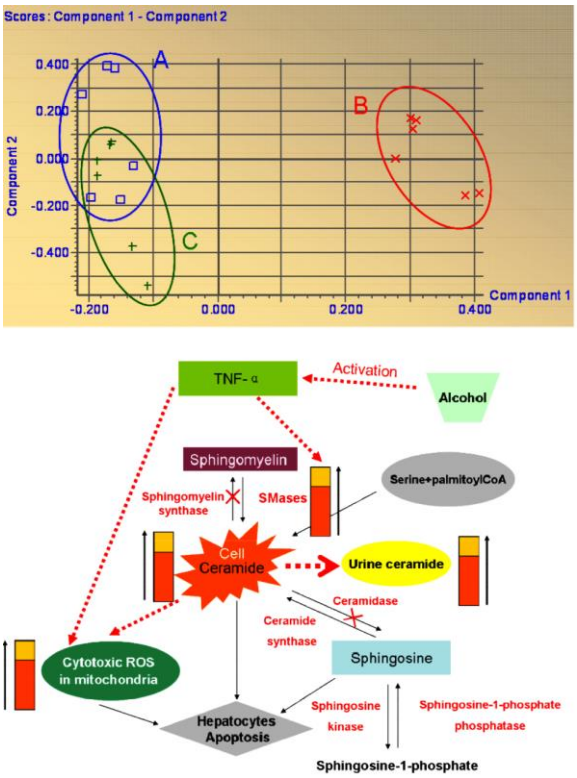


Figure 7. Mass spectrometry-based metabolomics is suitable to annotate the therapeutic action of Yin-Chen-Hao-Tang (YCHT) upon treatment of hepatotoxicity caused by drinking alcohol. Upper panel: Scores plot resulting from PCA of the data from reversed-phase UPLC-ESI-TOF-MS urine metabolomic determination from control rats (A); alcohol-treated rats (B) and rats treated with YCHT following an alcohol-treatment (C). Rats' urine samples were collected at day 7 from the alcohol treatments. Symbols indicate: control rats (□); alcohol-treated rats (×) and rats treated with alcohol followed by YCHT-treatment (+). **Lower panel:** Proposed metabolic pathway to explain the relationship between alcohol-induced hepatotoxicity and an increased concentration of ceramide (d18: 1/25:0) in the urine of the animals. The figure was reproduced from Wang, X., et al. (2008), with permission Copyright © 2008, Elsevier.

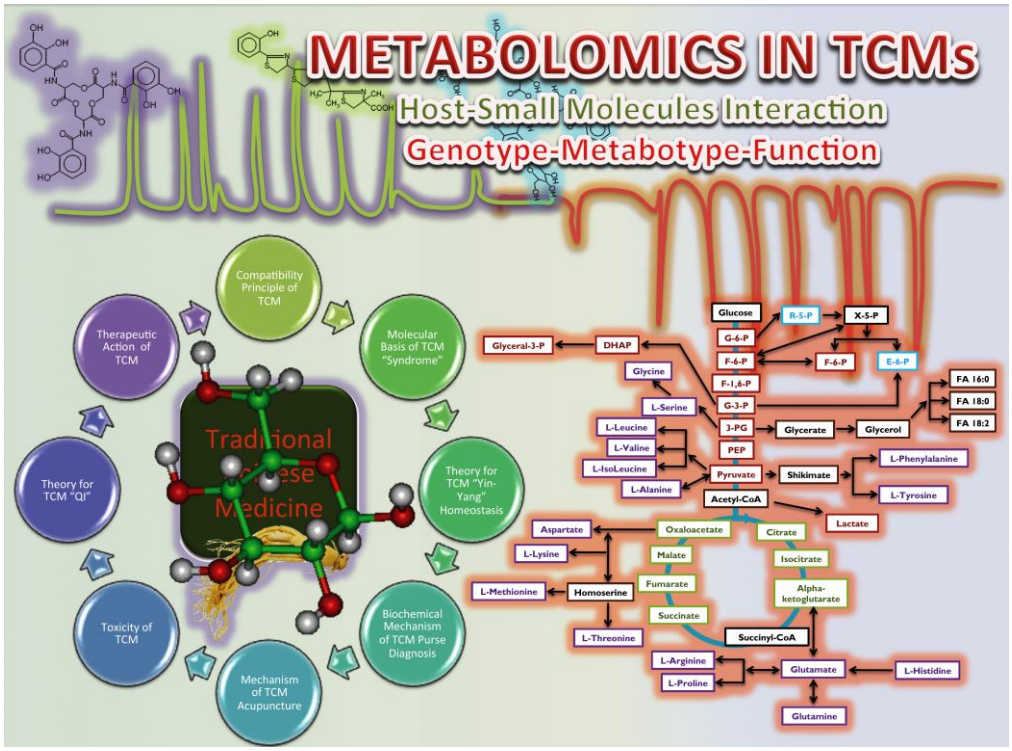
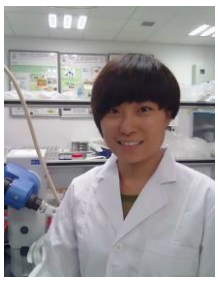


Figure 8. Schematic overview of the potential applicability of mass spectrometry-based metabolomics for dissection of a diversity of biological questions related to TCM disciplines. The metabolome is a down-stream consequence of gene expression; consequently, changes in expression patterns of diverse metabolites may allow the typification of functional regulation of the up-stream gene expression as a consequence of the utilization of TCM practices. Thus, well-defined mass spectrometry-based metabolomics strategies will permit the establishment of mechanistic links between genotype and metabotype. Moreover, it allows identification and annotation of meaningful bioactive compounds (effectors and products) via global profiling of small molecules in the host organism, as well as recognizing their interactions with the TCM treatment.

Authors' biographies and photographs

Mr Wenhu Liu acquired his B.SC (Pharmaceutical Sciences) from The Lanzhou University in 2004, and hecontinually pursued his Master Degree (Medicinal Chemistry) at the same university in 2009, then he started to take over a faculty position in The North Sichuan Medical College where his research concentrates on High throughput screening of bioactive lead compounds from natural resources. Since September 2014, he becomes a Ph.D student at the Laboratory for Functional Omics and Innovative Chinese Medicines in the Chongqing University Innovative Drug Research Centre (Chongqing, China). His research of interests is primarily centred on microbial metabolomics integrated with genetic technology targeting host-pathogen interaction, siderophore biology, and molecular phenotyping of urinary tract infections with the complex kidney disorders.



Miss Xiaojuan Guo finished her B.SC. with major in Pharmaceutical Sciences at The Lanzhou University in 2013, and then she starts to pursue her M.SC. in The Chongqing University Innovative Drug Research Centre, she currently works with Prof. Haitao Lu at The Laboratory for Functional Omics and Innovative Chinese Medicines. Her research projects were developed to investigate the pathogenesis of type 2 diabetes and urinary tract infections, and facilitate biomarker discovery for molecular phenotyping and diagnosis by employing the combined strategy of metabolomics and lipidomics, as well as dissects molecular mechanisms of the selected Chinese medicinal plants against those complex diseases.

Miss Qiang Li obtained B.SC with major in Food Quality and Safety from The East China University of Technology and Science, and she is currently pursuing the Master Degree (Pharmaceutical Sciences) at The Laboratory for Functional Omics and Innovative Chinese Medicines in the Chongqing University Innovative Drug Research Centre (Chongqing, China). Her Research focuses on Metabolomics combined with Chemical Biology approach as a general discovery tool for better understanding the pathogenesis of skin cell injury caused by chemical/biochemical factors as well as the therapeutic mechanisms of the selected Chinese medical plants against this healthy issue at a translational medicine setting.

Mr Guang Xu is a master student at the Laboratory for Functional Omics and Innovative Chinese Medicines at the Chongqing University Innovative Chinese Medicines. China Pharmaceutical University conferred his B.SC. With major in Pharmaceutical Sciences (Marketing), and then he moved to The Chongqing University immediately where he is engaging in his gradate study under supervision of Prof. Haitao Lu, and his projects are explored for the discovery and identification of novel siderophore compounds by combining omics technology with genetic approach, as well as therapeutic discovery of the selected natural compounds derived from Chinese medicinal plants or TCM formulae.

Miss Mengpei Feng is currently a graduate student at the Laboratory for Functional Omics and Innovative Chinese Medicines (FOCM-Future Opinion Creates Medicine), and her research is mostly interested in development of functional omics and its application to the study of molecular mechanisms of Hepatocelluar Carcinoma (HCC), discovery and characterization of functional compounds present in the defined traditional Chinese medicine. She finished her college study (B.SC. Majoring in Phytomedicine), at Hebei University (Baoding, China), and then she pursues her Master Degree with major in Pharmaceutical Sciences at the Chongqing University in China since September 2014.

Dr. Haitao Lv is a Professor of Chemical Biology, Metabolomics and Chinese Medicines at the Chongqing University Innovative Drug Research Centre since July 2012, after he finished his postdoctoral training at Albert Einstein College of Medicine and Washington University School of Medicine, MIT between 2009 and 2012, under supervision of Profs. Irwin J. Kurland, Jeffrey P. Henderson, and Peter Dedon. Dr Lv received his PhD in June 2009 at Heilongjiang University of Chinese Medicine. Dr. Lv is a high-profile young scientist with over 8-years research and management experience in the fields of metabolomics, chemical biology and traditional Chinese Medicines. Over past 5 years, he has authored 30 peer-reviewed papers in many high-profile journals, as well as 30+ conference publications. Dr. Lv has received two national competitive research grants and 5 research fellowships from different funding bodies and universities in China, USA, and Australia. Dr. Lv attends Editorial Board of journals as well: Bioanalysis, Current Metabolomics, and acts as a peer referee for National Natural Science Foundation of China, plus 20+ high-profile journals.