An overview of recent developments in metabolomics and proteomics – phytotherapeutic research perspectives

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ABSTRACT
In recent years, medicinal plants have gained much attention as potential source of bioactives for the development of novel herbal drugs for primary healthcare. However, phytotherapeutic mechanisms of action of phytomedicines need to be explored comprehensively. Out of the available strategies for the said purpose, metabolomics is one, which results in the production of inclusive metabolite profiles providing a clear understanding of diagnostic changes in the levels of metabolites, leading to therapeutic monitoring of drug targets through the elucidation of metabolic pathways. On the other hand, proteomics is also a powerful strategy to deal with systematic protein expression analysis and analyze different biomarkers compared to metabolomics during drug treatment. Currently, 1H nuclear magnetic resonance spectroscopy, mass spectrometry (MS), Fourier transform infrared spectroscopy, gas chromatography equipped with mass spectrometry, liquid chromatography-MS coupled with multivariate statistical techniques, such as principal component analysis, partial least square and orthogonal partial least square are among the widely applicable analytical tools for metabolite profiling, whereas two-dimensional electrophoresis and matrix-assisted laser desorption ionization time-of-flight -MS are the most accepted analytical methods for proteomic biomarker investigation. The present review summarizes the recent developments and perspectives of the biomarker investigation strategies so as to elaborate their imperative role for sustainable herbal drug developments.

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1. Introduction
Herbal medicines with diverse bioactivities are increasingly being used worldwide as alternative drugs in the management of infectious diseases and for health promotion (Patwardhan & Vaidya 2010; Na et al. 2011). Medicinal plants are known to consist of a wide array of bioactive compounds usually resulting from secondary metabolism based on enzyme-catalyzed reactions. The main enzymes involved in the metabolism include microsomal monooxygenases, mitochondrial and cytosolic oxidases, reductases, peroxidases, hydrolytic enzymes, monoamine oxidase, diamine oxidase, alcohol dehydrogenase, cyclooxygenase, aldehyde dehydrogenase, molybdenum hydroxylase, glucuronosyl transferases, N-acetyltransferases, sulfotransferases, methyltransferases and glutathione-S-transferases (Williams 2002; Dekant 2009; Deenen et al. 2011a; Deenen et al. 2011b; Roskar & Lusin 2012). The metabolome in a biological media consists mainly of all naturally occurring organic substances including xenobiotics along with their bio-transformation products, except polymers of biological origin (Tagore et al. 2014). With the escalating usage of herbal medicines, public health concerns based on drug–herb interactions are getting much attention among the scientific community. Moreover, modernization and development of herbal remedies require comprehensive evaluation of metabolites and underlying metabolic pathways and elucidation of their mechanism of actions. Metabolomics and proteomics are playing a key role toward meeting the above-said challenging tasks.

The qualitative and quantitative analysis of all the metabolites present in an organism at a particular
time is called metabolomics. Metabolomics is the most imperative approach that provides metabolite profiles for studying biochemical networks and gives a clear understanding regarding the effects of drug–herbal interventions (Patwardhan & Vaidya 2010; Na et al. 2011; Lao et al. 2014; Tagore et al. 2014). Comparatively, proteomics is a tool for the therapeutic monitoring of the altered proteins as possible drug targets, and provides a clear understanding of the herbal drug’s mechanism of actions (Lao et al. 2014). The present review, therefore, aims at a comprehensive elaboration of the strategical developments and perspectives of metabolomics and proteomics for herbal drugs development by explaining metabolite profiling of metabolic pathways and differentially expressed proteomes during the course of disease treatment.

2. Metabolomics

Metabolomics explores the ways by which living systems show multiparametric response toward a pathophysiological stimuli (Nicholson et al. 1999; Nicholson et al. 2002; Theodoridis et al. 2008). The metabolomic investigations result in the construction of comprehensive metabolite profiles, leading to a clear understanding of diagnostic changes in the levels of different organic metabolites (with low molecular weight) in samples, namely, organ extracts or biofluids, and hence generate metabolic phenotypes (Gavaghan et al. 2002). The metabolic phenotypes are considered to be highly emphatic in exploring unexpected insights of the biological processes, that is, progression of disease, ageing, response to therapy and toxicity, etc. In short, metabolomics is metabolome analysis under some specific conditions (Nicholson et al. 1999; Fiehn 2002; Beger et al. 2010). Metabolites are usually classified into endogenous and exogenous metabolites. Primary and secondary metabolites are subclasses of endogenous metabolites. Primary endogenous metabolites (such as amino acids or glycolysis intermediates) with broader distribution in living systems are basically involved in the primary life processes, for example, growth and reproduction, whereas with limited distribution are secondary metabolites (such as alkaloids or hormones), which are very much species-specific and their synthesis in living systems is specific toward a specific biological function (Herbert 1989; Roux et al. 2011).

Compared to endogenous metabolites, exogenous metabolites are recognized as bio-transformed products of exogenous compounds. These bio-transformations are involved in enzymatic modifications, during which an original molecule is modified with the introduction of some functional group, or may also be the result of conjugation (Sharge & Yu 1999). Metabolic studies involving herbal drugs are considered to be a formidable task because of their complicated constituents and complex metabolic pathways. In addition, metabolomics highlight functional outcomes related to the activities of an organism which are required for its growth and survival (Frederich et al. 2016). Factually, different reactions are involved in drugs metabolism and these are classified into two categories: phase I and phase II reactions. Oxidation, reduction and hydrolysis are the main phase I reactions. During drug metabolism, phase I reactions usually involve the introduction of new functional groups in a molecule and modification of a functional group, and sometimes also expose some functional groups to be used in phase II reactions as substrate. Phase I reactions result in increased drug hydrophillicity. On the other hand, conjugation reactions are considered to be phase II reactions, which result in further enhancement of the hydrophillicity, and assist in metabolites’ excretion from the body (Williams 2002; Roskar & Lusin 2012).

2.1. Metabolomics application in herbal drug metabolism and discovery

During recent years, metabolomic studies have been carried out worldwide by the scientific community to explore the metabolite profiles and metabolic pathways of different biofluids/herbal extracts (Liang et al. 2010; Prasad and Singh 2010; Zhang, Saif et al. 2010; Bai et al. 2011; Giorgi et al. 2013; Bony et al. 2014; Han et al. 2011; Kim et al. 2011; Ye et al. 2011; Shobha et al. 2012; Zhang et al. 2012; Li et al. 2013; Liu et al. 2013; Wang, Bai et al. 2013; Bertol et al. 2015; Cao et al. 2015; Hagel et al. 2015; Jia et al. 2015; Li, Cai et al. 2016; Meyer & Maurer 2015; Qiao et al. 2015; Wu et al. 2015; Zhang, Qia et al. 2015).

Recently, metabolites of orally administered kakkalide (isoflavone from Pueraria lobata flowers) have been investigated in rat urine, bile and feces (Wang, Bai et al. 2013). Seven metabolites, tectorigenin-7-O-glucuronide, tectorigenin-7-O-sulfate,
tectorigenin-4′-O-sulfate, 6-OH biochanin A-glucuronide (6-OH-BiA-6G), irisolidone-7-O-glucuronide (Ir-7G), tectorigenin and irisolidone, were identified in rat urine, whereas Ir-7G was found in bile, and irisolidone and kakkalide were found in the feces. Likewise, transformation of irisolidone (metabolite of kakkalide) in rat plasma has also been investigated (Zhang, Qia et al. 2015). Ir-7G and 6-OH-BiA-6G were the major metabolites in rat plasma. The presence of higher levels of conjugated metabolites in plasma ascertained the involvement of phase II metabolism. Decarbonylation, demethylation, reduction, dehydroxylation, demethoxylation, hydroxylation, glucuronidation and sulfation were depicted to be the main metabolic pathways of irisolidone in plasma. Ir-7G and 6-OH-BiA-6G were also found to be the main metabolites in rat plasma after the oral intake of kakkalide (Bai et al. 2011). The levels of Ir-7G and 6-OH-BiA-6G in the case of irisolidone administration were higher than those resulted from kakkalide administration. This difference may be either due to the difference in the sensitivity of the analytical techniques used for the metabolite profiling of irisolidone and kakkalide and/or may be because of the difference in administrated dose levels. In general, it was revealed that the metabolic pathways of irisolidone and kakkalide are almost similar.

In another study, chemical constituents of alkaloidal extract (from the leaves of *Alstonia scholaris* (AAS)) and their metabolism were evaluated in rats after oral administration (Cao et al. 2015). Thirty-five alkaloids were characterized from AAS extract. Out of these, 11 alkaloids were scholaricine-type, 12 picrinine-type, 9 vallesamine-type, and 3 tubotaiwine-type alkaloids. To evaluate the metabolic pathways of AAS alkaloids, representative compounds of scholaricine-type, vallesamine-type and picrinine-type alkaloids were administrated to rats. The order of their oral bioavailability was highly polarity dependent. The major metabolic reaction for scholaricine-type alkaloids was glucuronidation, whereas hydroxylation and glucuronidation were the main reactions involved in the metabolism of vallesamine-type alkaloids. On the other hand, major metabolic reactions for picrinine-type alkaloids were demethylation, hydroxylation and dehydrogenation. Moreover, scholaricine- and vallesamine-type alkaloids also undergo N-oxidation (Cao et al. 2015). Zhi-Zi-Da-Huang decoction (ZZDHD), a multiherb prescription consisting of four crude herbs: *Gardenia jasminoides* Ellis, *Citrus aurantium* L., *Rheum palmatum* L. and *Semen Sojae* Preparatum, has mostly been used to manage alcoholic liver disease. Metabolomics approach was used to investigate the metabolic pathways of ZZDHD in rats. A total of 61 ZZDHD-related metabolites including 34 prototype components and 27 metabolites were identified in rat plasma (Wu et al. 2015). Monoterpenoids and iridoid glycosides from *G. jasminoides* Ellis, whereas flavonoid glycosides and anthraquinones from *C. aurantium* L. and *R. palmatum* L., respectively, were the major absorbed metabolites. The main metabolic pathways of ZZDHD *in vivo* were hydrolysis, glucuronidation and sulfation. Metabolite profiling of some of the herbal extracts/ingredients reported recently by various researchers is described in Table 1.

*Antrodia cinnamomea* (a medicinal mushroom being used for cancer therapy) has also been investigated to elucidate its metabolism in rats. Eighteen triterpenoids and 8 bio-transformed metabolites were identified in rat plasma. Additionally, in the pharmacokinetic study of major bioactive constituents (ergostane and lanostane triterpenoids) of *A. cinnamomea*, ergostanes (relatively more polar than lanostanes) were absorbed and eliminated rapidly from rat plasma compared to lanostanes (Qiao et al. 2015). In another study, metabolomics approach was used to investigate the possible *in vivo* metabolites of geniposide (a major bioactive compound from *Fructus gardeniae*) in rats. Seventeen metabolites in plasma, 12 in liver, 31 in urine, 6 in heart, 3 in spleen, 12 in kidney, 4 in liver microsomes and 6 in the brain of rat were identified (Li, Cai et al. 2016). Moreover, genipinine as one of the metabolites of geniposide was undetected and it was assumed to be due to different rat species used (Han et al. 2011). Unlike the previous studies, more attention was paid toward major-to-trace metabolites of geniposide. Two possible metabolic pathways were involved in the metabolism of geniposide *in vivo*. The first pathway involves the hydrolysis of the C-1 hydroxyl groups immediately following taurine, sulfate and glucuronide conjugation and intramolecular dehydration. Contrarily, the second possible pathway includes metabolic reactions, that is, demethylation, methylation, cysteine conjugation, glucosylation and glucuronide conjugation.

Liu et al. (2013) evaluated the metabolic pathway of Xiang-Fu-Si-Wu decoction (XFSW-8) in rats. In total 15 constituents were reported. Among them, 6 were
## Table 1. Metabolite profiling of various herbal medicines/products/extracts.

| Medicinal Plants/Host/Drug | Target Analytes/Compounds | Characteristics | Metabolites | Possible metabolomics reaction/pathways | Major techniques applied | Comments/highlights | Reference |
|---------------------------|---------------------------|-----------------|-------------|----------------------------------------|--------------------------|---------------------|-----------|
| *Pueraria lobata* flowers | Irisolidone               | Well-recognized isoflavone | 15 metabolites along with irsloidone were identified.  
• Ir-7G and 6-OH-BiA-6G were the major metabolites | Decarboxylation  
• Demethylation  
• Demethoxylation  
• Reduction  
• Dehydroxylation  
• Sulfation  
• Glucuronidation  
• Hydroxylation | UHPLC-Q-TOF-MS  
• Metabolite profiling along with major metabolomic pathways for irsloidone isolated from *Pueraria lobata* flowers was performed | Metabolite profiling was based on elution order, mass spectral fragmentation patterns and comparison with reference standards  
• The metabolomic studies of *Pueraria lobata* flowers were performed | Zhang, Qia et al. (2015) |
| *Alstonia scholaris* leaves | Alkaloids                 | Alkaloids are major bioactive constituents | 35 alkaloids; 9 vallesamine types, 11 scholaricine  
• 3 tubotauiwine types and 12 picrinine types | Hydroxylation  
• Glucuronidation | UHPLC-Q-TOF-MS  
• *A. scholaris* leaves extract was characterized for metabolomics using high-accuracy mass spectral investigations of total of 35 alkaloids  
• *A. scholaris* leaves extract was orally administrated to rats and characterized 40 compounds in urine, 33 compounds in plasma and 38 compounds in feces | Cao et al. (2015) |
| Zhi-Zi-Da-Huang decoction (ZZDHD) | Xenobiotics               | Traditional prescription in China | 61 metabolites and xenobiotics including 27 metabolites and 34 prototype components | Hydrolysis  
• Glucuronidation  
• Sulfation | LC-TOF-MS  
LC-QqQ-MS  
• The metabolomic studies of Zhi-Zi-Da-Huang decoction revealed monoterprenoids and iridoid glycosides from *G. jasminoides* Ellis, whereas glycosides and anthraquinones were obtained from *C. aurantium* L. and *R. palmatum* L., respectively, as main components  
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| *Antrodia cinnamomea* | Ergostane and lanostane triterpenoids | Precious medicinal mushroom  
• Used in adjuvant cancer therapy | 18 triterpenoids along with 8 bio-transformed metabolites | Hydrogenation  
• Hydroxylation | UHPLC-Q-TOF-MS  
• Metabolism of *Antrodia cinnamomea* revealed 26 triterpenoids and metabolites in rat plasma after its oral administration  
• Different pharmacokinetic patterns were observed for different triterpenoids | Qiao et al. (2015) |

(continued)
| Medicinal Plants/Host/Drug | Target Analytes/Compounds | Characteristics | Metabolites | Possible metabolomics reaction/pathways | Major techniques applied | Comments/highlights | Reference |
|---------------------------|---------------------------|----------------|------------|----------------------------------------|-------------------------|---------------------|-----------|
| Fructus gardeniae         | Iridoid glucoside Geniposide | Exhibits antitumor, hepatoprotective and choleteric effects | 17 metabolites in rat plasma, 31 metabolites in urine, 6 metabolites in heart, 12 in liver, 3 in spleen, 12 in kidney, 6 in lung, 6 in brain and 4 in rat liver microsomes | Hydrolysis | UPLC-LTQ-Orbitrap-MS | Metabolite profiling of geniposide, an iridoid glucoside of Fructus gardeniae, was performed | Li, Cai et al. (2016) |
| Xiang-Fu-Si-Wu Decoction  | Alkloides                  | Used in gynecology diseases to treat blood stasis syndromes | 9 metabolites and 6 parent compounds in blood and organs | Methyltion | UPLC-Q-TOF-MS | The results provide significant insights and extensive guidance for safety monitoring and elucidation of side effect mechanism in the drug delivery system. Moreover, newly discovered metabolites of geniposide could be targets for future metabolism studies | Liu et al. (2013) |
| Rhizoma Anemarrhenae and Radix Astragali | Zhimu–Huangqi herb-pair  | Exhibit diuretic, tonic, immunostimulant, antianancer, hepatoprotective, antioxidant, antidiabetic and expectorant potential | 30 compounds from the herb-pair extract | Methyltion | LC-ESI-MSn | Xiang-Fu-Si-Wu decoction (XFSW-8 fraction) was investigated for the metabolism of its alkaloids | Li et al. (2013) |
| Forsythia suspensa        | Forsythiae Fructose        | Forsythiae Fructus fruits used in heat-clearing and detoxifying Chinese herbal medicines | 27 metabolites were detected, 8 of them were different in green fruit (GF) compared to ripened fruit (RF) groups. Phenyl ethanoid lignans, glycosides, flavonoids and cyclohexyl ethanol derivatives were major secondary metabolites. | Transamination | $^1$H NMR | Green and ripened Forsythia fructus fruits were investigated to compare their chemical compositions based on NMR-based metabolomics equipped with HPLC and UV spectroscopy. The antioxidant potential of GFS was greater than that of RFs, whereas the antibacterial activities differed nonsignificantly among GFS and RFs | Jia et al. (2015) |

(continued)
| Medicinal Plants/Host/Drug | Target Analytes/Compounds | Characteristics | Metabolites | Possible metabolomics reaction/pathways | Major techniques applied | Comments/highlights | Reference |
|----------------------------|---------------------------|-----------------|-------------|----------------------------------------|--------------------------|--------------------|-----------|
| Benzylisoquinoline (BIA)-accumulating plants from four families of the Ranunculales | Benzylisoquinoline alkaloid (BIA) | • Well recognized with antimicrobial, antimalarial, antiparasitic, cytotoxic and neurological properties | 91 metabolites with significant variation in amino acids, sugar and organic acid content | Extensive metabolite profiling using multiple analytical approaches was used for the evaluation of metabolism of 20 plants from four families of Ranunculales. Various alkaloids profiles (both universal and rare) were established | 1H NMR, DFI-MS/MS, HPLC, FTIR | | Hagel et al. (2015) |
| Glaucium flavum | Isoquinoline alkaloid Glaucine | • Exhibit antitussive and anti-inflammatory potential | 26 phase I and 21 phase II metabolites of isoquinoline alkaloid glaucine | | GC-MS and LC-HR-MSn | | Meyer and Maurer (2015) |
| Kinkeliba and Mitracarpus scaber Zucc. | Nonpolar metabolites | • Leaves of Kinkeliba are used for cholagogue, diuretic, antidiabetic, choleretic, and anti-inflammatory potential | 48 compounds (for Kinkeliba) and 51 compounds (for Mitracarpus scaber Zucc.) | Metabolites profiling based on nonpolar phytochemicals of M. scaber and C. micranthum’s dichloromethane extracts was carried out with HTGC-MS (high temperature gas chromatography–mass spectrometry) | HTGC-MS | | Bony et al. (2014) |

(continued)
Table 1. Continued.

| Medicinal Plants/Host/Drug | Target Analytes/Compounds | Characteristics | Metabolites | Possible metabolomics reaction/pathways | Major techniques applied | Comments/highlights | Reference |
|---------------------------|---------------------------|-----------------|-------------|----------------------------------------|--------------------------|---------------------|-----------|
| *Bixa orellana* L. (Bixaceae), Volatile organic compounds | • Popular aphrodisiac medicines • Remedy to manage inflammatory conditions, treat fevers and parasitic diseases | • Sesquiterpenes, monoterpenes and arene compounds • α-humulene as a major compound following D-germacrene, γ-elemene and caryophyllene. | | | GC-MS | • Volatile organic compound profiling of *Bixa orellana* L. seed extracts was carried out to evaluate the mosquito repellent capacity of *Bixa orellana* L. seed extracts | Giorgi et al. (2013) |
| *Radix aconiti* Lateralis (Shengfuzi (SF) decoction) | • Effective for the treatment of rheumatism, hypertension and heart failure | • 20 compounds including 4 metabolites and 16 prototype compounds | | | HPLC-ESI-Q-TOF-MS | • Spectrum–effect relationship model was employed for metabolite profiling and serum pharmaco-chemistry of *Radix aconiti* Lateralis after its oral administration in rats and established real cardio-toxic components in plasma | Zhang et al. (2012) |
| Indian formulations of glucocorticosteroids | Prednisolone and methylprednisolone’s metabolites | • Potent anti-inflammatory agents • Reduces pain | • 10 urinary metabolites and parent compound for prednisolone • 6 urinary metabolites and parent compound for methylprednisolone | | LC-MS/MS | • Analytical method with improved detection was developed for the characterization of prednisolone and methylprednisolone’s metabolites • The reported method has additional significant analytical applications for the detection of spiked homeopathic, ayurvedic and unani preparations. | Shobha et al. (2012) |
| *Aconitum napellus* | Mesaconitine, major *Aconitum* alkaloid | • Exhibit analgesic, neurological and antiarthritic effects | • 9 metabolites of mesaconitine • Demethylation • Dehydrogenation • Hydroxylation • Demethylation • Dehydrogenation | | UPLC-Q-TOF-MS/MS | • The metabolite characterization of mesaconitine, a major alkaloid of *Aconitum napellus*, was carried out. • The results provided comprehensive baseline chemical information to understand the metabolism of mesaconitine | Ye et al. (2011) |

(continued).
| Medicinal Plants/Host/Drug | Target Analytes/Compounds | Characteristics | Metabolites | Possible metabolomics reaction/pathways | Major techniques applied | Comments/highlights | Reference |
|---------------------------|---------------------------|----------------|-------------|--------------------------------------|-------------------------|---------------------|-----------|
| Angelica gigas            | Dang Gui                  | • Dang Gui is herbal medicine used to treat anemia, chronic bronchitis, rheumatism, hypertension, asthma, and CVDs | • Acetate, 1,3-dimethylurate, choline, fumarate, citrate, histamine, glucose, lactose, succinate, malate, valine and N-acetylgutamate were major primary metabolites, whereas decursin, marmesin, decursinol, nodakenin and 7-hydroxy-6-(2R-hydroxy-3-methylbut-3-ethyl) coumarin were among major secondary metabolites | ¹H NMR UPLC-MS | • Primary and secondary metabolites were investigated to discriminate various herbal medicines based on geographical origins based upon UPLC-MS and ¹H NMR metabolomics along with chemometric analysis | Kim et al. (2011) |
| Rifamycin                 | Rifabutin                 | • First choice drug to treat Tuberculosis | • 23 metabolites in rat urine | Hydroxylation | LC-MSn and LC-HR-MS | | Prasad and Singh (2010) |
| TCM                       | PHY906                    | • Used to treat diarrhea, nausea/vomiting and abdominal cramps | • 57 compounds parent PHY906 formulation and 27 metabolites in rat plasma | | LC-MS/MS | | Zhang, Saif et al. (2010) |
| Schisandra chinensis      | Lignans                   | • Exhibit antigastric ulcer, antihypertotoxic, antiasthmatic, anticarcinogenic, antioxidative and detoxification potential | • 44 metabolites in rat urine | Demethylation | LC-IT-TOF-MS | | Liang et al. (2010) |
parent compounds, whereas 9 compounds were identified in blood and organs. Methylates/demethylates sulfation, glucuronidation, sulfation and glucuronidation conjugation were identified as the major metabolic pathways involved in the metabolism of alkaloids of XFSW-8. Furthermore, an analysis was performed to study the metabolites of Zhimu–Huangqi herb-pair (consisting of Radix Astragali and Rhizoma Anemarrhenae) after oral administration to rats (Li et al. 2013). Zhimu–Huangqi herb-pair extract was actively metabolized in rat serum and urine. Four parent compounds along with 8 metabolites were identified in serum, whereas 7 parent compounds along with 23 metabolites were detected in urine. Glucuronidation and sulfation were found to be the key metabolic pathways of Zhimu–Huangqi herb-pair’s metabolism in rats.

The metabolic fate of glaucine (isoquinoline alkaloid and main component of Glaucium flavum) in rats has also been studied previously (Meyer & Maurer 2015). Twenty-six phase I and 21 phase II metabolites were found. Glaucine was O- and N-dealkylated, and then conjugated to sulfates or glucuronides. Moreover, N-oxidation and hydroxylation were also involved in its metabolism as additional metabolic pathways. Schisandra lignans extract was investigated for its metabolite profiling and associated metabolic pathways in vivo and in vitro. Five in vitro and 44 in vivo metabolites were identified. Demethylation and hydroxylation were reported as the primary metabolic pathways for in vitro metabolism, whereas hydroxylation was reported for in vivo metabolism (Liang et al. 2010). Recently, potential bioactive compounds with cardio-toxic effect in rat plasma after treatment with Radix aconiti. L-based Shengfuzi (SF) decoction have also been investigated (Zhang et al. 2012). Twenty compounds including 16 prototype constituents along with 4 metabolites were detected as potential bioactive components (Zhang et al. 2012). Cardio-toxic effect was evaluated based on heart ratio (HR) situation and electrocardiographic (ECG) parameters. Out of the 20 bioactive compounds, beiwutine, napelline, songorine and bikhaconine were characterized as potential cardio-toxic compounds based on their closest relationship with the results of cardio-toxic experiments. Bony et al. (2014) investigated African traditional herbal products for their nonpolar metabolites. They found 48 metabolites in Combretum micranthum and 51 metabolites in Mitracarpus scaber. The presence of a-polar phytochemicals, namely triterpenes, coumarins, quinones and phytosterols, was also revealed in the extracts of C. micranthum and M. scaber. In another study, the presence of volatile compounds, for example, monoterpenes, sesquiterpenes and arenas, in Bixa orellana L. (a shrub native to central and South America) has been ascertained. α-Humulene was found as the major volatile compound, while γ-elemene, D-germacrene and caryophyllene were revealed as the minor constituents (Giorgi et al. 2013).

In addition to herbal drug metabolism, metabolomics has also been widely used to study the therapeutic effect of single-compound or plant extracts on various diseases in different animal models. For example, ergone (ergosta-4,6,8(14),22-tetraen-3-one) has been identified in fungus and different mushroom species and is known for its antitumor and nephroprotective properties (Bok et al. 1999; Quang & Bach 2008; Zhao et al. 2009). Zhao, Chen et al. (2014) studied the nephroprotective effect of ergone in adenine-induced chronic kidney disease (CKD) rats. Ultra-performance liquid chromatography/high-sensitivity mass spectrometry (UPLC-HSMS)-based metabolomic profiling of kidney tissues was performed to analyze the metabolites regulated differently in adenine and adenine + ergone treated rats. Among 17 identified biomarkers, the perturbation of docosahexaenoic acid, 5-hydroxyeicosatetraenoic acid, xanthine, eicosapentaenoic acid, indoxyl sulfate and p-cresol sulfate was completely reversed in ergone-treated rats. Ergone treatment was found to be effective in delaying CKD by blocking the abnormal changes in metabolites in the kidney of adenine-treated rats. Serum and urine metabolomic study based on UPLC-HSMS was also performed in order to test the therapeutic effect of ergone in adenine-induced chronic renal failure (CRF) rats (Zhao, Cheng, Cui et al. 2012; Zhao, Shen et al. 2012). A significant difference in metabolites associated with amino acid and lecithin metabolism, that is, lysophosphatidylcholines, adenosine, dopamine, creatinine, aspartic acid and phenylalanine, was identified in the serum of CRF and CRF rats treated with ergone (Zhao, Cheng, Cui et al. 2012). Similarly, regulation of metabolites such as creatinine, proline, adrenosterone, taurine, creatine, phenylalanine, ornithine, dopamine, kynure-nine, kynurenic acid and 3-O-methylpda involved in energy and amino acid metabolism was also changed in urine of CRF rats compared to control groups (Zhao, Shen et al. 2012). In both cases, the level of most...
of the metabolites in CRF rats was restored back to normal state after treating with ergone. Zhao, Zhang, Long et al. (2013) studied the effect of ergone on the feces of CRF rats by using Ultra performance liquid chromatography-quadrupole-time-of-flight-high-sensitivity mass spectrometry (UPLC-Q-TOF/HSMS/MS(E))- based metabolic profiling. The identified metabolites, namely chenodeoxycholic acid, phytosphingosine, 3-Oxo-4,6-choladienoic acid, palmitic acid, cholic acid, 7-ketolithocholic acid and MG(24:1/0:0/0:0), were identified which are major components of bile acid and phospholipid metabolism. Compared to CRF rats, the concentration of all seven biomarkers in the ergone-treated group was close to that of the control group.

Along with pure compounds, natural products have also been widely used to study their mechanism of action against several diseases in animal-based models. UPLC–QTOF–HDMS-based metabonomic approach was used to study the renoprotective effects of *Poria cocos* extract in adenine-induced CKD rats (Zhao, Lei et al. 2013). A total of 19 metabolites were identified as potential biomarkers of CKD. Ten out of the 19 metabolites, namely, eicosapentaenoic acid, docosahexaenoic acid, lysophosphatidylcholine (20:4), lysophosphatidylcholine (18:2), lysophosphatidylcholine (15:0), lysophosphatidylcholine (20:0/0:0), indoxyl sulfate, hippuric acid, p-cresol sulfate and allantoin, were restored to the control level in *P. cocos*-treated groups. The renoprotective effect of *P. cocos* extract was also studied in the serum and urine of adenine-induced CKD rats (Zhao, Feng et al. 2013; Zhao, Li et al. 2013). Identified metabolites were correlated with progressive renal injury. The level of lysophosphatidylcholine (18:0), tetracosahexaenoic acid, lysophosphatidylcholine (18:2), creatinine, lysophosphatidylcholine (16:0) and lysophosphatidylcholine (22:0/0:0) in serum (Zhao, Feng et al. 2013), and that of adenine, 6-hydroxyadenine, hypoxanthine, creatine, methionine, phytosphingosine, acetylcarnitine and phenylalanine in urine (Zhao, Li et al. 2013) were reversed to normal state after treating CKD rats with *P. cocos* extract.

Chen, Chen, Tang et al. (2016) and Miao et al. (2016) evaluated the antihyperlipidemic effects of *P. cocos* extract in high-fat diet-induced hyperlipidemic rats. Urine and plasma samples were analyzed by UPLC-HDMS. Among 18 metabolites, arginine, aminoacipic acid and citric acid were found as potential biomarkers in urine (Chen, Chen, Tang et al. 2016); however, the level of seven fatty acids (palmitic acid, hexadecenoic acid, hexanoylcarnitine, tetra-cosahexaenoic acid, cervonoyl ethanolamide, 3-hydroxytetradecanoic acid and 5,6-dihydroxyicos-8,11,14-trienoic acid) and five sterols (cholesterol ester (18:2), cholesterol, hydroxysterosterone, 19-hydroxydeoxycorticosterone and cholic acid) (Miao et al. 2016) was altered in the plasma of hyperlipidemic rats compared to the control group. Treatment with *P. cocos* extract significantly improved the hyperlipidemia and partially ameliorated the abnormal regulation of metabolites in urine and serum of hyperlipidemic rats.

Lipidomics and metabolomics approaches were employed to study the nephroprotective effects of rhubarb in CRF rats. In total, 83 differential metabolites were found in CRF rats compared to that in the control group. Treatment with rhubarb extract improved the perturbed metabolites which were closely associated with glycerophospholipid, fatty acid and amino acid metabolisms (Zhang et al. 2016). In another study, rhubarb extract was shown to improve the renal function in CKD rats by reversing the disturbed urinary metabolites commonly involved in amino acid, purine, taurine and choline metabolisms (Zhang, Wei et al. 2015). Chen, Yuan et al. (2015) investigated the antihyperlipidemic activity of rhubarb extract in high-fat diet-induced hyperlipidemic rats. UPLC-QTOF-HDMS-based urinary metabolomic profiling was used to identify 29 metabolites which were altered in hyperlipidemic rats compared to control groups. Differential metabolites were mainly involved in fatty acid, amino acid and nucleoside metabolism and their concentrations were restored back to the normal level in rhubarb-treated rats. Likewise, Akhtar et al. (2016) reported the antidiabetic effect of *Andrographis paniculata* water extract in a Type 2 obese-diabetic (obdb) rat model. Nuclear magnetic resonance (NMR)-based metabolomic profiling of urine samples showed higher levels of glucose, choline and taurine, whereas low levels of lactate, formate, citrate, 2-oxoglutarate, succinate, dimethylamine, acetoacetate, acetate, allantoin and hippurate were found in obdb rats. Along with glucose, *A. paniculata* treatment successfully normalized the disturbed metabolism of obdb rats (Akhtar et al. 2016).

### 2.2. Analytical tools for metabolite profiling

More accurate assessment techniques are essentially required for clinical diagnostic research. Metabolomics exhibits a significant role in the discovery of novel biomarkers in complex disease states and
thus improves clinical diagnostics (Zhang, Sun et al. 2015). Initially in the past, compounds were subjected to metabolism studies after their discovery and the metabolites were characterized by conventional spectral techniques after their isolation from biological matrices (Prasad et al. 2011). Taking into account the high chemical diversity and variation in the concentrations of metabolites, it has now been well-established that metabolites cannot be efficiently characterized by a signal universal technique. Therefore, a combination of more than one analytical technique is required for reliable and reproducible studies, related to metabolic profiling (Theodoridis et al. 2008). A number of analytical tools have been employed by the scientific community in the recent years, out of which NMR spectroscopy and MS are the most frequently used techniques in metabolite profiling (Dunn et al. 2011). Both of these analytical approaches identify metabolites based upon their structural characteristics (Holmes et al. 1998; Aharoni et al. 2002; Lenz et al. 2003; Dettmer et al. 2007; Huang et al. 2007; Lindon & Nicholson 2008).

NMR spectroscopy is a highly popular technique among phytochemists, for the structural elucidation and functional characteristics/information of metabolites of interest based on the interpretation of NMR spectral features, namely, chemical shifts and coupling constants (Dunn et al. 2011; Mahrous & Farag 2015). Most of the metabolomics studies using NMR-based approaches are being carried out with the use of one-dimensional $^1$H NMR spectroscopy. Several analyses can be conducted with the same sample by NMR, due to its nondestructive nature. Minimal sample preparation is required for NMR studies. The sample is usually added into some deuterated solvent-like chloroform (CDCl$_3$) or deuterium oxide (D$_2$O) along with some reference compound such as TSP (trimethylsilyl propanoic acid). Sometimes, phosphate-based buffer is also required to be used to adjust the pH, and to avoid the pH-dependent chemical shift variations during NMR spectroscopic analysis (Beckwith-Hall et al. 1998).

The inherent less sensitive nature of NMR spectroscopic analysis is its largest disadvantage compared to other analytical techniques (Nicholson et al. 2002; Lenz et al. 2003; Lindon & Nicholson 2008; Roux et al. 2011). Moreover, as the signals for almost all the metabolites appear in NMR spectra, their interpretation is quite a tedious process (Roux et al. 2011). Dunn et al. (2011) summarized that using a simple one-dimensional $^1$H NMR pulse sequence, typically 30–100 metabolites can be identified in urine (Beckwith-Hall et al. 1998; Connor et al. 2004), 20–40 metabolites in tissue extracts (Griffin et al. 2001; Rooney et al. 2003) and 20–30 metabolites can be detected in blood plasma or serum (Brindle et al. 2002; Kirschenlohr et al. 2006).

In addition to the one-dimensional $^1$H NMR spectroscopic methods, two-dimensional NMR spectroscopic approaches including homotropic ($^1$H–$^1$H or $^1$C–$^1$C) as well as heteronuclear ($^1$H–$^{13}$C, $^1$H–$^{15}$N, etc.) experiments based on spatial or scalar dipolar coupling between similar nuclei and different nuclei, respectively, are also gaining importance in metabolomics for the structural evaluation of natural products (Lambert & Mazzola 2004; Noda 2014; Mahrous & Farag 2015). For the investigation of herbal extracts, where $^1$H NMR spectral information are highly congested, two-dimensional heteronuclear NMR ($^1$H–$^{13}$C) can be employed for efficient metabolite profiling. Therefore, for metabolomics studies of herbal extracts, in particular, for metabolites identification and their optimal structural characterization, the use of two-dimensional (2D) correlation spectroscopic approaches, namely, HSQC (heteronuclear single-quantum coherence) and HMBC (heteronuclear multiple-bond correlations), is highly recommended (Mahrous & Farag 2015).

Scognamiglio et al. (2015) applied NMR spectroscopic technique for the evaluation of metabolite changes in seven (07) aromatic Mediterranean plant species during different seasons. They targeted the detection and quantification of the both primary and secondary metabolites and revealed that flavonoids (quercetin, apigenin and kaempferol) and phenylpropanoid derivatives (rosmarinic and chlorogenic acid) were the principal identified polyphenols (Scognamiglio et al. 2015). Freitas et al. (2015) investigated the differences between the metabolite profiles of Huang Long Bing (HLB)-asymptomatic tissues and those of symptomatic tissues. They reported the use of NMR spectroscopy in combination with chemometry, and revealed that higher sucrose levels were present in the leaves of the symptomatic trees compared to the asymptomatic tissues, whereas no variation in sucrose levels was observed in their roots. Moreover, lower levels of betaine, proline and malate were observed in HLB-affected symptomatic leaves.
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(Freitas et al. 2015). Priori et al. (2015) reported the use of NMR-based metabolomics for the identification of targeted biomarkers relevant to their anticipated response toward biologics among rheumatoid arthritis patients. The studies revealed an increase in the levels of leucine, isoleucine, alanine, valine, glutamine, glucose and tyrosine levels, whereas a decrease in the levels of 3-hydroxybutyrate after treatment with etanercept for six (06) months relative to the baseline (Priori et al. 2015). Popescu et al. (2015) reported the use of $^1$H and $^{13}$C NMR spectroscopic techniques in combination with principal component analysis to differentiate 44 oil samples according to their origin, based upon the levels of saturated fatty acids, namely, oleic acid, linoleic acid, linolenic acid, compared to their iodine values. Close clustering of virgin olive oils was observed, whereas walnut oil showed significant variation with respect to their country of origin. In another study, Wu et al. (2014) compared two NMR approaches (completely relaxed spectra directly vs. partially relaxed spectra that were calculated with T1) for the quantitative metabolite profiling of seeds of mungbean. The authors reported a parameter-optimized procedure for NMR-based metabolomics analysis of plant seeds by maximizing their signal-to-noise ratio and extraction efficiency, but minimizing the degradations and chemical shift variations.

On the other hand, MS is also a popular analytical tool in metabolomics where metabolite identification is based on structural information. MS is a more costly, destructive, labor-intensive and time-consuming technique. Although metabolite profiling and its interpretation using MS are complex due to limited spectral databases (Want et al. 2010; Dunn et al. 2011; Lynn et al. 2015), still MS is considered as one of the key tools for effective metabolite identification, usually based on mass match of metabolites with the databases (Smith et al. 2005; Cui et al. 2008; Horai et al. 2010; Wishart et al. 2013).

MS works by the transformation of analytes of interest into charged species (ions), leading to their separation based upon their mass-to-charge ratio followed by their detection. A number of ionization methods are available, namely, EI (Electron Impact Ionization) and MALDI (Matrix-Assisted Laser Desorption Ionization), to convert analytes of interest into charged species at high pressure, in addition to Atmospheric Pressure Chemical Ionization and ESI (Electro Spray Ionization) to perform ionization at atmosphere pressure. The use of high vacuum is recommended during mass spectrometric separation and detection of understudy metabolites to achieve efficient sensitivity, mass resolution and mass accuracy by minimizing the number of collisions (ion–ion/ion–molecule). For metabolomics, a mass spectrometer is known to exhibit the ability for mass scan ranging from 20 to 1500 amu (Dunn et al. 2011). Lynn et al. (2015) deduced a protocol for metabolite identification based on mass spectrometric approach. Their proposed workflow was involved in peak grouping and annotation, leading to metabolite identification. They proposed grouping and annotation of the peaks associated with the same metabolite in the first step, followed by the use of multiple identification strategies for the peak groups on the basis of annotated ions content in each group, for the efficient evaluation of corresponding identification confidences (Lynn et al. 2015).

In addition to NMR and MS, a range of other analytical tools are also in use for metabolomics analysis, including GC-MS (gas chromatography equipped with MS), GCxGC-MS (two-dimensional gas chromatography coupled with MS), FTIR (Fourier transform infrared spectroscopy), LC-MS (liquid chromatography coupled with MS) and CE-MS (capillary electrophoresis coupled with MS) (Robertson et al. 2005; Vaidyanathan et al. 2005; Lenz & Wilson 2007; Theodoridis et al. 2008). However, the development of LC-MS proved to be highly significant for the metabolomics studies. Initially only gas chromatography was hyphenated to MS, and its usage for separation was limited to only volatile metabolites (Roux et al. 2011). In contrast, liquid chromatography hyphenated to mass spectrometry (LC-MS) is a comparatively more powerful and emphatic tool and plays significant contribution toward profiling of drug metabolism and bio-activation (Li et al. 2012).

Recently another imperative tool, that is, UPLC (ultra-performance liquid chromatography), has been introduced for the metabolomics research. UPLC has been ascertained to be more sensitive than LC with minimum band broadening, maximum signal-to-noise ratio and additional peak resolution (Lu et al. 2008). It has the ability to operate at pressures ranging from 6000 to 15,000 psi. Improved peak resolution of the UPLC analysis has also been proved to rectify the ion suppression problem linked with co-eluting peaks. Moreover, coupling UPLC
with oaTOF (orthogonal-acceleration time-of-flight) MS and with Q-TOF (quadrupole time of flight) has also gained much acceptance for trace constituents investigation in complex mixtures. UPLC-MS methods revealed improved potential toward the evaluation of differential metabolic pathway activities due to improved sensitivity and resolution (Lu et al. 2008). LC-MS-based metabolomic strategies provide more accurate, effective and comprehensive metabolite profiling compared to other traditional approaches, and have the ability to explore drug metabolism with indiscriminate metabolite identification and the ability to deal with large datasets of biomatrix (Li et al. 2012).

Li, Cai et al. (2016) developed the UPLC-linear trap quadrupole (LTQ)-Orbitrap method for the metabolite profiling of geniposide in rat blood plasma, liver microsomes, urine and various tissues. Based upon relevant drug bio-transformation information, accurate mass measurement, fragment ions diagnosis and bibliography data, 33 metabolites were detected Zhao, Zhang, Feng et al. (2013) employed UPLC-QTOF-MS for pharmacokinetic investigations of 2,3,5,4-tetrahydroxystilbene-2-O-β-D-glucoside (from Polygonum multiflori) in rats and reported the identification of three metabolites with the help of rapid resolution LC-MS(n). Dudzik et al. (2015) reported the use of LC-MS for the investigation of potential biomarkers of chorioamnionitis and to evaluate the associated perinatal neurological damage. It was found that the detected metabolites were involved in sphingolipids and glycerophospholipids metabolism. Taamalli et al. (2015) used RP-UHPLC (reversed phase-ultra performance liquid chromatography) equipped with ESI-QTOF-MS (electrospray ionization quadrupole time-of-flight MS) for the metabolite profiling of two different Lamiaceae medicinal plants and reported the identification of 85 metabolites, including organic acids and derivatives, nucleosides, amino acids, amino acids derivatives and phenolic compound. Gallo-catechin was depicted to be the major metabolite in the extract of Mentha pulegium, while quercetin dimethyl ether, dihydrokaempferide and jaceidin were among the major compounds in the extract of Orig-anum majorana. Tsogbaatar et al. (2015) carried out metabolite profiling of pennycress (Thlaspi arvense L.) based on GC-MS and LC-MS/MS. Organic acids, sugars/sugar alcohols and amino acids were depicted to be the three main metabolite families. Woldegiorgis et al. (2015) reported the use of LC/MS/MS equipped with a QTOF mass analyzer for the comparative metabolite profiling of seven edible mushroom varieties including P. ostreatus, A. campestris, L. edodes, L. sulphureus, T. microcarpus T. clypeatus and T. letestui, and identified biomarkers relevant to L. sulphureus. 18 α-Glycyr rhetinic acid was revealed to be one of the potential metabolites which might be responsible for the biological/pharmacological claim of understudy mushrooms by the local people.

In another study, Cajka et al. (2014) applied UHPLC-TOF-MS (ultrahigh performance liquid chromatography-time of flight MS) to differentiate control and Fusarium-infected barley samples based upon their metabolite profiles. Metabolomics analysis using positive mode provided higher molecular features compared to analysis conducted under negative mode. However, deoxynivalenol and deoxynivalenol-3-glucoside were depicted to be the main resistance-indicator metabolites of barley, when analysis was carried out under negative mode setup. Li et al. (2013) used a sensitive LC-ES-MSn (liquid chromatography–electrospray tandem mass spectrometry) approach for the detection of main compounds in the extract of Zhimu–Huangqi herb-pair along with their metabolites in rats. Thirty compounds were identified in the herb-pair extract. Out of these, 13 compounds were characterized by their retention times and mass spectra when compared with those of the reference standards, whereas 17 compounds were identified based upon their MSn fragmentation behaviors.

Zhao (2013), while summarizing metabolomic applications in chronic kidney diseases, described that UPLC-MS-based metabolomics has been used most widely for the investigation of kidney diseases and it has been revealed to be highly effective for the investigation of metabolic changes associated with this disease. Different mass analyzers being used to establish an interface with liquid chromatography for metabolomic research have also been reviewed. These include S/T-Q (single/triple-quadrupoles), ion traps, TOF (time of flight), Fourier transform ion cyclotron resonance and orbitrap along with many hybrid mass analyzers, that is, QTOF, Q-Trap (quadrupole linear ion traps) and ion trap FT. Out of these, quadrupole-based mass analyzer still remains the most widely employed mass analyzer with an analyzing capacity of 50–4000 m/z. Recently, MSE and QTOF MSE techniques have also been applied for the metabolomic
research (Le Blanc et al. 2003; Syka et al. 2004; Kawanishi et al. 2007; Rainville et al. 2007; Yao et al. 2009; Min et al. 2011; Zhao, Su et al. 2012, Zhao, Cheng, Wei et al. 2012; Zhao, Zhang, Long et al. 2013). In another review, Zhao, Cheng et al. (2014) highlighted the applications of UPLC-based metabolomics for biomarkers discovery in clinical chemistry. Cox et al. (2014) elaborated the significance of metabolomics for biomarkers characterization in natural product research and categorized metabolomics tools into five basic categories, that is, NMR, MS, LC × MS, GC × MS and integrated analytical strategies.

In a recent review, Chen, Chen, Chen et al. (2016) comprehensively described the applications of metabolomics for toxicological biomarker discovery related to the natural product research with special emphasis on sample preparation, data processing, interpretation and analysis. It has been revealed that although different analytical techniques including 1H NMR, GC-MS, CE-MS, HPLC-MS, UPLC-MS and solid phase extraction-NMR are in use for metabolomics research, for toxicological studies of natural products/plant extracts, metabolomics based on LC-MS has been utilized frequently to explore potential biomarkers and underlying mechanisms of toxicities. However, the use of metabolomics for toxicological studies still exhibits challenges, and it is difficult to build link with other experimental findings. Shi et al. (2016) in his review also described the applications of metabolomics techniques for efficacy and toxicological studies of traditional Chinese herb medicines (TCHM). They highlighted that NMR- and MS-based analytical methods are more commonly being used for TCHM research; however, the sensitivity and resolution of MS-based techniques (normally coupled with LC and GC) are much higher than that of NMR.

Moreover, separation modes including normal phase, reverse-phase liquid chromatography and hydrophilic interaction chromatography (HILIC) make LC-MS a more versatile analytical method compared to GC-MS. Recently, Li et al. (2016) reviewed the applications of two-dimensional liquid chromatography (2D-LC) for metabonomic investigations related to traditional Chinese medicines (TCMs), and reported that 2D-LC is more effective and versatile with the ability of improved separation and metabolite characterization as compared to 1D-LC due to its high resolution, selectivity and peak capacity. The use of 2D-LC-MS can be promising for better metabolomic analysis based on its multidimensional LC approach. Recently, different multidimensional chromatography approaches such as automated HILIC × RPLC-MS, 2D-LC-MS/MS and online 2D-HILIC × RPLC coupled with ESI-MS have been used by various researchers for metabolomics investigations (Yang et al. 2010; Klavins et al. 2014; Wang, Li et al. 2013). Therefore, 2D-LC systems significantly contributed in separation science related to metabolomics research. Taking into account the wide range of the metabolome levels, physicochemical diversity along with other associated complexities of herbal remedies, for accurate and reliable findings, the use of integrated analytical approaches is recommended.

All the methodologies that have been developed for metabolomic studies have distinct advantages and limitations. NMR-based metabolic platform offers the analyses of multicomponent mixtures with little or no sample preparation, nondestructive and noninvasive sample analyses provide highly reproducible results. Identification can be performed once the sample is placed in a magnetic field, and later by assigning the NMR spectral peaks to specific metabolites based on their chemical shifts or resonant frequency (Dona et al. 2016; García-Figueiras et al. 2016). This technique utilizes a combination of spectroscopic data along with multivariate data analysis and provides a wealth of information regarding the identification and quantification of a large number of metabolites in a single experiment (Edlund & Grahn 1991). Compared to MS, lower detection power is an inherent disadvantage of NMR spectroscopy which needs to be addressed. Metabolites can be detected from mM to μM concentration (Krishan et al. 2004). However, the lower NMR sensitivity issue can be overcome by using high-field magnets, and cryogenically cooled and microcoil probes to analyze small samples or metabolites present in a small quantity (Kovacs et al. 2005; Ravie et al. 2009).

Contrarily, MS having high sensitivity and specificity can detect the molecules even at the pictogram level and this feature makes it an important method in the field of metabolomics. It uses mass-to-charge ratio in charged particles and allows the identification of multiple metabolites present in a very low concentration in complex mixtures. Many of the MS-based methods are used in combination with separation techniques such as gas chromatography (GC) and liquid chromatography (LC) (Bajad & Shulaev 2011;
Garcia & Barbas 2011; Tsugawa et al. 2011; Zhou, Xiao et al. 2012). Compared to NMR, MS analyses require extensive sample preparation and further separation procedures make the analysis time-consuming. However, the limitation is minimized with the development of atmospheric sample introduction techniques, where no sample preparation or separation is required (Schaper et al. 2012).

In order to test the phytotherapeutic mechanism or for diagnostic purposes, biofluids (urine, serum or plasma) and intact tissues of organisms have been utilized for metabolomic studies. Urine samples contain relatively low percentage of proteins and higher amount of low molecular weight compounds and can easily be analyzed by NMR with minimum sample preparation and appear as high-quality narrow/sharp signals in NMR spectra (Emwas et al. 2015; Emwas et al. 2016). However, high salt contents in urine samples require pretreatment before MS analysis (Dettmer et al. 2007; Versace et al. 2012). Moreover, blood analyses provide an overall metabolic status of an organism as it perfuses all the living cells and carries important metabolic information regarding every cell. Unlike urine, blood samples contain higher protein and lipid contents, which along with narrow signals of small molecules produce broader signals in NMR spectra. Different spectral editing methods are used to deal with specific small and large signals in NMR spectra (Tang et al. 2004), whereas sample pretreatments such as derivatization and protein precipitation are performed before the analysis of serum samples on GC- and LC-MS, respectively (Dettmer et al. 2007; Versace et al. 2012). Moreover, blood analyses provide an overall metabolic status of an organism as it perfuses all the living cells and carries important metabolic information regarding every cell. Unlike urine, blood samples contain higher protein and lipid contents, which along with narrow signals of small molecules produce broader signals in NMR spectra. Different spectral editing methods are used to deal with specific small and large signals in NMR spectra (Tang et al. 2004), whereas sample pretreatments such as derivatization and protein precipitation are performed before the analysis of serum samples on GC- and LC-MS, respectively (Dettmer et al. 2007; Versace et al. 2012). Moreover, blood analyses provide an overall metabolic status of an organism as it perfuses all the living cells and carries important metabolic information regarding every cell. Unlike urine, blood samples contain higher protein and lipid contents, which along with narrow signals of small molecules produce broader signals in NMR spectra. Different spectral editing methods are used to deal with specific small and large signals in NMR spectra (Tang et al. 2004), whereas sample pretreatments such as derivatization and protein precipitation are performed before the analysis of serum samples on GC- and LC-MS, respectively (Dettmer et al. 2007; Versace et al. 2012). Moreover, blood analyses provide an overall metabolic status of an organism as it perfuses all the living cells and carries important metabolic information regarding every cell. Unlike urine, blood samples contain higher protein and lipid contents, which along with narrow signals of small molecules produce broader signals in NMR spectra. Different spectral editing methods are used to deal with specific small and large signals in NMR spectra (Tang et al. 2004), whereas sample pretreatments such as derivatization and protein precipitation are performed before the analysis of serum samples on GC- and LC-MS, respectively (Dettmer et al. 2007; Versace et al. 2012).

Together with biofluids, metabolomic profiling of intact tissue samples is becoming a popular tool for obtaining a biological understanding of a specific disease. It can aid in the early diagnosis and treatment of many diseases through the identification of biomarkers for the predictive interpretation of a disease state. Here, NMR offers an unmatchable advantage of analyzing intact tissues without any prior treatment or little sample preparation. Recent technological advancements have improved NMR sensitivity and allow the detection of biomarkers even in few mgs of samples (tissues) and provide high quality of spectra compared to spectra of samples in solution form (Imperiale et al. 2015). Although tissue extraction is required for MS analysis, very high sensitivity of MS makes it a suitable tool to detect early biomarkers. MS methods equipped with quadrupoles, triple quads, ion traps, TOF mass analyzers and tandem MS (MS/MS) methods are commonly used to identify unknown metabolites. Although Fourier transform ion cyclotron resonance is an expensive approach, it provides extremely high resolution and mass accuracy (Hird et al. 2014).

LC-MS and GC-MS are the most important contemporary MS-based metabolomic tools because of their high sensitivity and separation efficiency, respectively. Like NMR, urine samples can be analyzed on LC-MS with little sample preparation. However, extensive sample preparation (derivatization) is required for GC-MS before analysis. Additionally, inter-batch variation and separation process is a common limitation attributed to both LC-MS and GC-MS. Recently developed sample effusion and atmospheric sample introduction methods have been introduced as alternatives to chromatographic separation in MS-based metabolomic analysis (Kelly et al. 2008; Schaper et al. 2012). Furthermore, techniques such as EESI (extractive electrospray ionization) (Devenport et al. 2014), desorption electrospray atmospheric ionization (Roscioli et al. 2014) and DART (direct analysis in real time) (Lesiak et al. 2013) coupled with MS have been proved as good alternatives for minimizing the limitation of sample preparation or extraction.

In metabolomic studies, the major issue is to extract relevant information from a large data set as NMR and MS data consist of thousands of signals from hundreds of metabolites present in biofluids or intact tissues. The data obtained in metabolomics are huge and multivariate; therefore, a variety of chemometrics or pattern recognition methods are applied to identify trends and find significant information out of complex data (Madsen et al. 2010). A number of data pre-processing or alignment steps are involved before data are subjected to statistical or multivariate data analysis. These spectral processing techniques depend on the analytical methods (NMR, GC-MS and LC-MS) used to analyze the samples. Spectral processing is important to accurately identify the key metabolites present in the sample. It is also necessary to improve signal quality and remove biases present in raw data (Jacob et al. 2013).

In NMR- and MS-based methods, base line correction is required to reduce differences between the samples generated due to experimental and instrumental variations. In MS, peak-based methods are used to
identify key metabolites (Gika et al. 2014). However, the processing or alignment of MS data is more complicated because of the possibility of peaks to shift during chromatographic separation and a large size of data set involved. In NMR, binning-based methods (Vu & Laukens 2013) are used which sometimes capture multiple peaks from different metabolites in the same bin. This problem has been overcome with adaptive binning (Davis et al. 2007), adaptive intelligent binning (De Meyer et al. 2008) and Kernal-based methods (Anderson et al. 2008) for binning which are claimed to be more robust than traditional binning methods. Recent development in binning algorithm through the detection of optimal binning boundaries has also been introduced to resolve the issue of multiple peaks ending up in a single bin (Sousa et al. 2013). Overlapping peaks is a common issue in NMR- and MS-based methods which can be resolved by developing a deconvolution approach.

Currently, both methods (NMR and MS) have emerged as a powerful tool in the field of metabolomics with distinct advantages and limitations. Non-destructive, noninvasive, high reproducibility, minimal sample preparation, quick analysis, low cost and quantitative nature of NMR makes it a method of choice for targeted and untargeted metabolomic studies. On the other hand, extremely high sensitivity and specificity to particular metabolites and recent developments in MS-based methods endorse it as a good choice for targeted metabolomic analyses. In many cases, a combination of NMR and MS gives a more clear and global view of biosynthetic pathways, particularly in metabolic diseases, for example, diabetes and cancer.

2.3. Chemometric applications in metabolomics

Analytical techniques for metabolite profiling are being used in combination with multivariate statistical analysis. PCA and PLS (partial least square) are the two main chemometric methods that can be employed for dimension reduction while performing regression analysis. PCA is an unsupervised methodology, normally applied without taking into consideration the correlation among dependent and independent variables, whereas PLS (a supervised approach) is based on the correlation between variables (both dependent and independent variables). On the other hand, partial least square-discriminant analysis (PLS-DA) algorithm is a highly significant chemometric technique that deals with variable collinearity with additional ability to elaborate variables’ predictive capabilities in context with multivariate analysis (Maitra & Yan 2008). PCA is basically the simplest multivariate statistical analysis based on true eigenvector which envisages the underlying variable’s pattern during experiment. During PCA, the variables are usually arranged cumulatively in a simplified way to elaborate inclusive correlation among phytochemicals (Dutta et al. 2014). PCA, also called as ‘parsimonious summarization,’ is under use to minimize predictive variables and also provides solution for the multicollinearity problem (Bair Eric Hastie et al. 2006; Rosipal Roman Krämer, 2006).

PLS is a multipurpose algorithm and can be employed for the prediction of either continuous or discrete variables (Maitra & Yan, 2008). PLS-DA is normally used for discriminatory analysis. PLS-DA is a highly imperative algorithm for multivariate data. Orthogonal projections to latent structures discriminant analysis (O-PLS-DA) (orthogonal signal correction) is an extension of PLS-DA, by which explained variance is maximized among groups in a single dimension. O-PLS-DA also deals with the separation of within-group variance into orthogonal LVs (latent variables). The variable loadings based on a validated O-PLS-DA model can then be used for all under-study variables to rank them relative to their performance following discrimination between groups, and thus identify top predictors for a suitable model. The validation of PLS models can be achieved by cross-validation, external validation, variable importance and response permutation (Eriksson et al. 2001; Sun, 2004).

Hamad et al. (2015) reported PCA based on metabolites of date species, that is, amino acids, organic acids, sugars, phenolics, flavonoids, macro minerals, trace elements and antioxidants, and identified differences among the date cultivars. Freitas et al. (2015) evaluated the changes due to HLB in roots and leaves of citrus trees using PCA, such as natural clustering of the citrus trees and unsupervised pattern recognition. Bony et al. (2014) performed PCA and orthogonal-partial least square using metabolites of *Mitracarpus scaber* and *Combretum micranthum*, and reported a clear differentiation between the two herbal extracts. In another study, Uarrota et al. (2014)
described the discrimination of cassava samples based on PCA and PLS-DA for the post-harvest physiological deterioration period and also employed hierarchical clustering analyses for samples grouping as per their chemical compositions. Nkomo et al. (2014) correlated the antifungal potential and metabolite profiles of *Salvia africana-lutea* L. PCA was performed on NMR data, whereas PLS-DA was employed to integrate NMR and LC-MS data sets.

Rokaya et al. (2014) reported PCA based on pharmacological data, and discriminated selected medicinal plants of Nepal used to manage gastrointestinal disorders. Dutta et al. (2014) performed PCA to evaluate the contribution of 13 phytochemicals, namely, flavonoids, saponin, phenol, alkaloid, ascorbic acid, riboflavin, thiamine, total protein, lipid, tannin, ash content, soluble sugar and moisture, toward global phytochemical profile of *C. bonplandianus* stem extract. In another study, Pereira et al. (2014) described the use of HPLC-diode-array detection fingerprinting in combination with PCA and differentiated tea samples. Considerable variability based on total phenols and flavonoids was ascertained among different tea brands. Cook et al. (2013) also performed PCAs and k-nearest neighbor clustering analysis based on the phytochemical profile of *Equisetum arvense* extract and reported quantitative and qualitative differentiation among *Equisetum arvense* extracts based on their phytochemical profile in addition to their phytochemical origin. Metabolomics, a biomarkers-based approach, is therefore playing its pivotal role in the strengthening of existing pharmacological knowledge with new findings by exploring the metabolite profiles of various herbal drugs/products involved in the therapeutic treatment of certain diseases.

### 3. Proteomics

Proteomics deals with systematic protein expression analysis and analyzes different biomarkers compared to metabolomics during drug treatment; therefore, the use of information based on proteomics in combination with those obtained from metabolomics might be imperative for the evaluation of pharmacological response, novel biomarkers and biological pathways (Wang, Yan et al. 2013). The quantitative evaluation of differential protein expression in response to some variables is called expression proteomics. It is very helpful regarding the identification of differentially expressed proteins (in addition to the main proteins) in the diseased tissues/samples compared to the healthy ones (Davis et al. 2006). Normally, a cell regulates the levels and activities of its proteins in response to some internal and external change; therefore, variations in the proteome can provide a clear picture of a cell in action. Proteomics is an important tool for the investigation of physiological conditions and therapeutic monitoring of the altered proteins as possible drug targets, leading toward a clear understanding of the mechanism of action of herbal drugs (Lao et al. 2014). Investigations of the posttranslational modifications (i.e. glycosylation, phosphorylation, proteolysis, acetylation and amino acid polymorphisms), which occur during disease progression and drug treatment, and considerably affect the proteome, their structures and functions, are still other advantages of proteomics (Zhang & Ge 2011). Proteomics, therefore, helps to evaluate and understand the functions and interactions of the proteome in a specific organism.

#### 3.1. Analytical tools for proteomics

Protein separation, identification and characterization of the resolved proteins are the main aspects in proteomics research. There are numerous proteomic techniques for the separation of proteins/peptides. But out of these, two-dimensional gel electrophoresis (2DGE) with sufficiently high resolution is the most accepted technique for the separation and analysis of proteins in complex mixtures such as blood plasma and organ tissues, prior to the identification of proteins by MS (Ferber 2002; Frank & Hargreaves 2003; Hussain & Huygens 2012; Riaz 2015). 2DGE allows the separation of several thousand proteins with sufficiently high resolution. On the other hand, for the identification of separated proteins, highly sensitive mass spectrometric (MS) methods are being used worldwide with detection ability as low as $10^{-15}$ to $10^{-18}$ mole and accuracy ($0.1–0.01\%$). MALDI-TOF-MS (matrix-assisted laser desorption ionization time-of-flight MS) is typically being used for the identification of proteins and is now recognized as core technology in proteomics, because of its high accuracy and sensitivity (Riaz 2015). MALDI-TOF-MS is simple in operation, accurate, highly sensitive with significantly high resolution and offers a broad range of applications from the identification of protein biomarkers to their
characterization through mass-based fingerprinting of proteins/peptides (Aebersold & Mann 2003). Proteomic research using 2DGE coupled with MALDI-TOF-MS/MS thus exhibits the capability to investigate the proteins alteration during drug treatment and posttranslational modifications. The results of the proteomics then can easily be interpreted by comparison with the available toxicological data.

Lao et al. (2014) comprehensively reviewed the application of proteomics for the investigation of therapeutic targets and underlying mode of actions of TCM remedies for many diseases including neuronal disease, cardiovascular disease (CVD), diabetes, cancer and immunology-related disease. Proteomics has been successful in the discovery process of numerous TCM compounds. A general proteomics protocol has been described for the elucidation of mechanism of action of TCM extracts, starting from sample preparation following 2DGE, spots extraction, mass spectrometric (MS/MS) analysis, bioinformatics and, finally, candidate validation, both in vitro and in vivo. Alternatively, stable isotope labeling with amino acids in cell culture technique can be employed that integrates an isotopically labeled amino acid within the proteome of the cells, followed by the mixing of whole labeled proteome with that from unlabeled cells and the ultimate evaluation of differentially expressed proteins. Zhao and Lin (2014) briefly reviewed the application of UPLC-MS\textsuperscript{E} (Ultra performance liquid chromatography–mass spectrometry\textsuperscript{ElevatedEnergy}) in clinical proteomics for the discovery of novel proteome biomarkers, targeted therapeutics and disease diagnosis.

UPLC-MS\textsuperscript{E} is known to exhibit high resolution and better sensitivity for the investigation of cellular proteins. In another review, Sulistio and Heese (2015) summarized comparative proteomics, to elaborate the effectiveness of TCM treatment for Alzheimer’s disease. Three proteomic strategies have been reported to be used for the empirical understanding of the mechanism of action for TCM remedies including syndrome proteomics, screening proteomics and comparative proteomics. Syndrome proteomics is normally employed for translating a syndrome and can be attained by proteomics analysis of bodily fluids/organs related to specified TCM syndromes (Lu et al. 2010; Sun et al. 2010). Screening proteomics is being used to evaluate mechanisms of medicinal herbs based on the identification of binding partners of effective constituents. Comparative proteomics/differential proteomics, on the other hand, hold promise for the quantitative determination of proteins in control and TCM-treated groups and also explore key differentially expressed proteins (Fense-lau 2007; Firouzi et al. 2014). Ji et al. (2015) have also reviewed the applications of proteomics in diseases-TCM syndrome and summarized possible mechanisms of TCM treatments. Proteomic analysis normally requires the combination of several analytical approaches, including protein processing, separation and identification. Based on the literature, it has been revealed that as proteomics technology is capable of the identification of relatively fewer proteins and the reproducibility of its data is poor, hence for ideal and accurate results, the integrated usage of all proteomic techniques, that is, two-dimensional electrophoresis (2DE), HPLC, MALDI-TOF-MS, MS/MS, SELDI-TOF-MS, isobaric tags for relative and absolute quantitation (iTRAQ) and bioinformatics, is recommended.

Chemical proteomics is another rapidly evolving field of research that utilizes small molecule probes for the investigation of protein functions and can be applied for the unbiased and quantitative identification of protein-binding targets of small molecules. Recently, Wright and Sieber (2016) summarized chemical proteomics techniques for the identification of cellular targets based on natural products. Different chemical probes for targeted identification have been elaborated. These probes are capable of covalent linkage with their target proteins and can be employed directly in living systems. By using this technique along with a cell-permeable probe in combination with a two-step bio-orthogonal ligation procedure, endogenous protein levels in live cells can be investigated. Moreover, measurement of the half maximal inhibitory concentration of probe–protein binding, dose response or response to competitor can also be executed by using quantitative proteomics/well-characterized probes in combination with in-gel fluorescence detection. Typical proteomic procedures are described in Figures 1 and 2.

### 3.2. Proteomics application in herbal drug discovery

Recently different researchers have employed proteomics for the evaluation and identification of protein biomarkers as potential targets for the therapeutic
Figure 1. Typical sample preparation protocol for 2DGE for protein biomarker studies using a rat model (Fan et al. 2010; Guido & Oliva 2009; Kelleher et al. 2009; Wang et al. 2004; Yue et al. 2012; Gorg et al. 1988; Zhang, Sun et al. 2010; Amacher et al. 2005; Lee et al. 2009; Ye, Zhang et al. 2006; Ye, Chen et al. 2006).

monitoring of specific diseases. Different herbs/herbal constituents/preparations, such as *Cynodon dactylon* (Karthik et al. 2012), Tianqi Jiangtang Capsule (Zhang, Sun et al. 2010), Zi-Bu-Pi-Yin recipe (Shi et al. 2011), ShenSongYangXin (SSYX; Liu et al. 2015), Shuanglong Formula (SLF) (Fan et al. 2010), notoginsenoside and salvianolic acid (derived from *Panax notoginseng* and *Salvia miltiorrhiza*) (Yue et al. 2012), Buyang Huanwu decoction (BYHWD; Zhou, Liu et al. 2012; Chen, Shen et al. 2015), Yin-Chen-Hao-Tang (YCHT; Lee et al. 2009), periplocin (Lu et al. 2014), *Rosmarinus officinalis* (Çelebier et al. 2015), genistein (primary isoflavone component of soy) (Wang et al. 2011), triptolide (a diterpenoid triepoxide from *Tripterygium wilfordii*) (Liu et al. 2012), Tianma’s (TCM) (Manavalan et al. 2012), *Acanthopanax senticosus* (Jiang et al. 2015), *Scutellaria baicalensis* Georgi (Kim et al. 2014), huperzine A (lycopodium alkaloid from *Huperzia serrata*) (Tao et al. 2013) and Chinese 2-herbal formula (Tam et al. 2014), etc.,
Figure 2. A Typical stepwise protocol for 2DGE and MALDI-TOF-MS/MS (Gorg et al. 1988; Wang et al. 2004; Amacher et al. 2005; Ye, Zhang, et al. 2006; Ye, Chen, et al. 2006; Guido & Oliva 2009; Kelleher et al. 2009; Lee et al. 2009; Fan et al. 2010; Zhang, Sun et al. 2010; Yue et al. 2012).

have been investigated for their targeted proteome biomarkers during the treatment of specific disorders (Table 2).

Diabetes mellitus is a metabolic disorder associated with insulin deficiency (due to defects in insulin/insulin actions) leading to chronic hyperglycemia and irregularities in the metabolism of carbohydrates, fat and proteins (Kumar & Clark 2002; Bastaki 2005; Al-Zuaidy et al. 2016). Progression of the disease also results in adverse diabetic complications, such as neuropathy, retinopathy and cardiovascular complications (Lindberg et al. 2004; Moran et al. 2004; Svensson et al. 2004).
The proteomics approach has been employed by many researchers for the investigation of targeted proteomes of the body organs and serum during the treatment of diabetes mellitus and its associated complications by herbal products in vivo. The impact of antidiabetic herb *Cynodon dactylon* leaves extract on rat’s liver proteomes was investigated in one study. It was revealed that *Cynodon dactylon* exerted its impact on liver cells to control diabetes mellitus by regulating nucleophosmin, carbonic anhydrase III and L-xylulose reductase, and these proteins were involved in homoeostasis and cell proliferation of the liver tissues upon treatment with *Cynodon dactylon* leaves extracts (Karthik et al. 2012).

Ethanolic *Cynodon dactylon* leaves extract has also been investigated for its effects toward the prevention of heart failure linked with diabetes. The *Cynodon dactylon* leaves extract exerted effects by up-regulating the NTF4 (neurotrophic cascade protein) and ETFB (electron transport chain cascade protein). These proteins were found to be involved in the prevention of diabetic secondary complications (cardiomyopathy and diabetic polyneuropathy), and easy electron transfer to heart during diabetes, leading to reduced free-radical formation and oxidative stress (Karthik et al. 2014). In another study, the changes in rat serum proteins were studied during the treatment of diabetes mellitus (T2DM) by the TCM Tianqi Jiangtang Capsule. It was depicted that the induction of T2DM in rats resulted in the down-regulation of apolipoprotein A-I, apolipoprotein E and Ig gamma-2A chain C region, and the up-regulation of haptoglobin (Hp), transthyretin (TTR), serum amyloid P-component (SAP) and prothrombin. However, as a result of Tianqi Jiangtang Capsule treatment, the majority of differentially expressed proteins were restored to their normal levels and the antidiabetic effect was revealed to be associated with the reduction of hyperglycemia and improvement of lipid metabolism (Zhang, Sun et al. 2010). The effect of the Zi-Bu-Pi-Yin recipe toward diabetes has also been evaluated and novel protein biomarkers, that is, DRP-2 and PDHE1, were found to be the potential targets of the Zi-Bu-Pi-Yin recipe during the course of diabetes treatment (Shi et al. 2011).

A number of medicinal plants/herbs are being used for the treatment of CVDs worldwide. Several attempts have been made by the scientific community to elaborate the potential protein biomarkers and underlying mode of actions of different medicinal plants/herbs for the management of various CVDs (Fan et al. 2010; Yue et al. 2012; Zhou, Liu et al. 2012; Liu et al. 2015). In one study, differentially expressed proteins in bradycardia rabbits were characterized in response to the SSYX treatment. The SSYX effectively treated bradycardia by regulating the proteins involved in oxidoreductase activity, calcium ion-related proteins, electron carrier activity and structure proteins. Up-regulation of the calcium release channel (RyR2), voltage-dependent anion-selective channel (VDAC) and SRCa2+–ATPase (SERCA2) was found to be involved in the restoration of calcium ion homeostasis leading to enhanced cardiac function (Liu et al. 2015). In another study, the phytotherapeutic impact of herbal SLF on rat mesenchymal stem cells (MSCs) differentiation toward cardiomyocytes have been evaluated using proteomics. It was found that cardiac-specific proteins were expressed and around 36 proteins were regulated upon SLF treatment, which were mainly involved in cell tissue energy metabolism, cytoskeleton structure and signal transduction (Fan et al. 2010).

Proteomic analysis has also been carried out to evaluate the cardiovascular protective effects of notoginsengoside and salvianolic acid derived from *Panax noto ginseng* and *Salvia miltiorrhiza*, respectively. It was depicted that both notoginsengoside and salvianolic acid exerted their impact by inhibiting the ‘eukaryotic translation elongation factor 2’ involved in cell proliferation and also by inhibiting the activities of proteins, namely disulfide isomerase and prohibitin (Yue et al. 2012). The effect of BYHWD toward the improvement of ventricular remodeling caused by LAD (left anterior descending) artery ligation has also been investigated to elaborate targeted proteins. The atrial natriuretic factor was down-regulated, whereas peroxiredoxin-6 and heat shock protein beta-6 were up-regulated in BYHWD-treated rat group compared to the control. It was revealed that the BYHWD exerted its anti-remodeling effects by decreasing the apoptotic index, by reducing caspase 3 activity and escalating Bcl-2/Bax ratio, through up-regulated peroxiredoxin-6, by phosphorylation of heat shock protein beta-6 and by reduction of the atrial natriuretic factor (Zhou, Liu et al. 2012).

Some proteomic studies have also been carried out relevant to hepatic diseases. During a previous study, proteomics approach was employed to evaluate the
Table 2. Proteomics-based mechanism of action of various herbal drugs/extracts/ingredients.

| Medicinal herb/extracts/ingredient | Understudy disease state/effect | General description | Major analytical technique employed | Proposed mechanism | Comments/Highlights | References |
|-----------------------------------|---------------------------------|--------------------|-----------------------------------|--------------------|---------------------|------------|
| Cynodon dactylon                  | Diabetes mellitus               | • Effect of Cynodon dactylon (antidiabetic herb) leaves extract on rat liver proteome linked with diabetes mellitus was investigated using differential proteomics. | 2-DE MALDI-TOF-MS | Three proteins, that is, nucleophosmin, carbonic anhydrase III and L-xylulose reductase, were up-regulated in diabetic rats compared to controls. | • The results elaborated the molecular mechanism connected with insulin and liver functions during diabetes mellitus. | Karthik et al. (2012) |
| Tianqi Jiangtang Capsule          | Diabetes mellitus               | • Changes in the levels of serum proteins due to Tianqi Jiangtang Capsule (TCM) in diabetes mellitus (type 2) rat model were investigated. | 2-DE MALDI-TOF-MS ELISA | • Upon T2DM induction, apolipoprotein E, Ig gamma-2A chain C region and apolipoprotein A-I were down-regulated, while transthyretin (TTR), serum amyloid P-componen (SAP), haptoglobin (Hp) and prothrombin were up-regulated. | • Proteomics approach was employed and targeted proteins were identified. Interestingly, the levels of identified proteins were restored to the levels of healthy rats following treatment with Tianqi Jiangtang Capsule. | Zhang, Sun et al. (2010) |
| Cynodon dactylon                  | Heart failure linked with diabetes | • Ethanolic Cynodon dactylon leaves extract was investigated to evaluate its impact regarding the prevention of heart failure linked with diabetes in vivo. | 2DE MALDI-TOF-MS | • NTF4 (neurotrophic cascade protein) and ETFB (electron transport chain cascade protein) were up-regulated. | • Up-regulated proteins reduced CVDs in response to C. dactylon treatment | Karthik et al. (2014) |
| SSYX                              | Heart disease/bradycardia       | • Differentially expressed proteins in bradycardia rabbits were characterized in response to the SSYX treatment. | iTRAQ 2D LC-MS/MS | • Eighty-six proteins were differentially expressed. | • The SSYX effectively treated bradycardia by regulating the proteins involved in oxidoreductase activity, calcium ion-related proteins, electron carrier activity and structure proteins. | Liu et al. (2015) |

(continued)
| Medicinal herb/extracts/ingredient | Understudy disease state/effect | General description | Major analytical technique employed | Proposed mechanism | Comments/Highlights | References |
|-----------------------------------|---------------------------------|---------------------|-------------------------------------|--------------------|---------------------|------------|
| Herbal ingredients of SLF         | CVD                             | Effect of main herbal ingredients of SLF on rat MSCs proteome differentiation was investigated | 2-DE MALDI-TOF-MS  | Thirty six proteins were involved in cell tissue energy metabolism, signal transduction and cytoskeleton markedly regulated by SLF treatment | Proteome undergoes rearrangements during MSCs differentiation process following SLF treatment and offers the possible investigation of specific biomarkers driving differentiation of the stem cells | Fan et al. (2010) |
| Notoginsengnoside (Panaxnotoginseng) and salvianolic acid (from Salvia miltiorrhiza) | CVD                             | Proteomic analysis was applied using a rat model to elucidate the modes of action of notoginsengnoside and salvianolic acid | 2-DE MALDI-TOF-MS/MS | Notoginsengnoside and salvianolic acid inhibited eukaryotic translation elongation factor-2 which is mainly associated with cell proliferation | Proteome differentiation pattern of notoginsengnoside and salvianolic acid revealed that different protein targets were associated with both the compounds toward their cardiovascular protective potential | Yue et al. (2012) |
| Buyang Huanwu decoction (BYHWD)   | CVD                             | Differentially expressed proteins linked with BYHWD treatment were investigated through Proteomics approach using ischemia-induced ventricular rats remodel | 2-DE MALDI-TOF-MS/MS | Atrial natriuretic factor down-regulated | The results reflected the possible capability of BYHWD to alleviate ventricular remodeling, which is usually induced by the ligation of the LAD artery | Zhou, Liu et al. (2012) |
| Yin-Chen-Hao-Tang (YCHT)          | Hepatic disease                  | Proteomics approach was used to investigate changes in serum and hepatic proteins following Yin-Chen-Hao-Tang (YCHT) treatment using rat model | HPLC RT-PCR 2-DE MALDI-Q-TOF-MS/MS | The biomarker protein with major effect was plectin-1 (cytoskeleton-related protein) | The proteomic studies on Yin-Chen-Hao-Tang suggested its usage to treat liver fibrosis due to its antiapoptotic properties. | Lee et al. (2009) |
| Periplocin                         | Cancer                          | The molecular mechanisms involved in anticancer effect of periplocin based on altered protein profile of human lung cancer cell lines A549 was investigated | 2DE ESI/MALDI-Q-TOF-MS/MS | Thirty-nine proteins were differentially expressed, 29 were down-regulated and 10 were up-regulated | The therapeutic effects of Yin-Chen-Hao-Tang on liver diseases are possibly associated with lipid biosynthesis regulation. | Lu et al. (2014) |

(continued)
| Medicinal herb/extracts/ingredient | Understudy disease state/effect | General description | Major analytical technique employed | Proposed mechanism | Comments/Highlights | References |
|-----------------------------------|---------------------------------|---------------------|-----------------------------------|-------------------|---------------------|------------|
| Polyphenols enriched *Rosmarinus officinalis* L. | Antiproliferative effect | Antiproliferative effect of polyphenols enriched *Rosmarinus officinalis* L. extracts have been investigated in vitro using K562 and K562/R cells lines | 2DE MALDI-TOF/TOF MS | Annexin A1 and adenine phosphoribosyl transferase in K562/R cell lines, while tubulin alpha-1C chain in the case of K562 cell lines were down-regulated | Differentially expressed proteins were basically involved in antioxidant activity, tumorigenesis and cancer proliferation | Çelebier et al. (2015) |
| Genistein (isoflavone component of soy) | Cancer | Proteomic technology was employed to evaluate genistein mode of action regarding breast cancer protection | 2-DE MALDI-TOF/TOF LC-ESI-MS/MS | Annexin A2 was increased significantly at 21 days, but decreased at 50 days | Proteomics-based studies revealed that genistein (isoflavone component of soy) suppresses mammary cancer in rats, when prepubertally consumed in diet. | Wang et al. (2011) |
| Genistein & BPA | Cancer: | Protein biomarkers associated with BPA or genistein in rat serum were investigated using proteomics approach | TMT-MS | Expression of 63 and 28 proteins in the serum of rats altered at postnatal day 21, whereas of 9 and 18 proteins altered in rat serum at postnatal day 35 following prepubertal exposure of BPA and genistein | Changes in rat serum proteome were investigated using quantitative proteomic techniques and elaborated the potential cellular targets (involved in carcinogenesis) altered following BPA and genistein exposure | Betancourt et al. (2014) |
| Triptolide (diterpenoid triepoxide) from *Tripterygium wilfordii* | Cancer: | Proteome changes during colon cancer following triptolide treatment were investigated using proteomic technology | 2-DE MALDI-TOF/TOF-MS | The main protein (14-3-3 epsilon) of cell cycle arrest leading and the cell death was cleavage and peri-nuclear translocated | The investigation of triptolide (diterpenoid triepoxide) derived from medicinal herb *Tripterygium wilfordii* ascertained its significance as a therapeutic agent for the treatment of colon cancer by changes in 14-3-3 epsilon | Liu et al. (2012) |
| Medicinal herb/ extracts/ ingredient | Understudy disease state/effect | General description | Major analytical technique employed | Proposed mechanism | Comments/Highlights | References |
|------------------------------------|--------------------------------|---------------------|------------------------------------|--------------------|---------------------|------------|
| Gastrodia elata Blume (tianma)     | Neurological disorder          | • Proteomics approach was employed to investigate the mechanism of action and Tianma’s potential toward the treatment of neurodegenerative diseases such as Alzheimer’s | 2-DE-LC–MS/MS      | • Neuro-regenerative processes were promoted by stress-related protein inhibition and mobilization of neuroprotective genes/proteins, viz., Nxn, Mobkl3, Dbn1, Clic4, Bax and Mki67 with different regenerative modalities related to the neuro-synaptic plasticity | • Proteins with regenerative modalities relevant to neuro-synaptic plasticity of mouse N2A cells were investigated following Gastrodia elata Blume (a TCM) | Manavalan et al. (2012) |
| BYHWD                              | Neuroprotective effect         | • The effects of BYHWD have been investigated in the CIR-induced stroke mice using iTRAQ-based proteomics approach to unravel the underlying mechanism of action | iTRAQ nanoUHPLC-QTOF-MS/MS | • Numerous proteins were differentially expressed upon treatment with BYHWD. | • BHD treatment also up-regulated doublecortin (neurogenesis marker) and inhibited the activities of glycogen synthase kinase 3 and Tau revealing its neuroprotective effects. | Chen, Shen et al. (2015) |
| Acanthopanax senticosus            | Neuro-inflammatory effect      | • Molecular mechanism involved in neuro-inflammatory effect of Acanthopanax senticosus extract was investigated using proteomics approach | 2D-DIGE LC-ESIMS/ MS | • Seventeen proteins were altered significantly | • Acanthopanax senticosus extract inhibited LPS-induced nitric oxide production in BV-2 microglial cells with nonsignificant cell toxicity | Jiang et al. (2015) |

(continued)
| Medicinal herb/ extract/ ingredient | Understudy disease state/effect | General description | Major analytical technique employed | Proposed mechanism | Comments/Highlights | References |
|-----------------------------------|---------------------------------|---------------------|-------------------------------------|--------------------|---------------------|------------|
| Flavonoids isolated from *Scutellaria baicalensis* Georgi | Anti-inflammatory Response | Proteomics approach was employed to investigate anti-inflammatory effect of flavonoids isolated from *Scutellaria baicalensis* Georgi in lipopolysaccharide induced L6 skeletal muscle cells. | 2DE MALDI-TOF-MS | Twelve proteins were differentially expressed. Vimentin, annexin A1, annexin A2, TBX3 and annexin A5 proteins were involved in the inflammatory responses | Flavonoids isolated from *Scutellaria baicalensis* Georgi inhibited the expression of annexin A2, cyclooxygenase-2 (COX-2) and nitric oxide synthases (iNOS) proteins and protected the LPS-induced inflammation process of L6 skeletal muscle cells | Kim et al. (2014) |
| Huperzine A (main active ingredient of *Huperzia serrata*) | Anti-inflammatory responses | Proteomics approach was used to investigate the effect of huperzine A (main active ingredient of *Huperzia serrata*) toward nausea, vomiting, pain and dizziness | Nano-LC–MS/MS | Huperzine A down-regulated p53 levels and protected N2a cells from the amyloid β-induced death of cells | Using centrifugal proteomic reactor coupled with nano-LC-MS/MS strategies, 2860 proteins were characterized and moreover pathways analysis of 198 significantly altered proteins was also evaluated. | Tao et al. (2013) |

(continued)
| Medicinal herb/extracts/ingredient | Understudy disease state/effect | General description | Major analytical technique employed | Proposed mechanism | Comments/Highlights | References |
|-----------------------------------|--------------------------------|---------------------|------------------------------------|--------------------|---------------------|------------|
| Chinese 2-herb formula (Rehmanniae Radix & Astragali Radix) | Diabetic linked wound healing | • Proteomics analysis was employed for the investigation of the effect of Chinese 2-herb formula (Rehmanniae Radix & Astragali Radix) toward wound (associated with diabetic) healing using rats model Proteomic investigations were carried out on Shoseiryuto in Japan (Chinese herb prescription) to evaluate its potential for the treatment of allergic rhinitis, bronchial asthma, bronchitis and cold symptoms | 2-DE MALI-TOF/TOF-MS/MS | • Plasminogen activator inhibitor-1, annexin A1 and annexin A2 were the principal proteins associated with proangiogenesis in wound healing • Plasminogen activator inhibitor-1 was down-regulated in the static condition, whereas annexin A1 & A2 were up-regulated in the scratch condition | • Global proteome expression and molecular level differences were identified between scratch and static states associated with wound healing angiogenesis following the treatment of Chinese 2-herb formula (Rehmanniae Radix & Astragali Radix) | Tam et al. (2014) |
| Shoseiryuto | Anti-inflammatory responses | • Proteomic investigations were carried out on Shoseiryuto in Japan (Chinese herb prescription) to evaluate its potential for the treatment of allergic rhinitis, bronchial asthma, bronchitis and cold symptoms | 3D HPLC 2DE HPLC-ITMS/MS | • Protein spectrin 2 expression was reversely reduced in lung tissue following Shoseiryuto treatment | • Effects of Shoseiryuto, a Japanese herbal medicine, were evaluated toward bronchial asthma using a mouse model. Proteomic studies revealed that the treatment of Shoseiryuto reduced lung tissue inflammation and also minimized the airway hyperreactivity | Nagai et al. (2011) |
hepato-protective effect of YCHT (Lee et al. 2009). The hepato-protective effect was found to be associated mainly with the regulation of plectin-1, which is a cytoskeleton-related protein. The proteins involved in lipid metabolism (glycoprotein 330 and ApoA-I) were also affected. Moreover, YCHT treatment also caused significant up-regulation of keratin 8 and 19 in the liver tissue, whereas down-regulation of ‘monocyte chemotactrant protein-1’ (MCP-1) and ‘tissue inhibitor of metalloproteinase-1’ (TIMP-1) was also observed (Lee et al. 2009). Other proteomic studies have also been carried out to investigate the protein biomarkers in rat serum as potential indicators of hepatocellular necrosis, hepatomegaly and/or hepatobiliary injury (Amacher et al. 2005; Merrick et al. 2006).

Targeted proteome of various medicinal plants/herbs for the treatment of cancer and its related diseases has also been reported by researchers. The molecular mechanisms involved in the anticancer effect of periplocin based on the altered protein profile of ‘human lung cancer cell lines A549’ have been investigated recently. It was found that altered proteins were involved in proteolysis and transcription, and that the lung cancer growth was inhibited because of the down-regulation of aldehyde dehydrogenase 1, ATP synthase ecto-α-subunit, eukaryotic translation initiation factor 5A-1, proteasome subunit beta type-6 proteins, etc. However, the authors suggested further studies to evaluate the possible cross talk between the altered protein species and their relationship with proteolysis processes that were basically involved in the pleiotropic activity of periplocin (Lu et al. 2014). The antiproliferative effect of polyphenols-enriched Rosmarinus officinalis L. extracts has also been investigated in vitro using K562 and K562/R cell lines recently. The Rosmarinus officinalis L. extract exerted its antiproliferative effect by the down-regulation of annexin A1 and adenine phosphoribosyl transferase in K562/R cell lines, while tubulin alpha-1C chain in the case of K562 cell lines. Unexpectedly, D-3-phosphoglycerate dehydrogenase was up-regulated in K562 cell lines after Rosmarinus officinalis L. extracts treatment. The differentially expressed proteins were found to be involved in antioxidant activity, tumorigenesis and cancer proliferation (Celebier et al. 2015).

In another study, differentially expressed proteins as targeted biomarkers, responsible for genistein (primary isoflavone component of soy) breast cancer protection, have been investigated. The genistein treatment showed its impact regarding the prevention of breast cancer by significantly up-regulating annexin A2 and gelsolin, while down-regulating disulfide-isomerase A3 (Wang et al. 2011). Proteomics approach has also been employed to investigate the phytotherapeutic effect of bisphenol A (BPA) and genistein toward the suppression of mammary cancer recently (Bancourt et al. 2014). In another study, triptolide, which is a diterpenoid triepoxide from Tripterygium wilfordii (traditional Chinese medical herb), have been investigated for its effect toward the attenuation of colon cancer growth. Apoptosis and cell cycle-related protein (14-3-3 epsilon) were changed in colon cancer cells after triptolide exposure. The triptolide treatment, therefore, exerted its anticancer action by inducing cleavage and perinuclear translocation of the said protein involved in cell cycle arrest/cell death (Liu et al. 2012).

Studies have also been carried out to evaluate the mechanism of actions of various herbal treatments for neurological disorders such as dementia, neurodegenerative diseases and neuronal injury. A recent proteomics investigation evaluated Tianma’s (TCM) potential for the treatment of neurodegenerative diseases. Tianma exerted its impact by the inhibition of stress-related proteins such as nucleore-doxin (Nxn), Mps one binder kinase activator-like 3 (Mobkl3), drebrin-like protein (Dbn1) and Ki67 protein (Mki67) (Lao et al. 2014). The effects of BYHWD have been investigated in cerebral ischemia/reperfusion (CIR)-induced stroke in mice using iTRAQ-based proteomics approach to unravel the underlying mechanism of action (Chen, Shen et al. 2015). It was revealed that BYHWD treatment significantly preserved blood–brain barrier (BBB), albumin (Alb), fibrinogen alpha chain (Fga) and transferrin (Trf). The BYHWD treatment also escalated energy metabolism (Bdh) and suppressed excitotoxicity by regulating glutamate receptor metabotropic 5 (Grm5), guanine nucleotide binding protein (Gna1) and GDP-dissociation inhibitor (Gdi). Moreover, BYHWD treatment up-regulated doublecortin (neurogenesis marker) and inhibited the activities of glycogen synthase kinase 3 and Tau, revealing its neuroprotective effects (Chen, Shen et al. 2015). In another study, the molecular mechanism involved in the neuro-inflammatory effect of Acanthopanax senticosus extract has been investigated and it was depicted that Acanthopanax senticosus
extract inhibited lipopolysaccharide (LPS)-induced nitric oxide production in BV-2 microglial cells with nonsignificant cell toxicity. Seventeen proteins altered significantly upon *Acanthopanax senticosus* extracts treatment and contributed in free-radical scavenging, protein synthesis and cell death or survival. The possible underlying canonical pathways involved were superoxide radical’s degradation, nrf2-mediated oxidative stress response, pentose phosphate pathway, gap junction signaling and 14-3-3-mediated signaling. It was therefore revealed that *Acanthopanax senticosus* extract exerted its neuro-inflammatory effect by the suppression of nitrosative stress in BV-2 cells (Jiang et al. 2015).

The anti-inflammatory effect of flavonoids isolated from *Scutellaria baicalensis* Georgi has also been investigated in lipopolysaccharide-induced L6 skeletal muscle cells. Vimentin, annexin A1, annexin A2, T-box transcription factor (TBX3) and annexin A5 proteins were revealed to be involved in the inflammatory responses. It was also found that flavonoids isolated from *Scutellaria baicalensis* Georgi inhibited the expression of annexin A2, cyclooxygenase-2 (COX-2) and nitric oxide synthases (iNOS) proteins and protected the LPS-induced inflammation process of L6 skeletal muscle cells (Kim et al. 2014). In another study, proteomics approach was applied to investigate the neuroprotective effects of huperzine A (lycopodium alkaloid from *Huperzia serrata*) for Alzheimer’s disease on neuronal cells. It was depicted that huperzine A works by protecting N2a cells from cell death induced by amyloid-β by down-regulating p53 (cellular tumor antigen) expression (Tao et al. 2013). Protein biomarkers associated with Shoseiryuto (‘SST, Xiao-Qing-Long-Tang in Chinese’) during the treatment of bronchial asthma have also been investigated using an ovalbumin (OVA)-sensitized mice model. The oral administration of SST resulted in reduced inflammation in the lung tissue of mice and also decreased airway hyperreactivity. Spectrin α2 expression was down-regulated in the lung tissue of OVA-sensitized mice; however, SST administration recovered the spectrin α2 expression at the normal level in the lung tissue of OVA-sensitized mice. So, the ‘SST Xiao-Qing-Long-Tang decoction’ works by reducing spectrin α2 expression in the lung tissue (Nagai et al. 2011).

A Chinese 2-herbal formula (NF3) has also been investigated recently for its potential molecular targets involved in proangiogenic response during wound healing using ‘human umbilical vein endothelial cells (HUVEC)’ in both static and scratched conditions. The potential proteins involved in proangiogenesis during wound healing upon NF3 treatment were depicted to be plasminogen activator inhibitor-1, annexin A1 and annexin A2. It was found that NF3 treatment also involved reactive oxygen species defense, cell–cell interaction, transcription and translation in HUVEC (Tam et al. 2014). Recently Qing et al. (2015) comprehensively reviewed the applications of proteomics for research specific to traditional Chinese medicines. Proteomics-based biomarkers identification is, therefore, a significant diagnostic tool for the evaluation of protein–disease associations with potential futuristic applications of proteomics regarding the identification of pathways for specific drugs, associated targeted biomarkers, herbal drugs’ mechanisms of action and their therapeutic monitoring during the course of treatment.

4. Conclusions

Conclusively, as futuristic diagnostic tools, both metabolomics and proteomics can be the promising strategies for the efficient translation and understanding of metabolite–disease and protein–disease associations leading to a revolution in therapeutic monitoring. These biomarkers-based approaches are pivotal toward the strengthening of existing pharmacological knowledge with new findings by exploring the mechanisms of action of various herbal drugs/products involved in the therapeutic treatment of certain diseases. However, to cope with the challenges regarding the generation of comprehensive, inclusive and quantitative biomarker profiles of biofluids under different therapeutic conditions, the existing analytical strategies still possess considerable drawbacks, viz., low sensitivity associated with NMR spectroscopic analysis coupled with difficulty in interpretation of complex NMR and mass spectra, lack of global database for the accurate identification of metabolites in plant extracts, etc.. Dealing accurately with differentially altered proteins during different disease states of herbal drug treatment, accurate data analysis and structural characterization of the significant biomarkers are still other challenges. However, to cope with these challenges in the future, coordinated efforts are required for technical and methodological advancements to create a significant impact on biomarker
investigations. Advancements in terms of increased NMR sensitivity, improved mass accuracy and resolution in the case of mass spectrometers, and accurate monitoring of altered proteomes during proteomics will result in effective biomarker characterization. Moreover, effective collaboration among the relevant scientific communities will further assist biomarker studies by the construction of freely available and easily accessible global databases.

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References
Aebersold R, Mann M. 2003. Mass spectrometry-based proteomics. Nature. 422:198–207.
Aharoni A, Ric DV, Verhoeven HA, Maliepaard C, Kruppa G, Bino RJ. 2002. Nontargeted metabolome analysis by use of Fourier transform ion cyclotron mass spectrometry. OMICS. 6:217–234.
Akhtar MT, Bin Mohd Sarib MS, Ismail IS, Abas F, Ismail A, Lajis NH, Shaari K. 2016. Anti-Diabetic activity and metabolic changes induced by Andrographis paniculata plant extract in obese diabetic rats. Molecules. 21:1026.
Al-Zaaidy MH, Hamid AA, Ismail A, Mohamed S, Razis A, Faizal A, Mumtaz MW, Salleh SZ. 2016. Potent anti diabetic activity and metabolite profiling of Melicope Lunu-ankenda Leaves. J Food Sci. 81:C1080–C1090.
Amacher DE, Adler R, Herath A, Townsend RR. 2005. Use of proteomic methods to identify serum biomarkers associated with Rat liver toxicity or hypertrophy. Clin Chem. 51:1796–1803.
Anderson PE, Reo NV, DelRaso NJ, Doom TE, Raymer ML. 2008. Gaussian binning: A new kernel-based method for processing NMR spectroscopic data for metabolomics. Metabolomics. 4:261–272.
Asche CV, McAdam-Marx C, Shane-McWhorter L, Sheng X, Plauschinat CA. 2008. Association between oral anti diabetic use, adverse events and outcomes in patients with type 2 diabetes. Diabetes Obes Metab. 10:638–645.
Bai X, Qu J, Lu J, Kano Y, Yuan D. 2011. Isolation and identification of urinary metabolites of kakkalide in rats. J Chromatogr B. 879:395–402.
Bair Eric Hastie T, Debashis P, Tibshirani R. 2006. Prediction by supervised principal components. J Amer Statist Assoc. 101:119–137.
Bajad S, Shulaev V. 2011. LC-MS-based metabolomics. Methods Mol Biol. 708:213–228.
Bastaki S. 2005. Diabetes mellitus and its treatment. Int J Diabetes Metab. 13:111–134.
Beckwith-Hall BM, Nicholson JK, Nicholls AW, Foxall PJD, Lindon JC, Connor SC, Abdi M, Connelly J, Holmes E. 1998. Nuclear magnetic resonance spectroscopic and principal components analysis investigations into biochemical effects of three model hepatotoxins. Chem Res Toxicol. 11:260–272.
Beger RD, Sun J, Schnackenberg LK. 2010. Metabolomics approaches for discovering biomarkers of drug-induced hepatotoxicity and nephrotoxicity. Toxicol Appl Pharmacol. 243:154–166.
Bertol E, Vaiano F, Milia MGD, Mari F. 2015. In vivo detection of the new psychoactive substance AM-694 and its metabolites. Forensic Sci Int. 256:21–27.
Betancourt A, Mobley JA, Wang J, Jenkins S, Chen D, Kojima K, Russo J, Lamartiniere CA. 2014. Alterations in the rat serum proteome induced by prepubertal exposure to bisphenol a and genistein. J Proteome Res. 13:1502–1514.
Bok JW, Lermmer L, Chilton J, Klingeman HG, Towers GH. 1999. Antitumor sterols from the mycelia of cordyceps sinensis. Phytochemistry. 51:891–898.
Bony NF, Libong D, Solgadi A, Bleton J, Champy P, Malan AK, Chaminade P. 2014. Establishing high temperature gas chromatographic profiles of non-polar metabolites for quality assessment of African traditional herbal medicinal products. J Pharm Biomed Anal. 88:542–551.
Brindle JT, Antti H, Holmes E, Tranter G, Nicholson JK, Bethell HWL, Clarke S, Schofield PM, McKilligin E, Mosedale DE, Grainger DJ. 2002. Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomics. Nat Med. 8:1439–1445.
Cajka T, Vaclavikova M, Dzuman Z, Vaclavik L, Ovesna J, Hajslova J. 2014. Rapid LC–MS-based metabolomics method to study the Fusarium infection of barley. J Sep Sci. 37:912–919.
Cao J, Shen H-M, Wang Q, Qian Y, Guo H-C, Li K, Qiao X, Guo Z-A, Luo X-D, Ye M. 2015. Characterization of chemical constituents and rats metabolitesof an alkaloidal extract of Alstonia scholaris leaves by liquidchromatography coupled with mass spectrometry. J Chromatogr B. 195:45:1–13.
Çelebiy M, Simöl C, Alunouz S, Cifuentes A. 2015. Comparative proteomics to investigate the in vitro antiproliferative effect of dietary polyphenols against K562 leukemia cells. Turk J Biochem. 40:95–104.
Chen D-Q, Chen H, Chen L, Tang D-D, Miao H, Zhao Y-Y. 2016. Metabolomic application in toxicity evaluation and toxicological biomarker identification of natural product. Chem Biol Interact. 252:114–130.
Chen H, Chen L, Tang DD, Chen DQ, Miao H, Zhao YY, Ma SC. 2016. Metabolomics reveals hyperlipidemic biomarkers and antihyperlipidemic effect of Poria cocos. Curr Metabolomics. 4:104–115.
Chen H-J, Shen Y-C, Shiao Y-J, Liou K-T, Hsu W-H, Hsieh P-H, Lee C-H, Chen Y-R, Lin Y-L. 2015. Multiplex brain proteomic analysis revealed the molecular therapeutic effects...
of Buyang Huanwu decoction on cerebral ischemic stroke mice. PLoS ONE. 10:1–27.

Chen H, Yuan B, Miao H, Tan Y, Bai X, Zhao YY, Wang Y. 2015. Urine metabolomics reveals new insights into hyperlipidemia and the therapeutic effect of rhubarb. Anal. Methods. 7:3113–3123.

Connor SC, Wu W, Sweatman BC, Manini J, Haselden JN, Crowther DJ, Waterfield CJ. 2004. Effects of feeding and body weight loss on the 1H-NMR-based urine metabolic profiles of male Wistar Han rats: implications for biomarker discovery. Biomarkers. 9:156–179.

Cook R, Hennell JR, Lee S, Khoo CS, Carles MC, Higgins VJ, Govindaraghavan S, Sucher NJ. 2013. The Saccharomyces cerevisiae transcriptome as a mirror of phytochemical variation in complex extracts of Equisetum arvense from America, China, Europe and India. BMC Genomics. 14:445–418.

Cox DG, Oha J, Keasling A, Colson KL, Hamann MT. 2014. The utility of metabolomics in natural product and biomarker characterization. Biochim. Biophys. Acta. 1840: 3460–3474.

Cui Q, Lewis IA, Hegeman AD, Anderson ME, Li J, Schulte AS, Ghabalia HR, Sussman MR, Markley JL. 2008. Metabolite identification via the Madison Metabolomics Consortium database. Nat Biotechnol. 26:162–164.

Davies MA, Hinerfeld D, Joseph S, Hui Y-H, Huang NH, Leszyk J, Rutherford-Bethard J, Tam SW. 2006. Proteomic analysis of rat liver phosphoproteins after treatment with protein kinase inhibitor H89 (N-(2-[p-bromocinnamylamino-]ethyl)-5-isooquinolinesulfonamide). J Pharmacol Exp Ther. 318: 589–595.

Davis RA, Charlton AJ, Godward J, Jones SA, Harrison M, Wilson JC. 2007. Adaptive binning: An improved binning method for metabolomics data using the undecimated wavelet transform. Chemometr. Intell Lab Syst. 85:144–154.

Deenen MJ, Cats A, Beijnen JH, Schellens JH. 2011a. Part 3: pharmacogenetic variability in drug transport and phase I anticancer drug metabolism. Oncologist. 16: 820–834.

Deenen MJ, Cats A, Beijnen JH, Schellens JH. 2011b. Part 3: pharmacogenetic variability in phase II anticancer drug metabolism. Oncologist. 16:992–1005.

Dekant W. 2009. The role of biotransformation and bioactivation in toxicity. EXS. 99:57–86.

Dettmer K, Aronov PA, Hammock BD. 2007. Mass spectrometry-based metabolomics. Mass Spectrom Rev. 26:51–78.

Davenport NA, Blenkhorn DJ, Weston DJ, Reynolds JC, Creaser CS. 2014. Direct determination of urinary creatinine by reactive-thermal desorption-extractive electrospray ionization mobility-tandem mass spectroscopy. Anal Chem. 86: 357–361.

Dona AC, Kyriakides M, Scott F, Shephard EA, Varshavi D, Veselkov K, Everett JR. 2016. A guide to the identification of metabolites in NMR-based metabolomics/metabolomics experiments. Comput Struct Biotechnol J. doi:10.1016/j.csbj.2016.02.005.

Dudzik D, Revello R, Barbas C, Bartha JL. 2015. LC–MS-based metabolomics identification of novel biomarkers of choorioamnionitis and its associated perinatal neurological damage. J Proteome Res. 14:1432–1444.

Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. 2011. Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. Chem Soc Rev. 40:387–426.

Dutta S, Dey P, Chaudhuri TK. 2014. Phytochemical investigation and correlation study of Croton bonplandianus Baill stem. J Pharmacogn Phytochem. 3:142–148.

Edlund U, Grahn H. 1991. Multivariate data analysis of NMR data. J Pharm Biomed Anal. 9:655–658.

Emwas AH, Luchinat C, Turano P. 2015. Standardizing the experimental conditions for using urine in NMR-based metabolic studies with a particular focus on diagnostic studies: a review. Metabolomics. 11:872–894.

Emwas AH, Roy R, Mckay RT. 2016. Recommendations and standardization of biomarker quantification using NMR-based metabolomics with particular focus on urinary analysis. J Proteome Res. 15:360–373.

Eriksson L, Hagberg P, Johansson E, Rannar S, Whelehan O. 2001. Multivariate process monitoring of a newsprint mill. Application to modelling and predicting COD load resulting from de-inking of recycled paper. J Chemometr. 15: 337–352.

Fan X, Li X, Lv S, Wang Y, Zhao Y, Luo G. 2010. Comparative proteomics research on rat MSCs differentiation induced by Shuanglong Formula. J Ethnopharmacol. 131:575–580.

Fenselau C. 2007. A review of quantitative methods for proteomic studies. J Chromatogr B. Analyt Technol Biomed Life Sci. 855:14–20.

Ferber G. 2002. Biomarkers and proof of concept. Methods Find Exp Clin Pharmacol. 24:35–40.

Fiehn O. 2002. Metabolomics – the link between genotypes and phenotypes. Plant Mol Biol. 48:155–171.

Firouzi Z, Lari P, Rashedinia M, Ramezani M, Iranshahi M, Abnous K. 2014. Proteomics screening of molecular targets of curcumin in mouse brain. Life Sci. 98:12–17.

Frank R, Hargreaves R. 2003. Clinical biomarkers in drug discovery and development. Nat Rev Drug Discov. 2:566–580.

Friedrich M, Pirotte B, Fillet M, Tullio P. 2016. Metabolomics as a challenging approach for medicinal chemistry and personalized medicine. J Med Chem. 59:8649–8666.

Freitas DS, Carlos EF, Gil MCSS, Vieira LGE, Alcantara GB. 2015. NMR-based metabolomic analysis of huanglongbing-asymptomatic and-symptomatic citrus trees. J Agric Food Chem. 63:7582–7588.

Garcia A, Barbas C. 2011. Gas chromatography-mass spectrometry (GC-MS)-based metabolomics. Methods Mol Biol. 708:191–204.
García-Figueiras R, Baleato-González S, Padhani AR, Oleaga L, Vilanova JC, Luna A, Gómez JCC. 2016. Proton magnetic spectroscopy in oncology: the fingerprints of cancer. Diagn Interv Radiol. 22:75–89.

Gavaghan CL, Wilson ID, Nicholson JK. 2002. Physiological variation in metabolic phenotyping and functional genomic studies: use of orthogonal signal correction and PLS-DA. FEBS Lett. 530:191–196.

Gika HG, Theocharidis GA, Plumb RS, Wilson RD. 2014. Current practice of liquid chromatography-mass spectrometry in metabolomics and metabonomics. J Pharm Biomed Anal. 87:12–25.

Giorgi A, Marinis PD, Granelli G, Chiesa LM, Panseri S. 2013. Secondary metabolite profile, antioxidant capacity, and mosquito repellent activity of Bixa orellana from Brazilian Amazon region. J Chem. 2013:1–10.

Gorg A, Postel W, Gunther S. 1988. Two-dimensional electrophoresis with immobilized pH gradients of leaf proteins from barley (hordeum vulgare): method, reproducibility and genetic aspects. Electrophoresis. 9:531–546.

Griffin JL, Williams HJ, Sang E, Nicholson JK. 2001. Abnormal lipid profile of dystrophic cardiac tissue as demonstrated by one- and two-dimensional magic-angle spinning 1H NMR spectroscopy. Magn Reson Med. 46:249–255.

Guido RVC, Oliva G. 2009. Structure-based drug discovery for tropical diseases. Curr Top Med Chem. 9:824–843.

Hagel JM, Mandal R, Han B, Han J, Dinsmore DR, Borchers CH, Wishart DS, Facchini PJ. 2015. Metabolome analysis of 20 taxonomically related benzyloisquinoline alkaloid-producing plants. BMC Plant Biol. 15:1–17.

Hamad I, Abd Elgawad H, Al Jaouni S, Zinta G, Asard H, Hassan S, Hegab M, Hagagy N, Selim S. 2015. Metabolic analysis of various date palm fruit (Phoenix dactylifera L.) cultivars from Saudi Arabia to assess their nutritional quality. Molecules. 20:13620–13641.

Han H, Yang L, Xu Y. 2011. Identification of metabolites of geniposide in rat urine using ultra-performance liquid chromatography combined with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. Rapid Commun Mass Spectrom. 25:3339–3350.

Herbert RB. 1989. The biosynthesis of secondary metabolites. 2nd ed. Brossura: Chapman and Hall. ISBN: 10:0412277204.

Hird SJ, Lau BPY, Schuhmacher R, Krska R. 2014. Liquid chromatography-mass spectrometry for the determination of chemical contaminants in food. TrAC – Trend Anal Chem. 59:59–72.

Holmes E, Nicholls AW, Lindon JC, Ramos S, Spraul M, Neidig P. 1998. Development of a model for classification of toxin-induced lesions using 1H NMR spectroscopy of urine combined with pattern recognition. NMR Biomed. 11:235–244.

Horai H, Arita M, Kanaya S, Nihei Y, Ikeda T, Suwa K, Ojima Y, Tanaka K, Tanaka S, Aoshima K, et al. 2010. Massbank: a public repository for sharing mass spectral data for life sciences. J Mass Spectrom. 45:703–714.

Huang G, Chen H, Zhang X, Cooks RG, Ouyang Z. 2007. Rapid screening of anabolic steroids in urine by reactive desorption electrospray ionization. Anal Chem. 79:8327–8332.

Hussain MA, Huygens F. 2012. Proteomic and bioinformatics tools to understand virulence mechanisms in Staphylococcus aureus. Current Proteomics. 9:2–8.

Imperiale A, Moussallieh FM, Roche P. 2015. Metabolome profiling by HRMAS NMR spectroscopy of pheochromocytomas and paragangliomas detects SDH deficiency: clinical and pathophysiological implications. Neoplasia. 17:55–65.

Jacob D, Deborde C, Moing A. 2013. An efficient spectra processing method for metabolite identification from 1H-NMR metabolomics data. Anal Bioanal Chem. 405:5049–5061.

Ji Q, Zhu F, Liu X, Li Q, Su S-B. 2015. Recent advance in applications of proteomics technologies on traditional Chinese medicine research. Evid Based Complement Alternat Med. 2015:1–13.

Jia J, Zhang F, Li Z, Qin X, Zhang L. 2015. Comparison of fruits of forsythia suspensa at two different maturation stages by NMR-based metabolomics. Molecules. 20:10065–10081.

Jiang T, Wang Z, Shenren Xia T, Zhao X, Jiang L, Teng L. 2015. Quantitative proteomics analysis for effect of Acanthopanax senticosus extract on neuroinflammation. Pak J Pharm Sci. 28:313–318.

Karthik D, Ilavenil S, Kaleeswaran B, Ravikumar S. 2012. Analysis of modification of liver proteome in diabetic rats by 2D electrophoresis and MALDI-TOF-MS. Ind J Clin Biochem. 27:221–230.

Karthik D, Vijayakumar R, Pazhanichamy K, Ravikumar S. 2014. A proteomics approach to identify the differential protein level in cardiac muscle of diabetic rat. Acta Biochim Pol. 61:285–293.

Kawanishi H, Toyo’oka T, Ito K, Maeda M, Hamada T, Fukushima T, Kato M, Inagaki S. 2007. Hair analysis of histamine and several metabolites in C3H/HeNCrj mice by ultra-performance liquid chromatography with electrospray ionization time-of-flight mass spectrometry (UPLC–ESI-TOF-MS): influence of hair cycle and age. Clin Chim Acta. 378:122–127.

Kelleher MT, Fruhwirth G, Patel G, Ofo E, Festy F, Barber PR, et al. 2009. The potential of optical proteomic technologies to individualize prognosis and guide rational treatment for cancer patients. Targeted Oncol. 4:235–252.

Kelly RT, Page JS, Zhao R. 2008. Capillary-based multi nano-electrospray emitters: improvements in Ion transmission efficiency and implementation with capillary reversed-phase LC-ESI-MS. Anal Chem. 80:143–149.

Kim HJ, Cho CW, Hwang JT, Son N, Choi JH, Shim GS, Han CK. 2013. LC-MS based metabolomic analysis of serum and livers from red ginseng-fed. J Ginseng Res. 37:371–378.

Kim EJ, Kwon J, Park SH, Park C, Seo Y-B, Shin X, Kim HK, Lee K-S, Choi S-Y, Ryub DH, Hwang G-S. 2011. Metabolite profiling of angelica gigas from different geographical origins using 1H NMR and UPLC-MS analyses. J Agric Food Chem. 59:8806–8815.

Kim JA, Nagappan A, Park HS, Saralamma VVG, Hong GE, Yunnam S, Lee HJ, Raha S, Kim EH, Young PS, Kim GS.
2014. Proteome profiling of lipopolysaccharide induced L6 rat skeletal muscle cells response to flavonoids from *Sclerola bicaulesis* Georgi. BMC Complement Altern Med. 14:1–10.

Kirschenlohr HL, Griffin JL, Clarke SC, Rydwren R, Grace AA, Schofield PM, Brindle KM, Metcalfe JC. 2006. Proton NMR analysis of plasma is a weak predictor of coronary artery disease. Nat Med. 12:705–710.

Klavins K, Chu DB, Hannon S, Koelensperger G. 2014. Fully automated on-line two-dimensional liquid chromatography in combination with ESI MS/MS detection for quantification of sugar phosphates in yeast cell extracts. Analyst. 139:1512–1520.

Kovacs H, Moskau D, Spraul M. 2005. Cryogenically cooled probes—a leap in NMR technology. Prog Nucl Mag Res Sp. 46:131–155.

Krishan P, Kruger NJ, Ratcliffe RG. 2004. Metabolite fingerprinting and profiling in plants using NMR. J Exp Bot. 56:255–265.

Kumar PJ, Clark M. 2002. *Textbook of clinical medicine*. London: Saunders; p.1099–1121.

Lambert J, Mazzola E. 2004. Nuclear magnetic resonance spectroscopy: an introduction to principles, applications and experimental methods. NJ: Pearson Education Inc.

Lao Y, Wang X, Xu N, Zhang H, Xu H. 2014. Application of proteomics to determine the mechanism of action of traditional Chinese medicine remedies. J Ethnopharmacol. 155:1–8.

Le Blanc JC, Hager JW, Ilisiu AM, Hunter C, Zhong F, Chu I. 2003. Unique scanning capabilities of a new hybrid linear ion trap mass spectrometer (Q TRAP) used for high sensitivity proteomics applications. Proteomics. 3:859–869.

Lee T-Y, Chang H-H, Kuo J-J, Shen J-J. 2009. Changes of hepatic proteome in bile duct ligated rats with hepatic fibrosis following treatment with Yin-Chen-Hao-Tang. Int J Mol Med. 23:477–484.

Lenz EM, Bright J, Wilson ID, Morgan SR, Nash AF. 2003. A *1H* NMR-based metabolic study of urine and plasma samples obtained from healthy human subjects. J Pharm Biomed Anal. 33:1103–1115.

Lenz EM, Wilson ID. 2007. Analytical strategies in metabolomics. J Proteome Res. 6:443–458.

Lesiak AD, Musah RA, Cody RB, Domin MA, Dane AJ, Shepard JR. 2013. Direct analysis in real time mass spectrometry (DART-MS) of “bath salt” cathinone drug mixtures. Analyst. 138:3424–3432.

Li Y, Cai W, Cai Q, Che Y, Zhao B, Zhang J. 2016. Comprehensive characterization of the in vitro and in vivo metabolites of geniposide in rats using ultra-high-performance liquid chromatography coupled with linear ion trap–orbitrap mass spectrometer. Xenobiota. 46:357–368.

Li Z, Chen K, Guo M, Tang D. 2016. Two-dimensional liquid chromatography and its application in traditional Chinese medicine analysis and metabolomic investigation. J Sep Sci. 39:21–37.

Li F, Gonzalez FJ, Ma X. 2012. LC–MS-based metabolomics in profiling of drug metabolism and bioactivation. Acta Pharmacuetica Sinica B. 2:118–125.

Li Z, Song X, Fu Z, Wu B, Ling Y, Sun Z, Chen M, Xu D, Huang H. 2013. Identification of the major constituents in Zhimu–Huangqi. herb-pair extract and their metabolites in rats by LC–ESI–MSn. Chromatographia. 76:767–780.

Liang Y, Hao H, Xie L, Kang A, Xie T, Zheng X, Dai C, Hao K, Sheng L, Wang G. 2010. Development of a systematic approach to identify metabolites for herbal homologs based on liquid chromatography hybrid ion trap time-of-flight mass spectrometry: gender-related difference in metabolism of Schisandra lignans in rats. Drug Metab Dispos. 38:1747–1759.

Lindberg G, Lindblad U, Melander A. 2004. Sulfonylureas for treating type 2 diabetes mellitus. Cochrane Database Syst Rev 3:254.

Lindon JC, Nicholson JK. 2008. Spectroscopic and statistical techniques for information recovery in metabolomics and metabolitomics. Annu Rev Anal Chem. 1:45–69.

Liu P, Duan J-A, Guo J-M, Shang E-X, Qian D-W, Su S-L, Tang Y-P. 2013. Identification of major chemical constituents and their metabolites in Rat plasma And various organs after oral administration Of effective Xiang–Fu–Si–Wu decoction fraction By uplc–Q-ToF–Ms And metabolynx. J Liq Chromatogr Relat Tech. 36:1736–1749.

Liu Z, Huang J, Huo Y, Gong J, Zhang Y, Wei C, Pu J. 2015. Identification of proteins implicated in the increased heart rate in ShenSongYangXin-treated bradycardia rabbits by iTRAQ-based quantitative proteomics. Evid Based Complement Alternat Med. 2015:1–7.

Liu Y, Song F, Wu WKK, He M, Zhao L, Sun X, Li H, Jiang Y, Yang Y, Peng K. 2012. Triptolide inhibits colon cancer cell proliferation and induces cleavage and translocation of 14-3-epsilon. Cell Biochem Func. 38:1747–1759.

Liu P, Duan J-A, Guo J-M, Shang E-X, Qian D-W, Su S-L, Tang Y-P. 2013. Identification of major chemical constituents and their metabolites in Rat plasma And various organs after oral administration Of effective Xiang–Fu–Si–Wu decoction fraction By uplc–Q-ToF–Ms And metabolynx. J Liq Chromatogr Relat Tech. 36:1736–1749.

Lu C-L, Qv X-Y, Jiang J-G. 2010. Proteomics and syndrome of Chinese medicine. J Cell Mol Med. 14:2721–2728.

Lu Z, Song Q, Yang J, Zhao X, Zhang Y, Yang P, Kang J. 2014. Comparative proteomic analysis of anti-cancer mechanism by periplocin treatment in lung cancer cells. Cell Physiol Biochem. 33:859–868.

Lu X, Zhao X, Bai C, Zhao C, Lu G, Xu G. 2008. LC–MS-based metabolomics analysis. J Chromatogr B. 866:64–76.

Lynn K-S, Cheng M-L, Chen Y-R, Hsu C, Chen A, Lih TM, Chang H-Y, Huang C-J, Shiao M-S, Pan W-H, et al. 2015. Metabolite identification for mass spectrometry-based metabolomics using multiple types of correlated Ion information. Anal Chem. 87:2143–2151.

Madsen R, Lundstedt T, Trygg J. 2010. Chemometrics in metabolomics-A review in human disease diagnosis. Anal Chim Acta. 659:23–33.

Mahrous EA, Farag MA. 2015. Two dimensional NMR spectroscopic approaches for exploring plant metabolome: A review. J Adv Res. 6:3–15.

Maitra S, Yan J. 2008. Principal Component Analysis and Partial Least Squares: Two Dimension Reduction Techniques for Regression. Casualty Actuarial Society. Discussion Paper Program, p.79–90.
Manavalan A, Ramachandran U, Sundaramurthi H, Mishra M, Sze SK, Hu JM, Feng ZW, Heese K. 2012. Gastrodia elata Blume (tianania) mobilizes neuro-protective capacities. Int J Biochem Molecular Biol. 3:219–241.

Merrick BA, Bruno ME, Madenspacher JH, Wetmore BA, Foley J, Pieper R, Zhao M, Makusky AJ, McGrath AM, Zhou JX, et al. 2006. Alterations in the Rat serum proteome during liver injury from acetaminophen exposure. J Pharmacol Exp Ther. 318:792–802.

Meyer GMJ, Maurer HH. 2015. In vivo and in vitro metabolism studies of glauicine, a new herbal high by GC-MS, LC-MS, LC-HR-MS, and NMR techniques. Toxicim Krimtech. 82:323.

Miao H, Zhao YH, Vaziri ND, Tang DD, Chen H, Chen H, Khazaeli M, Tarbiat-Boldaji M, Hatami L, Zhao YY. 2016. Lipidomics biomarkers of diet-induced hyperlipidemia and its treatment with Portia cocos. J Agric Food Chem. 64:969–979.

Min JZ, Yano H, Matsumoto A, Yu HF, Shi Q, Higashi T, Inagaki S, Toyo-o’ka T. 2011. Simultaneous determination of polyamines in human saliva as 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole derivatives by nano-flow chip LC coupled with quadrupole time-of-flight tandem mass spectrometry. Clin Chim Acta. 412:98–106.

Moran A, Palmas W, Field L. 2004. Cardiovascular autonomic neuropathy is associated with microalbuminuria in older patients with type 2 diabetes. Diabetes Care. 27:972–977.

Na DH, Ji HY, Park EJ, Kim MS, Liu KI, Lee HS. 2011. Evaluation of metabolism-mediated herb–drug interactions. Arch Pharm Res. 34:1829–1842.

Nagai T, Nakao M, Shimizu Y, Kodera Y, Oh-Ishi M, Maeda T, Yamada H. 2011. Proteomic analysis of anti-inflammatory effects of a Kampo (Japaneseherbal) medicine “Shoseiryuto (Xiao–Qing–Long–Tang)” on airway inflammation in a mouse model. J Evid Based Complementary Altern Med. 2011. Article ID 604196.

Nicholson JK, Connelly J, Lindon JC, Holmes E. 2002. Metabonomics: a platform for studying drug toxicity and gene function. Nat Rev Drug Discov. 1:153–161.

Nicholson JK, Lindon JC, Holmes E. 1999. ‘Metabonomics’: understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica. 29:1181–1189.

Nkomo M, Katerere DR, Vismer HF, Cruz T, Balayssac M, Malet-Martino M, Makunga NP. 2014. Fusarium inhibition by wild populations of the medicinal plant salvia africana-lutea L. linked to metabolomic profiling. BMC Complement Altern Med. 14:99. doi:10.1186/1472-6882-14-99.

Noda I. 2014. Frontiers of two-dimensional correlation spectroscopy. Part I. New concepts and noteworthy developments. J Mol Struct. 1069:3–22.

Olokoba AB, Obateru OA, Olokoba LB. 2012. Type 2 diabetes mellitus: A review of current trends. Oman Med J. 27:269–273.

Patwardhan B, Vaidya AD. 2010. Natural products drug discovery: accelerating the clinical candidate development using reverse pharmacology approaches. Indian J Exp Biol. 48:220–227.

Pereira VP, Knor FJ, Vellosa JCR, Beltranme FL. 2014. Determination of phenolic compounds and antioxidant activity of green, black and white teas of Camellia sinensis (L.) Kuntze, Theaceae. Rev Bras Pl Med. 16:490–498.

Popescu R, Costinel D, Dinca OR, Marinescu A, Stefanescu I, Ionete RE. 2015. Discrimination of vegetable oils using NMR spectroscopy and chemometrics. Food Control. 48:84–90.

Prasad B, Garg A, Takwani H, Singh S. 2011. Metabolite identification by liquid chromatography-mass spectrometry. Trend Anal Chem. 30:360–387.

Prasad B, Singh S. 2010. Identification of rat urinary metabolites of rifabutin using LC–MSn and LC–HR-MS. Eur J Pharm Sci. 41:173–188.

Priori R, Casadei L, Valerio M, Scrivo R, Valesini G, Manetti C. 2015. 1H-NMR-BasedMetabolomic study for identifying serum profiles associated with the response to etanercept in patients with rheumatoid arthritis. PLoS ONE. 10:1–14.

Qiao X, Wang Q, Li Q, Huang Y, Liu K, Zhang Z-X, Bo T, Zheng Y-M, Guo D-A, Ye M. 2015. Metabolites identification and multi-component pharmacokinetics of ergostane and lanostane triterpenoids in the anticancer mushroom Antrodia cinnamomea. J Pharm Biomed Anal. 111:266–276.

Qing J, Fangshi Z, Xuan L, Qi L, Shi-bing S. 2015. Recent advance in applications of proteomics technologies on traditional Chinese medicine research. J Evid Based Complementary Altern Med. 2015:1–10.

Quang DN, Bach DD. 2008. Ergosta-4,6,8(14), 22-tetraen-3-one from Vietnamese Xylaria sp. possessing inhibitory activity of nitric oxide production. Nat Prod Res. 22:901–906.

Rainville PD, Stumpf CJ, Shockcor JP, Plumb RS, Nicholson JK. 2007. Novel application of reversed-phase UPLC–oaTOFMS for lipid analysis in complex biological mixture: a new tool for lipidomics. J Proteome Res. 6:552–558.

Ravi KC, Henry ID, Park GHK, Raftey D. 2009. Design and construction of a versatile dual volume heteronuclear double resonance microcoil NMR probe. J Magn Reson. 197:186–192.

Riaz S. 2015. Study of protein biomarkers of diabetes mellitus type 2 and therapy with vitamin B1. J Diabetes Res. 2015:1–10.

Robertson D, Lindon J, Nicholson JK, Holmes E. 2005. Metabonomics in toxicity assessment. Boca Raton, FL: CRC Press.

Rokaya MB, Upreti Y, Poudel RC, Timsina B, Münzbergová Z, Asselin H, Tiwari A, Shrestha SS, Sigdel SR. 2014. Traditional uses of medicinal plants in gastrointestinal disorders in Nepal. J Ethnopharmacol. 158:221–229.

Roscioli KM, Tufariello JA, Zhang X, Li SX, Goetz GH, Cheng G, Siems WF, Hill HH Jr. 2014. Desorption electrospray ionization (DESI) with atmospheric pressure ion mobility spectrometry for drug detection. Analyst. 139:1740–1750.

Rosipal Roman Krämer N. 2006. Overview and recent advances in partial least squares. In: Saunders C, et al. editor. Subspace,
latent structure and feature selection, Vol. 3940. Heidelberg: Springer-Verlag; p. 34–51.

Roskar R, Lusin TT. 2012. Analytical methods for quantification of drug metabolites in biological samples. In: Calderon, L, editor. Chromatography - the most versatile method of chemical analysis. Intech. doi:10.5772/51676.

Roux A, Lison D, Junot C, Heilier JF. 2011. Applications of liquid chromatography coupled to mass spectrometry-based metabolomics in clinical chemistry and toxicology: a review. Clin Biochem. 44:119–135.

Rooney OM, Troke J, Nicholson JK, Griffin JL. 2003. High resolution diffusion and relaxation-edited magic angle spinning $^1$HNMR spectroscopy of intact liver tissue. Magn Reson Med. 50:925–930.

Schaper JN, Pfeuffer KP, Shelley JT, Bings NH, Heflje GM. 2012. Drop on demand sample introduction system coupled with the flowing atmospheric-pressure after glow for direct molecular analysis of complex liquid microvolume samples. Anal Chem. 84:9246–9252.

Scognamiglio M, Abrosca BD, Esposito A, Fiorentino A. 2015. Chemical composition and seasonality of aromatic Mediterranean plant species by NMR-based metabolomics. J Anal Meth Chem. 2015:1–9.

Sharge L, Yu A. 1999. Applied biopharmaceutics and pharmacokinetcs. 4th ed. McGraw-Hill.

Shi J, Cao B, Wang X-W, Aa J-Y, Duan J-A, Zhu X-X, Wang H, Liu C-X. 2016. Metabolomics and its application to the evaluation of the efficacy and toxicity of traditional Chinese herb medicines. J Chromatogr B. 1026:204–216.

Shi X, Lu XG, Zhan LB, Qi X, Liang LN, Hu SY, Yan Y, Zhao SY, Sui H, Zhang FL. 2011. The effects of the Chinese medicine Zi Bu Pi Yin recipe on the hippocampus in a rat model of diabetes-associated cognitive decline: a proteomic analysis. Diabetologia. 54:1888–1899.

Shobha A, Sachin D, Awanish U, Rakesh YS, Rahul P, Alka B. 2012. Identification of prednisolone, methylprednisolone and their metabolites in human urine using HPLC (+) ESI-MS/MS and detection of possible adulteration in Indian herbal drug preparations. Ibonsina J Med BS. 4: 44–52.

Smith CA, O’Maille G, Want EJ, Qin C, Trauger SA, Brandon TR, Custodio DE, Abagyan R, Siuizdak G. 2005. METLIN: a metabolite mass spectral database. Ther Drug Monit. 27:747–751.

Sousa SAA, Magalhães A, Ferreira MMC. 2013. Optimized bucketing for NMR spectra: three case studies. Chemomert. Intell Lab Syst. 122:93–102.

Sulistio YA, Heese K. 2015. Proteomics in traditional Chinese medicine with an emphasis on Alzheimer’s disease. J Evid Based Complementary Altern Med. 2015:1–17.

Sun H. 2004. A universal molecular descriptor system for prediction of LogP, LogS, LogBB, and absorption. J Chem Info and Comput Sci. 44:748–757.

Sun X-G, Zhong X-L, Liu Z-F, Cai H-B, Fan Q, Wang Q-R, Liu Q, Song Y-H, He S-Q, Zhang X-F, Lu Z-P. 2010. Proteomic analysis of chronic restraint stress-induced Gan-stagnancy syndrome in rats. Chin J Integ Med. 16:510–517.

Svensson M, Eriksson JW, Dahlquist G. 2004. Early glycemic control, age at onset, and development of microvascular complications in childhood-onset type 1 diabetes: a population-based study in northern Sweden. Diabetes Care. 27:955–962.

Syka JE, Marto JA, Bai DL, Horning S, Senko MW, Schwartz JC, Ueberheide B, Garcia B, Busby S, Muratore T, et al. 2004. Novel linear quadrupole ion trap/FT mass spectrometer: performance characterization and use in the comparative analysis of histone H3 post-translational modifications. J Proteome Res. 3:621–626.

Taamalli A, Arráez-Román D, Abaza L, Iswaldi I, Fernández-Gutiérrez A, Ziarouk M, Segura-Carretero A. 2015. LC-MS-based metabolite profiling of methanolic extracts from the medicinal and aromatic species Mentha pulegium and Origanum majorana. Phytochem Anal. 26:320–330.

Tagore S, Chowdhury N, De RK. 2014. Analyzing methods for pathmining with applications in metabolomics. Gene. 534:125–138.

Tam JCW, Ko CH, Zhang C, Wang H, Lau CP, Chan WY, Leung PC, Fung KP, Zhang JF, Lau CBS. 2014. Comprehensive proteomic analysis of a Chinese 2-herb formula (Astragal Radix and Rehmnanniae Radix) on mature endothelial cells. Proteomics. 14:2089–2103.

Tang H, Wang Y, Nicholson JK, Lindon JC. 2004. Use of relaxation-edited one-dimensional and two dimensional nuclear magnetic resonance spectroscopy to improve detection of small molecules in blood plasma. Anal Biochem. 325:260–272.

Tao LM, Fang L, Yang YM, Jiang HL, Yang HY, Zhang H, Zhou H. 2013. Quantitative proteomic analysis reveals the neuro protective effects of huperzine A for amyloid beta treated neuro blasting N2a cells. Proteomics. 13:1314–1324.

Theodoridis G, Gika HG, Wilson ID. 2008. LC-MS-based methodology for global metabolite profiling in metabolomics/metabolomics. Trend Anal Chem. 27: 251–260.

Tsogtbaatar E, Cocuron J-C, Sonera MC, Alonso AP. 2015. Metabolite fingerprinting of pennycress (Thlaspi arvense L.) embryos to assess active pathways during oil synthesis. J Exp Bot. 2015:1–11.

Tsugawa H, Tsujimoto Y, Arita M, Bamba T, Fukusaki E. 2011. GC/MS based metabolomics: development of a data mining system for metabolite identification by using soft independent modeling of a class analogy (Simca). BMC Bioinformatic. 12:1–13.

Uarrota VG, Moresco R, Coelho B, Nunes EC, Peruch LAM, Neubert EO, Rocha M, Maraschin M. 2014. Metabolomics combined with chemometric tools (PCA, HCA, PLS-DA and SVM) for screening cassava (Manihot esculenta Crantz) roots during postharvest physiological deterioration. Food Chem. 161:67–78.

Vaidyanathan S, Harrigan GG, Goodacre R. 2005. Metabolome analyses: strategies for systems biology. New York: Springer.

Versace F, Sporkert F, Mangin P, Staub C. 2012. Rapid sample pre-treatment prior to GC-MS and GC-MS/MS urinary toxicology screening. Talanta. 101:299–306.
Vu TN, Laukens K. 2013. Getting your peaks in line: A review of alignments methods for NMR spectral data. Metabolites. 3:259–276.

Wang H, Bai X, Sun J, Kano Y, Makino T, Yuan D. 2013. Metabolism and excretion of kakkalide and its metabolites in rat urine, bile, and feces as determined by HPLC/UV and LC/MS/MS. Planta Med. 79:1552–1557.

Wang J, Betancourt AM, Mobley JA, Lamantiniere CA. 2011. Proteomic discovery of genistein action in the rat mammary gland. J Proteomic Res. 10:1621–1631.

Wang SY, Li J, Shi XZ, Qiao LZ, Lu X, Xu GW. 2013. A novel stop-flow two-dimensional liquid chromatography–mass spectrometry method for lipid analysis. J Chromatogr A. 1321:65–72.

Wang D, Park JS, Chu JS, Krakowski A, Luo K, Chen DJ, Li S. 2004. Proteomic profiling of bone marrow mesenchymal stem cells upon transforming growth factor beta-1 stimulation. J Biol Chem. 279:43725–43734.

Wang XJ, Yan GL, Zhang AH, Sun H, Piao CY, Li WY, Sun C, Wu XH, Li XH, Chen Y. 2013. Metabolomics and proteomics approaches to characterize and assess proteins of bear bile powder for hepatitis C virus. Chin J Nat Med. 11:653–665.

Want EJ, Wilson ID, Gika H, Theodoridis G, Plumb RS, Shockcor J, Holmes E, Nicholson JK. 2010 Global metabolic profiling procedures for urine using UPLC–MS. Nat Protoc. 5:1005–1018.

Williams DA. 2002. Drug metabolism. In: Troy D, editor. Foye’s principles of medicinal chemistry. 5th ed. Baltimore: Lippincott Williams & Wilkins; p. 174–233.

Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, Djoumbou Y, Mandal R, Aziat F, Dong E, et al. 2013. HMDB 3.0 – the human metabolome database in 2013. Nucleic Acids Res. 41:D801–D807.

Woldegiorgis AZ, Abate D, Haki GD, Ziegler GR. 2015. LC–MS/MS-based metabolomics to identify biomarkers unique to leatiporus sulphureus. Int J Nut Food Sci. 4:141–153.

Wright MH, Sieber SA. 2016. Chemical proteomics approaches for identifying the cellular targets of natural products. Nat Prod Rep. 33:681–708.

Wu X, Li N, Li H, Tang H. 2014. An optimized method for NMR-based plant seed metabolomic analysis with maximized polar metabolite extraction efficiency, signal-to-noise ratio, and chemical shift consistency. Analyst. 139:1769–1778.

Wu H, Li X, Yan X, An L, Luo K, Shao M, Jiang Y, Xie R, Feng F. 2015. An untargeted metabolomics-driven approach based on LC–TOF/MS and LC–MS/MS for the screening of xenobiotics and metabolites of Zhi-Zi Da-Huang decoction in rat plasma. J Pharm Biomed Anal. 115:315–322.

Yang Q, Shi XZ, Wang Y, Wang WZ, He HB, Lu X, Xu GW. 2010. Urinary metabolomic study of lung cancer by a fully automatic hyphenated hydrophilic interaction/RPLC-MS system. J Sep Sci. 33:1495–1503.

Yao M, Ma L, Duchoslav E, Zhu M. 2009. Rapid screening and characterization of drug metabolites using multiple ion monitoring dependent product ion scan and postacquisition data mining on a hybrid triple quadrupole-linear ion trap mass spectrometer. Rapid Commun Mass Spectrom. 23:1683–1693.

Ye L, Tang L, Gong Y, Lv C, Zheng Z, Jiang Z, Liu Z. 2011. Characterization of metabolites and human P450 isoforms involved in the microsomal metabolism of mesacoline. Xenobiotica. 41:46–58.

Ye NS, Chen J, Luo GA, Zhang RL, Zhao YF, Wang YM. 2006. Proteomic profiling of rat bone marrow mesenchymal stem cells induced by 5-azacytidine. Stem Cells Dev. 15:665–676.

Ye NS, Zhang RL, Zhao YF, Feng X, Wang YM, Luo GA. 2006. Effect of 5-azacytidine on the protein expression of porcine bone marrow mesenchymal stem cells in vitro. Genomics Proteomics Bioinformatics. 4:18–25.

Yue QX, Xie FB, Song XY, Wu WY, Jiang BH, Guan SH, Yang M, Liu X, Guo DA. 2012. Proteomic studies on protective effects of salvianolic acids, notoginsenosides and combination of salvianolic acids and notoginsenosides against cardiac ischemicreperfusion injury. J Ethnopharmacol. 141:659–667.

Zhang J-M, Fu C-M, Hu Y-C, Li Y, Qing S-H, Gao F. 2012. Screening out potential cardio-toxic components of Chinese herb radix aconiti lateralis in rat dosed plasma by high performance liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry. Anal Lett. 45:1695–1712.

Zhang H, Ge Y. 2011. Comprehensive analysis of protein modifications by top-down mass spectrometry. Circ Cardiovasc Gen. 4:1–21.

Zhang G, Qia W, Xu L, Kano Y, Yuan D. 2015. Pharmacokinetics of irisolidone and its main metabolites in rat plasma determined by ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. J Chromatogr B. 1005:23–29.

Zhang W, Saif MW, Dutschman GE, Li X, Lama W, Bussoma S, Jiang Z, Yea M, Chub E, Cheng YC. 2010. Identification of chemicals and their metabolites from PHY906, a Chinese medicine formulation, in the plasma of a patient treated with irinotecan and PHY906 using liquid chromatography/tandem mass spectrometry (LC/MS/MS). J Chromatogr A. 1217:5785–5793.

Zhang S-X, Sun H, Sun W-J, Jiao G-Z, Wang X-J. 2010. Proteomic study of serum proteins in a type 2 diabetes mellitus rat model by Chinese traditional medicine Tianqi Jiangtang capsule administration. J Pharm Biomed Anal. 53:1011–1014.

Zhang A, Sun H, Yan G, Wang P, Wang X. 2015. Metabolomics for biomarker discovery: moving to the clinic. BioMed Res Int. 2015:1–6.

Zhang ZH, Vaziri ND, Wei F, Cheng XL, Bai X, Zhao YY. 2016. An integrated lipidomics and metabolomics reveal nephroprotective effect and biochemical mechanism of rheum officinale in chronic renal failure. Scient Rep. 6:1–18.

Zhang ZH, Wei F, Vaziri ND, Cheng XL, Bai X, Lin RC, Zhao YY. 2015. Metabolomics insights into chronic kidney disease and modulatory effect of rhubarb against tubulointerstitial fibrosis. Scient Rep. 5:1–17.
Zhao Y-Y. 2013. Metabolomics in chronic kidney disease. Invited Crit Rev Clin Chim Acta. 422:59–69.
Zhao YY, Chen H, Tian T, Chen DQ, Bai X, Wei F. 2014. A pharmaco-metabonomic study on chronic kidney disease and therapeutic effect of ergone by UPLC-QTOF/HDMS. Plos One. 9:1–18.
Zhao YY, Cheng XL, Cui JH, Yan XR, Wei FR, Bai XU, Lin RC. 2012. Effect of ergosta-4,6,8(14),22-tetraen-3-one (ergone) on adenine-induced chronic renal failure rat: A serum metabonomic study based on ultra performance liquid chromatography/high-sensitivity mass spectrometry coupled with MassLynx i-FIT algorithm. Clin Chim Acta. 413:1438–1445.
Zhao Y-Y, Cheng X-L, Vaziri ND, Liu S, Lin R-C. 2014. UPLC-based metabonomic applications for discovering biomarkers of diseases in clinical chemistry. Clin Biochem. 47:16–26.
Zhao YY, Cheng XL, Wei F, Bai X, Lin RC. 2012. Ultra performance liquid chromatography coupled with electrospray and atmospheric pressure chemical ionization-quadrupole time-of-flight mass spectrometry with novel mass spectrometry elevated energy data collection technique: determination and pharmacokinetics, tissue distribution and biliary excretion study of ergone in rat. J Sep Sci. 35:1619–1629.
Zhao YY, Feng YL, Bai X, Tan XJ, Lin RC, Mei Q. 2013. Ultra performance liquid chromatography-based metabonomic study of therapeutic effect of the surface layer of Poria cocos on adenine-induced chronic kidney disease provides new insight into anti-fibrosis mechanism. PLoS One. 8:1–10.
Zhao YY, Lei P, Chen DQ, Feng YL, Bai X. 2013. Renal metabolic profiling of early renal injury and renoprotective effects of Poria cocos epidermis using UPLC Q-TOF/HSMS/MSE. J Pharm Biomed Anal. 81–82:202–209.
Zhao YY, Li HT, Feng YL, Bai X, Lin RC. 2013. Urinary metabonomic study of the surface layer of poria cocos as an effective treatment for chronic renal injury in rats. J. Ethanopharmacol. 148:403–410.
Zhao Y-Y, Lin R-C. 2014. UPLC–MSE application in disease biomarker discovery: The discoveries in proteomics to metabolomics-mini review. Chem Biol Int. 215:7–16.
Zhao YY, Shen X, Cheng XL, Feng W, Bai XU, Lin RC. 2012. Urinary metabolomics study on the protective effects of ergosta-4,6,8(14),22-tetraen-3-one on chronic renal failure in rats using UPLC Q-TOF/MS and a novel MSE data collection technique. Process Biochem. 47:1980–1987.
Zhao YY, Su Q, Cheng XL, Tan XJ, Bai X, Lin RC. 2012. Pharmacokinetics, bioavailability, and metabolism of rhaponticin in rat plasma by UHPLC–Q-TOF/MS and UHPLC–DAD-MSn. Bioanalysis 4:713–723.
Zhao YY, Zhang L, Feng YL, Chen D-Q, Xi ZH, Du X, Bai X, Lin R-C. 2013. Pharmacokinetics of 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside in rat using ultra-performance LC–quadrupole TOF-MS. J Sep Sci. 36:863–871.
Zhao YY, Zhang L, Long FY, Cheng XL, Bai X, Wei F, Lin RC. 2013. UPLC-Q-TOF/HSMS/MS(E)-based metabolomics for adenine-induced changes in metabolic profiles of rat faeces and intervention effects of ergosta-4,6,8(14),22-tetraen-3-one. Chem Biol Interact. 201:31–38.
Zhao YY, Zhao Y, Zhang YM, Lin RC, Sun WJ. 2009. Qualitative and quantitative analysis of the diuretic component ergone in poly porous umbellatus by HPLC with fluorescence detection and HPLC-APCI-MS/MS. Pharmazie. 64:366–370.
Zhou YC, Liu B, Li YJ, Jing LL, Wen G, Tang J, Xu X, Lv ZP, Sun XG. 2012. Effects of buyang huanwu decoction on ventricular remodeling and differential protein profile in a rat model of myocardial infarction. Evid Based Complementary Alternat Med. 2012:1–11.
Zhou B, Xiao JF, Tuli L, Ressom HW. 2012. LC-MS-based metabolomics. Mol Biosyst. 8: 470–481.