Chapter 11
Metabolomics of Important Medicinal Plants

Jyotsna Baby, Toji Thomas, and T. Dennis Thomas

1 Introduction

According to Food and Agricultural Organization, more than 50,000 plant species have medicinal values across the globe (Schippmann et al. 2002). The biological diversity of medicinal plants and their vivid chemical capabilities have been used by man for the treatment of various diseases. This brought forth to traditional medicinal practices such as Ayurveda, Unani, Chinese, Middle Eastern, and African systems of medicine (Mamedov 2012). The conventional detection methods of plant metabolites mainly relied on phytochemical screening tests followed by simple techniques of separation such as thin layer chromatography (TLC). Isolation of metabolites by these methods is often tedious and not much efficient. Also the poor and low selectivity of detection makes hard the detection of trace amount of metabolites in the sample (Srivastava et al. 2014). A thorough understanding of the entire metabolites of plants became inevitable for discovering novel metabolites, along with their concerned biosynthetic genes. Thus, metabolomics, an ongoing extension of "omics", has emerged as a powerful tool (Sumner 2010).

Currently, there is an increased attraction toward herbal remedies, as the green medicine is assumed to be safe and eco-friendly. Rising incidences of harmful side effects from using synthetic drugs also persuade human beings to utilize natural products for various ailments (Chanda 2014). Along with this, there is an increased attention toward natural products; novel approaches are also developed to make a clear understanding of the mode of action of these green remedies, as demonstrated by the integrated "omics" approaches, which provides a holistic approach in drug research (Gonulalan et al. 2020).

J. Baby · T. Thomas
Department of Botany, St. Thomas College Palai, Pala, Kerala, India

T. Dennis Thomas (✉)
Department of Plant Science, Central University of Kerala, Kasaragod, Kerala, India

M. K. Swamy, A. Kumar (eds.), *Phytochemical Genomics*,
https://doi.org/10.1007/978-981-19-5779-6_11

A large number of phytochemicals with pharmacological value have been isolated and play predictable therapeutic roles in the clinical world (Gonulalan et al. 2020). The therapeutic effects exhibited by many of the medicinal plants are not always due to a single major compound, but in many cases due to synergistic or antagonistic activity of various compounds. If active compounds are present in very minute quantities in plants, it can affect its chemical characterization, and the process is very challenging also (Williamson 2001). In order to address this task, metabolomics can contribute an important role; it provides insight into the metabolome of plants. Metabolomics is observed as extension of the ongoing high-throughput technologies to the comprehensive analysis of small molecule metabolites of biological system (Sumner 2010). Besides, metabolomics confers several benefits as compared to other "omics;" this is because the biochemical phenotypes are not always represented by transcriptomic or proteomic approaches (Fernie and Stitt 2012). Metabolomics aims to measure the metabolites in the sample and provides information about the interconnection of metabolic pathways and the phenotype of the biological system (Johnson et al. 2016).

Metabolomics is also employed for obtaining valuable data to aid in discovering novel genes and the concerned pathways. The advancements in the sequencing technologies and metabolome-based genome-wide association study (mGWAS) are effectively utilized to unveil the genetic mechanisms behind diverse metabolome and how they are associated to the complex traits seen in plants (Hong et al. 2016). Wen et al. (2014) conducted mGWAS study in maize kernels, through which they identified 1459 locus-trait associations among three environments. Resequencing and analysis of association among candidate genes led to the identification of causal variants of five genes concerned with metabolic traits. Likewise, by combining metabolomics and transcriptomics, all genes of seco-iridoid pathway in *Catharanthus roseus* have been identified (Miettinen et al. 2014; Salim et al. 2014).

As per the estimates of the World Health Organization (WHO), about 80% of world's population use traditional medicines at a certain period of their lifetime (Chintamunnee and Mahomoodally 2012). Traditional herbal medicines are widely distributed across the globe and have been used for centuries without any rigorous rules (Efferth and Greten 2012). Together with this, there are chances for adulteration as products of medicinal plants are marketed and distributed (Perini et al. 2018). The bioactive compounds responsible for the therapeutic properties also need to be identified in order to do semi-synthesis and further development of novel new drug candidates from these lead compounds (Plazas et al. 2019).

In this context, metabolomics is a promising tool that can be effectively used to mine various bioactive metabolites from different medicinal plants (RaoGajula and Nanjappan 2021). In this chapter, we present how metabolomics approach can effectively be employed for medicinal plants. The chapter also discusses various strategies, technical advancements, data processing methods, and databases. In addition to this, various applications of metabolomics in connection with medicinal plants are also addressed.

2 Strategies Employed in Plant Metabolomics

Before the advent of "omics" era, investigation and extraction of bioactive metabolites from plants mainly relied on conventional extraction techniques like Soxhlet extraction, hydrodistillation, and maceration. The efficiency of these methods mainly depends on the solvents selected, polarity of selected compound, etc. (Azmir et al. 2013). Also, traditional methods of separation and bioassay-guided fractionation are too laborious as well as expensive. Hence, they are not cost-effective in an industrial approach for drug development (Yuliana et al. 2013). Revolutionizing changes have been made by the metabolomics technologies, which aim for targeted and global profiling of metabolites of the sample. The metabolomic profiling of medicinal plants is becoming crucial and aids in the development of novel phytotherapeutics (Shyur and Yang 2008). As herbal formulations are multi-compound medicines, metabolomic approach is vital for screening of multi-compounds. Hence, metabolomic approaches are gaining an upper hand in medicinal plant research (Lee et al. 2017). In addition to this, the technical advancements in mass spectrometry (MS) and nuclear magnetic resonance (NMR) make possible the estimation of a wide array of compounds and comparing the support data of novel compounds with natural products library (RaoGajula and Nanjappan 2021). The entire process of metabolomic analysis to derive meaningful data from the metabolome of medicinal plants is outlined in Fig. 11.1. Some of the major strategies employed in metabolomics are briefly discussed below.

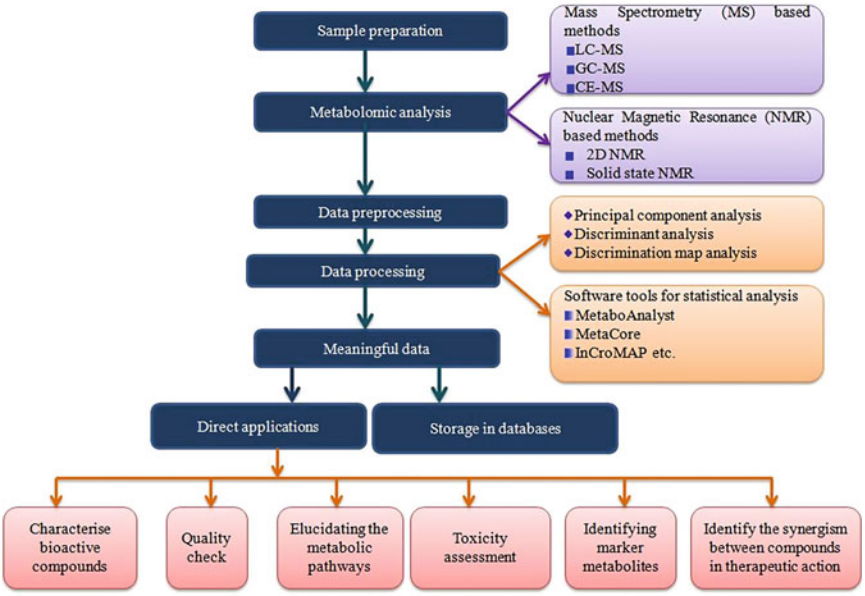


Fig. 11.1 The overall process of metabolomic analysis to mine useful data from medicinal plants

2.1 Metabolite Profiling

This involves the identification and quantification of a large group of compounds, sharing similar chemistry or related by metabolic pathways (Shafi and Zahoor 2021). Metabolic profiling includes nontargeted and targeted approaches.

2.1.1 Nontargeted Approach

The nontargeted/untargeted approach involves simultaneous measurement of a possible large number of metabolites in the sample (Shah et al. 2012; Schrimpe-Rutledge et al. 2016). This approach is appropriate for biomarker discovery and is employed for generating hypothesis. It is also called undirected or unbiased metabolomics (Wang et al. 2010). However, it cannot be regarded as truly unbiased because the researcher has to choose a combination of ionization mode and stationary phase, which in turn facilitates the detection of certain compounds and lowers the detection of some others (Ribbenstedt et al. 2018). Together with, it faces certain other challenges such as complicated protocols, longtime consumption to handle huge amount of raw data, problems of identification and characterization of unknown metabolites, reliability of the platform chosen, and greater chances of detecting more abundant metabolites (Roberts et al. 2012).

2.1.2 Targeted Approach

Metabolic profiling of known metabolites with distinct identities (already defined compounds) is done for the targeted approach (Shah et al. 2012). Also called directed or biased metabolomics, it mainly focuses on metabolites of a particular chemical class or any other predetermined group of compounds. This approach is hypothesis-driven and aims to verify biological pathways or authenticate an untargeted metabolomic study (Wang et al. 2010). Here, analysis can be carried out either in a quantitative or semiquantitative manner. Some advantages of targeted analyses like the downstream analysis are almost set free from analytical artifacts, and novel metabolic associations can be deciphered at particular physiological states (Roberts et al. 2012). Major metabolomic studies in medicinal plants, which employed targeted/untargeted approaches, are given in Table 11.1.

2.2 Metabolite Fingerprinting

This involves an untargeted approach, in which extensive identification and quantification of individual metabolites are not done. The data obtained were analyzed to recognize patterns specific to the fingerprint of metabolites at a given biological

Table 11.1 List of metabolomic studies of medicinal plants via targeted/untargeted approaches (from 2010 onward)

Sl. No.	Plant name	Metabolomic approach (targeted/ untargeted)	Major metabolites (identified/ discovered)/major findings	Reference
1	*Acanthopanax senticosus* (Rupr. et Maxim.) Harms	Targeted metabolomics	Compounds of C_6C_1 type greater in 9-year-old, $C_6C_3C_6$ type greater in 5-year-old and C_6C_3 type greater in 3-year-old plants	Xu et al. (2020)
2	*Aconitum pendulum* N. Busch	Targeted metabolomics	80 metabolites identified and 19 compounds were selected as biomarkers for the collected samples	Wang et al. (2022)
3	*Bryophyllum* spp.	Untargeted metabolomics	Phenolic compounds with therapeutic activity	García-Pérez et al. (2021)
4	*Centella asiatica* (L.) Urban	Targeted metabolomics	Confirmed the effect of methyl jasmonate in inducing the production of madecassic acid, madecassoside, asiaticoside and asiatic acid	James et al. (2013)
5	*Chrysanthemum morifolium* Ramat.	Targeted metabolomics	661 metabolites were identified among which 46 different metabolites are found simultaneously during different growth stages with flooding stress	Wang et al. (2019a)
6	*Cistanche salsa* (C. A. Mey.) G. Beck, *C. sinensis* G. Beck, *C. tubulosa* (Schenk) R. Wight, and *C. deserticola* Y.C. Ma	^{1}H NMR-based nontargeted to LC–MS-based targeted metabolomics	8-*epi*-loganic acid, acteoside, echinacoside, betaine, sucrose, mannitol, and 6-deoxycatalpol are identified as markers for discrimination among these four species	Liu et al. (2019)
7	*Citrus sinensis* L.	Untargeted metabolomics	Leaves have more flavonoid, condensed tannin, and phenol content, whereas flavedo has more carbohydrates	Lamine and Mliki (2021)
8	*Clausena lansium* (Lour.)	Untargeted metabolomics	364 metabolites identified, and 64 potential biomarkers were selected	Fan et al. (2020)
9	*Cuminum cyminum* L.	Untargeted metabolomics	45 metabolites with differential expression including quercetin, luteolin, kaempferol, and salvianolic acid	Pandey et al. (2015)
10	*Curcuma elata* Roxb., *C. aromatica* Salisb., *C. longa* L., *C. phaeocaulis* Val. and *C. caesia* Roxb.	Targeted metabolomics	*C. longa* has the highest quantity of curcuminoids, but some of the bioactive compounds like 6-gingerol was found the lowest in *C. longa*	Ye et al. (2022)

(continued)

Table 11.1 (continued)

Sl. No.	Plant name	Metabolomic approach (targeted/ untargeted)	Major metabolites (identified/ discovered)/major findings	Reference
11	*Ephedra sinica* Stapf.	Untargeted metabolomics	Identified that 22 chemical markers differ between stem and root	Lv et al. (2015)
12	*Eucommia ulmoides* Oliver	Untargeted metabolomics	2373 metabolites were identified, and 116 metabolites discovered	Chen et al. (2022)
13	*Fritillaria* spp.	Untargeted metabolomics	21 species specific markers were identified	Liu et al. (2020)
14	*Gleditsia sinensis* Lam.	Targeted metabolomics	728 metabolites identified from epidermis, xylem, and pith of thorn	Ya et al. (2022)
15	*Grammatophyllum speciosum* Blume	Untargeted metabolomics	721 metabolites were identified with vitexin and orientin being the most abundant	Yingchutrakul et al. (2021)
16	*Ligusticum canbyi* (J.M. Coult & Rose)	Targeted and untargeted metabolomics	Detected 34,000 compounds with 70 phthalide metabolites. Ferulic acid was found responsible for antioxidant activity	Turi and Murch (2013)
17	*Maytenus aquifolium* Mart. and *Maytenus ilicifolia* Mart. ex Reiss	Untargeted metabolomics	Differentiated their chemical composition and analyzed the effect of environment in metabolome	Antunes et al. (2020)
18	*Mikania glomerata* Spreng. and *M. laevigata* Sch.Bip. ex Baker	Untargeted metabolomics	Identified that coumarin is present only in *M. laevigata* and volatile compounds like pinenes are more abundant in plants during drought	Ueno and Sawaya (2019)
19	*Persea americana* Mill.	Targeted metabolomics	Quantified 8 acetogenins in peel, pulp, and seeds of the fruit	Rodríguez-López et al. (2015)
20	*Phyllanthus amarus* Schum. & Thonn., *P. acidus* L., *P. emblica* L., *P. urinaria* L., *P. debilis* Linn., *P. virgatus* G. Forst., *P. reticulates* Poir., *P. myrtifolius* (Wight) Mull. Arg. and *P. lawii* J.Graham	Untargeted metabolomics	Identified the differential expression of 14 metabolites from nine *Phyllanthus* spp.	Kiran et al. (2021)
21	*Polygonum multiflorum* Thunb.	Untargeted and targeted metabolomics	Revealed the appropriate processing of its radix for medicinal use	Liang et al. (2018)

(continued)

Table 11.1 (continued)

Sl. No.	Plant name	Metabolomic approach (targeted/ untargeted)	Major metabolites (identified/ discovered)/major findings	Reference
22	*Rehmannia glutinosa* Libosch.	Targeted metabolomics	Detected 228 metabolites from roots of cultivated and wild variety out of which 170 metabolites were unchanged and 58 were differential metabolites	Zhou et al. (2021)
23	*Rosmarinus officinalis* L.	Untargeted metabolomics	Rosmarinic acid, rosmaridiphenol, carnosol, carnosic acid, quercitin, luteolin, etc.	Salem et al. (2020b)
24	*Salsola collina* Pall	Targeted metabolomics	637 metabolites were identified	Li et al. (2021)
25	*Salvia miltiorrhiza* Bunge	Untargeted metabolomics	Identified 23 specific metabolites out of which 11 metabolites changed during "sweating" process	Cao et al. (2020)
26	*Suaeda salsa* (L.) Pall.	Targeted metabolomics	Identified 521 metabolites out of which 165 are differential metabolites of different leaf phenotypes	Wang et al. (2019b)

state, for example, stress response, etc., provided in a living system (Wolfender et al. 2015; Shafi and Zahoor 2021). It mainly finds applications in comparing fingerprints or patterns of metabolites that vary with response to a particular disease condition, environmental changes, etc. (Barderas et al. 2011). The major platforms employed here include NMR spectroscopy, Fourier transform infrared spectroscopy (FTIR), and Raman spectroscopy (Ellis et al. 2007). Gray and Heath (2005) examined cold acclimation effects on the metabolome of *Arabidopsis* using metabolite fingerprinting.

2.3 Metabolite Footprinting

This approach comprises the profiling of extracellular metabolites alone (Pope et al. 2007). It mainly focuses on the biochemical and chemical changes brought about by organisms due to the effect of its immediate environment. Every living cell modifies its medium by the secretion of various enzymes and other metabolites; later, a subsequent interaction of the secreted components with the constituents present in the medium happens. This will generate metabolic profiles that are specific to particular species and/or their genetic makeup (Villas-Bôas et al. 2006). As it

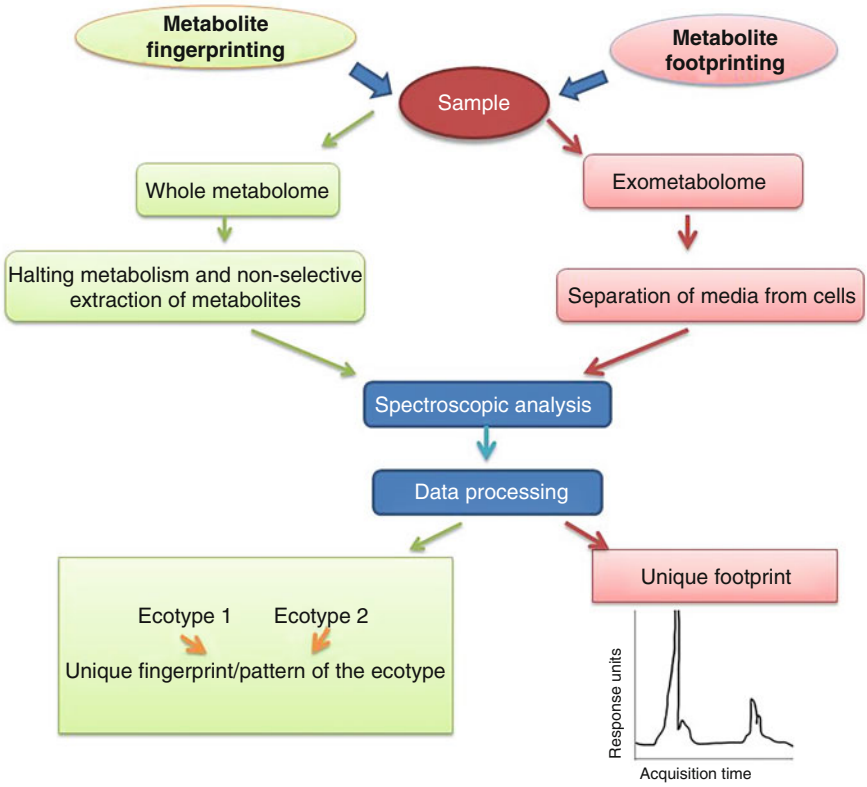


Fig. 11.2 Workflow depicting the procedure and differences of metabolite fingerprinting and footprinting

involves secreted metabolites, sampling is relatively simple in which it only requires the procedure of separating the media from the cell/tissue. Further analysis was done by means of NMR (Filloux and Ramos 2014). The procedure and differences in metabolite footprinting and fingerprinting are outlined in Fig. 11.2.

2.4 Metabonomics

Metabonomics implies extensive evaluation of metabolic changes in a living system. It does quantitative measurement of the metabolic variations of a biological system in response to genetic modification or pathological/physiological stimuli (Bjerrum 2015).

2.5 Chemoprofiling

The chemical constitution of plants needs to be understood to ensure sufficient therapeutic evaluation (Efferth and Greten 2012). Chemoprofiling includes pattern-oriented/multicompound approach and compound-oriented/marker approach. Pattern-oriented approach focuses on all detectable compounds in a plant extract that forms a unique fingerprint, and it does not characterize all the compounds in a plant extract. In the case of compound-oriented approach, some of the bioactive or major constituents are considered for obtaining specific chemoprofiles (Govindaraghavan et al. 2012).

2.6 Metabolic Fluxomics

Rates of metabolic reactions (fluxes) provide information about metabolic phenotypes and cellular regulation mechanisms. The direct measurement of metabolic fluxes is not possible, and it needs to be obtained by measuring other observables like changes in concentration of enzymes, metabolites, carbon balance, etc. (Niedenführ et al. 2015). Flux analysis by means of ^{13}C fluxomics (using ^{13}C, ^{14}C, ^{2}H, ^{15}N isotopic traces) has become a method of choice to decipher the regulation and constitution of metabolic networks (Zamboni 2011; Niedenführ et al. 2015).

3 Technological Advancements in Plant Metabolomics

3.1 Mass Spectrometry Imaging (MSI)

MSI works by ionizing the peptides/protein or metabolites from the biological sample in a two-dimensional or three-dimensional coordinate (Fletcher et al. 2008; Seeley and Caprioli 2012).

3.1.1 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS is routinely used for the analysis of organic compounds that are volatile or can be derivatized to make them volatile (Hall 2006). Separation of compounds happens due to the difference in partition coefficients between stationary (solid) phase and mobile (gas) phase (Smedsgaard 2007; Roessner and Beckles 2009). By employing electron impact ionization (EI), analytes are ionized; it results in a unique fragmentation pattern for every constituent phytochemical. Identification of compounds can be made by comparing the GC-MS fragmentation patterns and retention time, along with the available information in the databases of GC-MS (Kopka et al. 2004). GC-MS method gains popularity because it helps in the determination of

amino acids, sugars, and organic acids. One of the limitations of GC-MS is that the overlapping peaks of GC-MS chromatogram render the detection of individual metabolite signals much more difficult (Saito and Matsuda 2010).

3.1.2 Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS forms a valuable tool to unveil the immense wealth of phytochemicals including both primary and secondary metabolites. As opposed to GC-MS, LC-MS can handle a wide range of compounds with different chemical properties, for example, they are either volatile or not (Hill and Roessner 2015). In addition to this, partially purified crude extracts can be directly fed into the LC/MS system. This eliminates various steps of sample treatment. The introduction of ultra performance liquid chromatography (UPLC) together with high-resolution Fourier transform (FT) MS, time-of-flight (TOF) MS, and Orbitrap-based MS made beneficial advancements in LC-MS-based metabolomics (Salem et al. 2020a).

3.1.3 Capillary Electrophoresis-Mass Spectrometry (CE-MS)

Capillary electrophoresis employs high voltage for the electrophoretic separation of different ions in a narrow-bore capillary (Ren et al. 2018). The initial separation of metabolites takes place according to their charge-to-size ratio followed by mass-to-charge ratio-based separation (Zhao et al. 2012; Klepárník 2015). Even though CE-MS is considered as a novel method in metabolomics, it suffers from drawbacks of having poor stability, and there are chances for capillary blockage by salt (Ren et al. 2018). However, it performs the ability to separate low volume of biological fluids, simple sample preparation protocol, capability of concentrating analytes and better separation efficiency, making it as a method of choice (Klepárník 2015; Ren et al. 2018).

3.2 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is a chief tool for studying plant metabolomics. It gains an upper hand over other techniques by facilitating high-throughput analysis, easy sample preparation, rapid performance, and easy quantitation (Kim et al. 2011). NMR makes possible the analysis of profuse primary metabolites and heterogeneous secondary metabolites such as alkaloids, flavonoids, terpenoids, etc. As the signals in the NMR spectrum are indicative of the molar concentrations of the component compounds, concentrations of these compounds can be compared with other samples, and it eliminates the need for calibration curves for each compound (Kim et al. 2010). NMR also suffers from certain disadvantages. Its low sensitivity demands larger amount of sample than other methods. Also, there is considerable overlapping

of signals in the NMR spectra impeding accurate signal identification and peak integration (Kim et al. 2010; Halabalaki et al. 2014).

3.2.1 2D NMR

Signal overlap is a real problem in 1D NMR, hindering both the identification and quantification of compounds in complex plant extracts. Hence, 2D NMR spectroscopy can be employed, which provides good signal resolution by distributing the resonance in a second axis. One limitation of this method is the lengthy acquisition time as compared to 1D NMR (Kim et al. 2011). *J*-resolved spectroscopy and heteronuclear single quantum coherence (HSQC) are the major useful 2D methods (Ludwig and Viant 2010; Salem et al. 2020a). HSQC is particularly employed as a confirmation tool to detect whether the usual suspected compounds (common primary and secondary plant metabolites) are present or not and for the quantification of metabolites. ^{1}H-^{1}H COSY (correlation spectroscopy), NOESY (nuclear overhauser effect spectroscopy), HMBC (heteronuclear multiple bond correlation), etc. are few important 2D NMR-based methods (Kim et al. 2011; Salem et al. 2020a).

3.2.2 Solid-State NMR

Solid-state NMR allows the examination of semisolid samples such as tissue samples via the use of high-resolution magic-angle spinning (HRMAS). This technique involves mixing of the sample with a minimal solvent volume followed by rotating the sample at 54.74° with high spinning rates (Pérez et al. 2010; Kruk et al. 2017). Better resolution can be achieved by HRMAS. Nevertheless, the faster spinning and high temperature may cause tissue distortion. Also, the quantification of metabolites poses a challenge in HRMAS, which can be overcome by a method called ERETIC (Electronic REference To access In vivo Concentration) (Kruk et al. 2017).

4 Data Processing Methods

Large amount of data is generated in metabolomics analysis through the analytical platforms such as NMR and MS (Liland 2011). Meaningful data can be mined from this complex data set through multivariate analysis (MVA) methods like discriminant analysis, discrimination map analysis, and principal component analysis (PCA). These methods are mainly focused on the reduction of data such as *m*/*z* values from MS analysis, chromatography data, NMR data, etc. The reduced data are presented as discrimination maps, score pots, load plots, etc. according to the MVA method employed. The raw data from the analysis are mathematically and statistically processed, and that is represented in the form of vectors in score plot. These vectors

correspond to the metabolite fingerprint of the tested sample in the case of score plot, whereas in the loading plot, each signal's contribution is represented. The discrimination map, in addition to visualizing the contribution of each signal, also represents the variation in signals. Thus, the marker metabolites can be selected by statistical means by connecting the loaded factor and the data from chemical analysis (Okada et al. 2010).

Another strategy to handle this enormous metabolomic data involves the utilization of software tools. These software packages process the raw data of spectra and also perform statistical analysis in order to find out the metabolites, which are significantly expressed in the sample; later, the metabolites are compared with metabolite databases, and subsequently, multiple "omics" data is integrated and analyzed; finally, it helps to visualize molecular interactions also (Krastanov 2010; Sugimoto et al. 2012). MetaboAnalyst, MetaCore™, and InCroMAP are some of the versatile software tools used for this purpose (Cambiaghi et al. 2016). Among these, only MetaboAnalyst is a comprehensive tool that can perform both the pre-processing of data and its statistical analysis (Xia et al. 2015). MetaCore™ is an integrated database, which makes possible the visualization and functional analyses of various kinds of omics data together with options for biological pathway analyses. This versatile tool also finds applications in biomarker identification to drug discovery process (Cambiaghi et al. 2016). InCroMAP is user-friendly software, which can generate global maps of metabolic processes in cell from the metabolic analysis (Wrzodek 2012; Wrzodek et al. 2012). It suffers a drawback, as it can't perform data pre-processing (Cambiaghi et al. 2016).

5 Databases for Handling Metabolomics Data

The field of plant metabolomics is advancing rapidly, the ultimate aim of which is a holistic understanding of various functions and healing potential of medicinal plants (Afendi et al. 2012). Therefore, to achieve profile management, metabolite identification, effective data mining, and efficient platforms are required (Ferry-Dumazet et al. 2011). For making the metabolomics data meaningful, it has to be organized in a standard form that allows cross-referencing with other datasets. Several specialized metabolomics databases for plants are available, and these databases provide updated and comprehensive information (Shafi and Zahoor 2021). In order to make metabolomics as a valuable tool for functional genomics, it requires the availability of annotated metabolomics data, and it can be accessed through internet. Visualization tools (such as error and ratio plots), if integrated into the databases, would help in the comparison of metabolome at various conditions such as environmental changes, genetic disturbances, and variation in experimental parameters to which the biological system is subjected and would find the effective analytical platform that can effectively communicate the maximum metabolomic changes according to these changing conditions (Bais et al. 2010). The different databases, which handle metabolomics data of medicinal plants, are indicated in Table 11.2

Table 11.2 List of metabolomics databases of medicinal plants (from 2010 onward)

Sl. No.	Database	Description	References
1	CathaCyc	It contains metabolic pathway database of *Catharanthus roseus* containing its RNA-seq data and metabolomics data	Van Moerkercke et al. (2013)
2	HerbalDB 2.0	The updated version of HerbalDB, Indonesian medicinal plant database. It contains 3D structure of 1405 herbal compounds from Indonesian plants to aid in in silico drug design	Syahdi et al. (2019)
3	IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics)	It consists of 1742 (Indian) medicinal plants, 1124 therapeutic uses and 9599 phytochemicals. It includes a library with 9596 phytochemicals	Mohanraj et al. (2018)
4	InDiaMed (Indian Medicinal Plants for Diabetes)	It is a database for the information of medicinal plants in India with antidiabetic activity. Also lists antidiabetic poly herbal formulations	Tota et al. (2013)
5	KNApSAcK family databases	This database describes the connection between the species and the corresponding metabolites they encode. The relation of medicinal plants with their geographical zones is documented along with their activities and their herbal formulations	Afendi et al. (2012)
6	MassBank	First public database containing mass spectral data of small molecules for life sciences (<3000 Da)	Horai et al. (2010)
7	Medicinal Plant Metabolomic Resource (MPMR)	Comprised of detailed RNA-seq and associated metabolomics data from 14 medicinal plants	Wurtele et al. (2012)
8	MED-PDB	Contains details of 147 plants, 53 botanical families and subfamilies, 435 disease types, and 369 active compound types	Sargia et al. (2018)
9	MeRy-B	It consists of ^{1}H NMR metabolic profiles and includes the data from description of the plant to metabolite identification and determining its concentration	Ferry-Dumazet et al. (2011)
10	MetaboLights	It aims at developing cross-species and cross-platform research in metabolomics. It provides both experimental and raw data from metabolomics experiments	Kale et al. (2016)
11	MPD3 (Medicinal Plants Database for Drug Designing)	It provides database merging activities and is a comprehensive database that aids in computer aided drug design (CADD) by providing information about phytochemical bioactivities, targets of phytochemicals and their literature references. Over 5000 phytochemicals reported from ~1000 plants are included with targets over 200 and literature references over 900	Mumtaz et al. (2017)

(continued)

Table 11.2 (continued)

Sl. No.	Database	Description	References
12	Plant Metabolome Database (PMDB)	It helps to visualize the 3D structure of plant metabolites. It contains external and internal links to KEGG, CAS NUMBER, and PUBCHEM	Udayakumar et al. (2012)
13	PlantMetabolomics.org (PM)	It is a combined database and web portal which integrates metabolomics data from multiple laboratories employing various analytical methods. It includes visualization tools such as error and ratio plots	Bais et al. (2010)
14	ReSpect	Repository of MS/MS-based metabolomics data specific for plants which contain 3595 metabolites. Enables the narrowing down of phytochemical structure to candidate structures	Sawada et al. (2012)
15	RIKEN Plant Metabolome MetaDatabase (RIKEN PMM)	Compiles GC-MS-based metabolomics data from plants along with their experimental metadata	Fukushima et al. (2018)
16	SHPIS (Saudi Herbal Plants Information System)	Contains about 120 varieties of unique Saudi Arabian medicinal plants	Syed and Khan (2017)
17	SoyMetDB (The Soybean Metabolome Database)	Metabolomic database for *Glycine max*, which aims to integrate its metabolomics data (LC-MS and GC-MS based data). Include metabolomics data from *Arabidopsis* to enable cross-species comparisons	Joshi et al. (2010)
18	Super Natural II	Consisting of ~326,000 molecules along with their 2D structures, physicochemical and structural properties, probable toxicity (of about 170,000), and vendor information	Banerjee et al. (2015)
19	Uttarakhand Medicinal Plants Database (UMPDB)	Contains details of 1127 medicinal plants belonging to 153 families distributed along 13 Uttarakhand districts	Kumar et al. (2018)

(i.e., from 2010 onward). Various applications of databases in medicinal plant research are schematically represented in Fig. 11.3.

6 Applications of Metabolomics in Medicinal Plant Research

6.1 Investigating the Bioactive Compounds in Medicinal Plants

Inability to efficiently identify potential bioactive compounds from medicinal plants is a bottleneck in their extensive clinical applications, masking the wealth of efficient

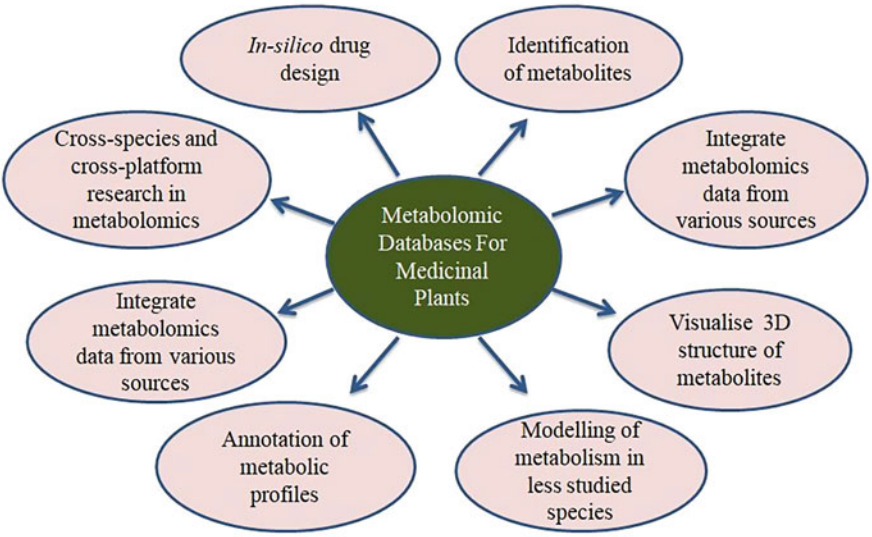


Fig. 11.3 Various applications of plant metabolomics databases

lead compounds for drug industry (Plazas et al. 2019). Metabolomics finds immense applications in unveiling the bioactive compounds responsible for the therapeutic effects of medicinal plants (Yuliana et al. 2013), and some of the examples are briefly outlined. The treatment of Alzheimer's disease is posing a great challenge, because of the absence of efficient drug candidates (Rahman and Choudhary 2015). Only viable option is to focus on drugs that act as cholinesterase inhibitors. To screen for anti-cholinesterase alkaloids found in *Zanthoxylum* members of Rutaceae, metabolomics profiling of its nine species has been performed along with chemometric analysis. This experiment resulted in the detection of 11 isoquinoline alkaloids with potential anti-cholinesterase activity (Plazas et al. 2019). To explore the potential of *Camptotheca acuminata* for antineoplastic treatment, metabolite fingerprinting has been performed, and they identified the alkaloids related to camptothecin having antineoplastic action. Leaves at different growth stages were analyzed to find the optimum stage of growth for harvesting. Various camptothecin-related alkaloids revealed through the study offer promising compounds to be utilized as precursors in the synthesis of semisynthetic derivatives of camptothecin (Montoro et al. 2010). Li et al. (2013) performed metabolic profiling of *Tussilago farfara* to investigate the compounds responsible for its expectorant and antitussive activities. They also analyzed different plant parts to optimize the part with maximum activity; it revealed that flower buds and leaves possessed maximum bioactive principles. The study confirmed the role of 3,5-dicaffeoylquinic acid, rutin, and chlorogenic acid in the therapeutic properties of the plant.

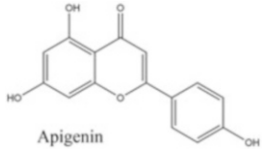
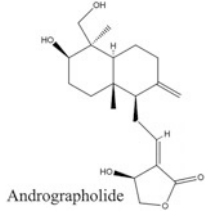
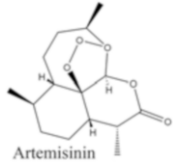
Carica papaya has broad spectrum of therapeutic applications such as anti-inflammatory, antibacterial, anticancer, antioxidant, antimalarial, vasodilatory

properties, etc. Metabolic fingerprinting performed through 1D and 2D ^{1}H NMR revealed that high concentration of secondary metabolites occupied in younger leaves and UPLC-ESI-MS analysis verified and confirmed the active metabolites. Apart from attributing the therapeutic effects to one or two major compounds, the role of synergism of metabolites in its diverse clinical applications was revealed in this study (Gogna et al. 2015). Taha et al. (2020) investigated the antimicrobial, anticancer, and antioxidant activities of a desert medicinal plant *Hyphaene thebaica*. Past studies in this plant were mainly concentrated on the clinical applications of the fruit, while this study utilized leaves, fruit, and male parts of the plant. These three parts were evaluated in three groups according to the anthocyanin, flavonoid, flavonol, phenolic, saponin, and tannin content. Significant increase in antioxidant activity was found in male parts and leaves than fruits. Metabolic profiling by HPLC confirmed the dominance of chrysin, *p*-hydroxybenzoic acid, *p*-coumaric acid, protocatechuic acid, syringic acid, rosmarinic acid, and vanillic acid in male parts; chlorogenic acid in fruit; and apigenin-7-glucosides, catechins, and rutin in leaves. Some of the important bioactive compounds from medicinal plants studied using metabolomics are given in Table 11.3.

6.2 Elucidate the Mode of Action of Herbal Medicine

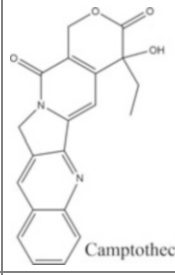
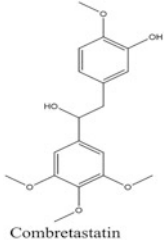
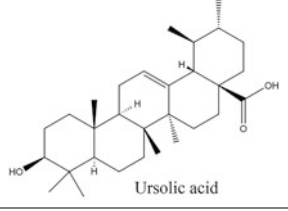
There are concerns over toxicity, efficacy, quality, etc. of many traditional medicinal herbs. Metabolomics is a valuable tool to answer these concerns. Herbal formulations of traditional Chinese medicine (TCM) have wide clinical applications in treatment of liver diseases, which were largely limited due to queries over safety and quality. Metabolomics has been employed to decipher the mode of action of three herbal remedies (yin chen hao tang, xiaozhang tie, and silymarin) for liver disease in TCM through studies in cell culture systems, animal models, and clinical studies. This study provides insights to the efficacy and safety of herbal remedies and also underlines the importance of combinations of herbal medicines in efficient treatment methods (Beyoğlu and Idle 2020). Likewise, the ability of Suanzaoren decoction to treat insomnia has been delineated by assessing the metabolic changes taking place in a model insomnia drosophila after administering Suanzaoren decoction. This decoction is a formulation of five herbal medicines (Poria, seed of *Ziziphus jujuba*; roots and rhizome of *Glycyrrhiza uralensis*, *G. inflata*, and *G. glabra*; rhizome of *Ligusticum chuanxiong*; and rhizome of *Anemarrhena asphodeloides*). The analysis revealed that Suanzaoren decoction could significantly increase sleep activity. In addition to this, the hypnotic effect of this formulation is found to affect the global metabolomics of the test organism (Yang et al. 2012).

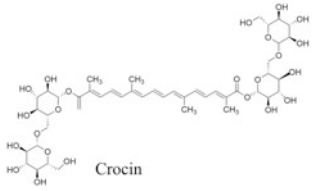
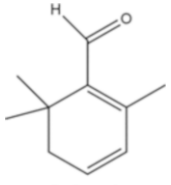
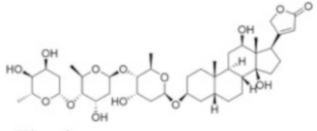
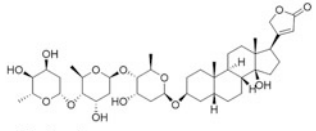
Table 11.3 Major bioactive compounds from medicinal plants studied using metabolomics

Sl. No	Plant name	Major metabolites under study	Medicinal use	Metabolomic technique/s employed	References
1	*Allium sativum* L.	 Apigenin	Its hydroxylated form prevents proliferation of tumor cells and angiogenesis	LC-MS	Afsheen et al. (2018)
2	*Andrographis paniculata* (Burm. f.) Nees	 Andrographolide	Anticancer, anti-inflammatory, anti-HIV, antimalarial activities	^{1}H NMR	Tajidin et al. (2019)
3	*Artemisia afra* Jacq.	 Artemisinin	Anti-plasmodial activity	NMR	Liu et al. (2010)

(continued)

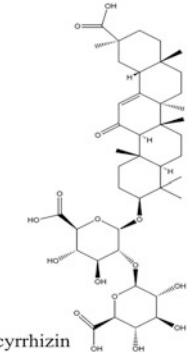
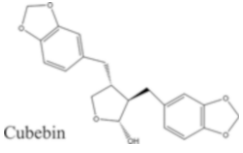
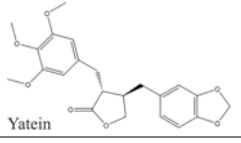
Table 11.3 (continued)

Sl. No	Plant name	Major metabolites under study	Medicinal use	Metabolomic technique/s employed	References
4	*Camptotheca acuminata* Dence.	 Camptothecin	Anticancer activity	HPLC-MS/MS	Cho et al. (2018)
5	*Combretum caffrum* (Eckl. & Zeyh.) Kuntze	 Combretastatin	Anticancer activity	Liquid chromatography high-resolution mass spectrometry platform (LC-HRMS)	Jaroch et al. (2018)
6	*Crataegus oxyacantha* L.	 Ursolic acid	Inhibits angiotensin converting enzyme, cardioactive potential	LC-MS	Afsheen et al. (2018)

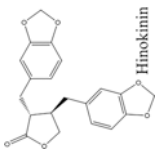
7	*Crocus sativus* L.	 Crocin	Treatment of aging and age associated neurodegenerative disorders	High-resolution MS	Gikas et al. (2021), Rezaee and Hosseinzadeh (2013)
		 Safranal	Antidepressant, anticonvulsant, treatment of withdrawal syndrome		
8	*Digitalis lanata* Ehrh. and *D. purpurea* L.	 Digoxin  Digitoxin	Treatment of atrial fibrillation systolic heart disease	LC-MS/MS	Ravi et al. (2020)

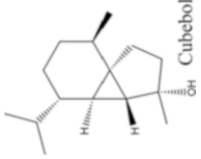
(continued)

Table 11.3 (continued)

Sl. No	Plant name	Major metabolites under study	Medicinal use	Metabolomic technique/s employed	References
9	*Glycyrrhiza glabra* L., *G. uralensis* Fisch. and *G. inflata* Bat.	 Glycyrrhizin	Antioxidant, anti-inflammatory activities	High resolution MS	Rizzato et al. (2017)
10	*Piper cubeba* L.	 Cubebin  Yatein	Anticancerous activity	^{1}H-NMR	Mazlan et al. (2018)

(continued)





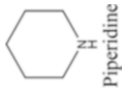
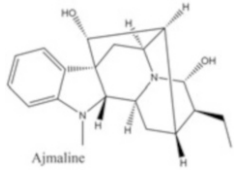
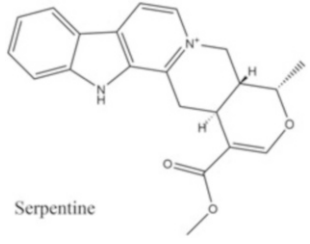
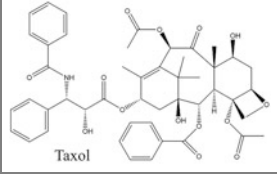


Table 11.3 (continued)

Sl. No	Plant name	Major metabolites under study	Medicinal use	Metabolomic technique/s employed	References
11	*Rauvolfia serpentina* (L.) Benth. ex Kurz.	 Ajmaline	Sodium channel blocker, stimulate intestinal movements and respiration	LC-MS	Afsheen et al. (2018)
		 Serpentine	Used to treat hypertension, neurological and cardiovascular disorders		
12	*Taxus fuana* Nan Li & R.R. Mill and *T. yunnanensis* Cheng et L. K. Fu.	 Taxol	Anticancer activity	Comparative metabolomics via targeted UPLC-MS/MS method to quantify the production of bioactive metabolites	Yu et al. (2018)

6.3 Quality Assessment of Herbal Products

The capabilities of natural product derived drugs can be utilized in full potential only if the chemical composition of herbal products is standardized to check the proper quality of natural product (Heyman and Meyer 2012). Quality of herbal formulations has been assessed earlier based on one or two major compounds present in it. However, herbal medicines comprise multiple compounds, and metabolic profiling can be performed to evaluate the multiple components, thereby meeting adequate quality standards (Lee et al. 2017). Metabolomics is gaining wide acclaim nowadays to assess phytochemical constituents, thus providing an acceptable method for quality control of herbal medicine. By employing chromatographic and spectroscopic methods, a valid metabolite fingerprint can be obtained, and further metabolite profiling helps to identify the individual constituents that form the unique fingerprint (RaoGajula and Nanjappan 2021).

For even properly authenticated plant material, there may be difference in quality between different batches due to a variety of factors such as inter or intra-species variations, environmental factors, harvesting stage, plant parts used, post-harvesting factors, etc. (Nafiu et al. 2017). Xiang et al. (2011) employed metabonomic analysis to characterize ecotypic variation in three species of *Curcuma*, namely, *Curcuma kwangsiensis*, *C. phaeocaulis*, and *C. wenyujin* as part of quality control measures. This study was focused on the essential oil composition, and PCA efficiently distinguished samples according to differences in species and ecotypes. Some of the medicinal herbs are difficult to distinguish between varieties as in the case of *Ficus deltoidea* (a popular medicinal herb of Malaysia), whose seven varieties are laborious to identify morphologically due to extensive heterophylly. The difficulty to identify the desired variety and variations of their chemical constitution is posing challenges in their commercialization. Untargeted metabolomics is performed by ultra-high-performance liquid chromatography time-of-flight mass spectrometry (UHPLC-TOFMS), and the subsequent data analysis was able to distinguish three chemotypes on the basis of differences in flavonoid content. The study also identified 15 glycosylated flavones and 1 furanocoumarin as chemical marker (Afzan et al. 2019). Saposhnikoviae Radix (a common crude drug) obtained from rhizome and root of *Saposhnikovia divaricata* was investigated for the differences in metabolome using the specimens from China and Mongolia. Metabolic profiling confirmed that these two regional groups are clearly distinct with respect to *O*-glucosylcimifugin, being more abundant in Mongolian group. They can also be distinguished based on the differences in content of eight chromones (Batsukh et al. 2020). Rastogi et al. (2020) investigated the interspecies variation among three medicinally important species of *Ocimum*, namely, *O. gratissimum*, *O. kilimandscharicum*, and *O. sanctum* by analyzing the temporal changes in metabolite composition.

Adulteration of medicinal herbs with certain other plants or any other foreign substance is a common practice and a major challenge of natural drug industry (Chanda 2014). Wallace et al. (2018) evaluated the adulteration in commercial plant-derived products by using supplements of *Hydrastis canadensis* (goldenseal) as the

test case. An untargeted metabolomics analysis employing UPLC-MS helped to detect adulteration in 3 test samples among the 35 test samples. This analysis revealed the potential applications of untargeted metabolomics in detecting possible adulteration. Extensive adulteration is observed in highly priced oil of *Serenoa repens* (saw palmetto), which triggered the investigation for methods to detect designer blends of cheap fatty acids as adulterants. The combined use of metabolomic analysis and isotopic fingerprinting suggested the possible adulteration and whose source is mostly animal based fatty acids (Perini et al. 2018).

6.4 Safety and Toxicity Assessment of Natural Products

One of the factors that hinder the clinical applications of traditional phytomedicine into mainstream is their limited molecular level characterization. If there is proper recording of the side effects, reaction with other medicines, safety, hypersensitivity, tolerance, problems of overdose, etc. of natural products, then only the product can be commercialized (Cordell 2015). Nowadays, traditional Chinese medicine (TCM) is attracting wide attention due to debates over safety concerns. Even though TCM contains natural formulations, their complex nature and mechanism of action remain as a barrier to assess it by traditional methods. For this, metabolomics helps to get an idea about the possible toxicity of bioactive compounds in TCM. Cardiotoxicity, hepatotoxicity, nephrotoxicity, and reproduction toxicity have been revealed as the side effects many of the components of TCM by metabolomic studies (Duan et al. 2018). Traditional African medicine (TAM) also has much popularity among African communities with wide acceptance among both rural and urban populations. In this context, metabolomics provides an opportunity to make a holistic analysis of phytochemicals, biomarkers, and the mechanism by which TAM modifies metabolic pathways (Quansah and Karikari 2016).

In order to investigate the different levels of toxicity exhibited by *Senecio scandens* and *S. vulgaris*, a metabolomics study employing UPLC-MS was performed. *Senecio scandens*, which is an approved medicine of Chinese medicine, did not show toxic effects, while *S. vulgaris* belonged to the same genera exhibited significant hepatotoxicity. The metabolomics analysis revealed that senecionine, a particular marker of *S. vulgaris* remained the cause for its toxic effects (Xiong et al. 2012). *Polygonum multiflorum* has been a part of TCM, whose dry roots are widely used for therapeutic properties. Hepatotoxicity is a concern over its use, and different processing methods are documented for its processing to reduce the toxicity. With the aim of finding the most suitable method of its processing, variations in metabolomic profiles of *P. multiflorum* with respect to various processing methods were analyzed by UHPLC/Q-Orbitrap-MS. The study identified emodin-8-*O*-glucoside and torachrysone-*O*-hexose as the toxic markers of *P. multiflorum*. The toxic effects were decreased with every processing method and the best results being obtained by steaming with black soybean; the method was suggested by Chinese Pharmacopoeia (Han et al. 2019).

7 Conclusions and Future Prospects

A large proportion of world's population still relies on medicinal plants for therapeutics and our traditional medicinal systems use herbal formulations, it was tested and evaluated by trial-and-error mechanisms. Even though these medicinal plants have such a history of long-term association with human therapeutics, systematic characterization of their active compounds and the intricate mechanisms underlying their action have been achieved with the advent of metabolomics. Metabolomics is a promising approach, which can revitalize the researches in herbal medicine by characterizing the active compounds in medicinal plants responsible for their clinical action. Metabolomics helps to study the synergism between various compounds in attaining the therapeutic effects, assessing the quality, safety, and toxicity of herbs and herbal formulations, etc. The rapid analysis of large number of metabolites in metabolomics is what makes it distinct from other analytical methods. It can also contribute much to the upcoming era of personalized medicine by metabolic profiling of the concerned individual in response to the herbal drug administration; this helps to monitor the efficacy and toxicity of the drug to each individual. Efficient analytical platforms, data handling methods, and associated databases are crucial to the success of metabolomics. Recent advancements in MS- and NMR-based methods have contributed much to the progress of medicinal plant metabolomics. If these two platforms are employed together, metabolomics has much potential to provide a non-biased quantification and characterization and can provide an integrated picture of component metabolites in the sample. Such a comprehensive knowledge regarding the medicinal plants and herbal formulations can open new avenues in herbal clinical industry. Thus, the treasure trove of phytochemicals as potential drug leads can be explored in future.

References

Afendi FM, Okada T, Yamazaki M, Hirai-Morita A, Nakamura Y, Nakamura K, Ikeda S, Takahashi H, Altaf-Ul-Amin M, Darusman LK, Saito K, Kanaya S (2012) KNApSAcK family databases: integrated metabolite-plant species databases for multifaceted plant research. Plant Cell Physiol 53:1–12. https://doi.org/10.1093/pcp/pcr165

Afsheen N, Khalil-Ur-Rehman, Jahan N, Ijaz M, Manzoor A, Khan KM, Hina S (2018) Cardioprotective and metabolomic profiling of selected medicinal plants against oxidative stress. Oxid Med Cell Longev 2018:17. https://doi.org/10.1155/2018/9819360

Afzan A, Kasim N, Hadiani N, Norfaizura I, Abdul A, Ali M, Mat N (2019) Differentiation of *Ficus deltoidea* varieties and chemical marker determination by UHPLC-TOFMS metabolomics for establishing quality control criteria of this popular Malaysian medicinal herb. Metabolomics 15: 35. https://doi.org/10.1007/s11306-019-1489-2

Antunes ERM, Duarte RS, Moritz T, Sawaya ACHF (2020) Differentiation of two *Maytenus* species and their hybrid via untargeted metabolomics. Ind Crop Prod 158:113014. https://doi.org/10.1016/j.indcrop.2020.113014

Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MHA, Ghafoor K, Norulaini NAN, Omar AKM (2013) Techniques for extraction of bioactive compounds from plant materials: a review. J Food Eng 117:426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

Bais P, Moon SM, He K, Leitao R, Dreher K, Walk T, Sucaet Y, Barkan L, Wohlgemuth G, Roth MR, Wurtele ES, Dixon P, Fiehn O, Lange BM, Shulaev V, Sumner LW, Welti R, Nikolau BJ, Rhee SY, Dickerson JA (2010) Plantmetabolomics.org: a web portal for plant metabolomics experiments. Plant Physiol 152(4):1807–1816. https://doi.org/10.1104/pp.109.151027

Banerjee P, Erehman J, Gohlke BO, Wilhelm T, Preissner R, Dunkel M (2015) Super Natural II-a database of natural products. Nucleic Acids Res 43:D935–D939. https://doi.org/10.1093/nar/gku886

Barderas MG, Laborde CM, Posada M, De La Cuesta F, Zubiri I, Vivanco F, Alvarez-Llamas G (2011) Metabolomic profiling for identification of novel potential biomarkers in cardiovascular diseases. J Biomed Biotechnol 2011:1–9. https://doi.org/10.1155/2011/790132

Batsukh Z, Toume K, Javzan B, Kazuma K, Cai SQ, Hayashi S, Kawahara N, Maruyama T, Komatsu K (2020) Metabolomic profiling of Saposhnikoviae Radix from Mongolia by LC–IT–TOF–MS/MS and multivariate statistical analysis. J Nat Med 74:170–188. https://doi.org/10.1007/s11418-019-01361-0

Beyoğlu D, Idle JR (2020) Metabolomic insights into the mode of action of natural products in the treatment of liver disease. Biochem Pharmacol 180:114171. https://doi.org/10.1016/j.bcp.2020.114171

Bjerrum JT (2015) Metabonomics: analytical techniques and associated chemometrices at a glance. In: Bjerrum JT (ed) Metabonomics, methods and protocols, Methods in molecular biology, vol 1277, p 2. https://doi.org/10.1007/978-1-4939-2377-9

Cambiaghi A, Ferrario M, Masseroli M (2016) Analysis of metabolomic data: tools, current strategies and future challenges for omics data integration. Brief Bioinformatics 18:498–510. https://doi.org/10.1093/bib/bbw031

Cao M, Liu Y, Jiang W, Meng X, Zhang W, Chen W, Peng D, Xing S (2020) UPLC/MS-based untargeted metabolomics reveals the changes of metabolites profile of *Salvia miltiorrhiza* bunge during Sweating processing. Sci Rep 10:1–10. https://doi.org/10.1038/s41598-020-76650-w

Chanda S (2014) Importance of pharmacognostic study of medicinal plants: an overview. J Pharmacogn Phytochem 2:69–73

Chen J, Wang W, Kong J, Yue Y, Dong Y, Zhang J, Liu L (2022) Application of UHPLC-Q-TOF MS based untargeted metabolomics reveals variation and correlation amongst different tissues of *Eucommia ulmoides* Oliver. Microchem J 172. https://doi.org/10.1016/j.microc.2021.106919

Chintamunnee V, Mahomoodally MF (2012) Herbal medicine commonly used against non-communicable diseases in the tropical island of Mauritius. J Herb Med 2:113–125. https://doi.org/10.1016/j.hermed.2012.06.001

Cho JY, Park MJ, Ryu DH, Kang Y (2018) Biological activities and the metabolite analysis of *Camptotheca acuminata* Dence. Proc Plant Resour Soc Korea Conf 2018:14

Cordell GA (2015) Phytochemistry and traditional medicine - the revolution continues. Phytochem Lett 10:xxviii–xi. https://doi.org/10.1016/j.phytol.2014.06.002

Duan L, Guo L, Wang L, Yin Q, Zhang CM, Zheng YG, Liu EH (2018) Application of metabolomics in toxicity evaluation of traditional Chinese medicines. Chin Med 13:60. https://doi.org/10.1186/s13020-018-0218-5

Efferth T, Greten HJ (2012) Quality control for medicinal plants. Med Aromat Plants 1:10–13. https://doi.org/10.4172/2167-0412.1000e131

Ellis DI, Dunn WB, Griffin JL, Allwood JW, Goodacre R (2007) Metabolic fingerprinting as a diagnostic tool. Pharmacogenomics 8:1243–1266. https://doi.org/10.2217/14622416.8.9.1243

Fan R, Peng C, Zhang X, Qiu D, Mao G, Lu Y, Zeng J (2020) A comparative UPLC-Q-Orbitrap-MS untargeted metabolomics investigation of different parts of *Clausena lansium* (Lour.) Skeels. Food Sci Nutr 8:5811–5822. https://doi.org/10.1002/fsn3.1841

Fernie AR, Stitt M (2012) On the discordance of metabolomics with proteomics and transcriptomics: coping with increasing complexity in logic, chemistry and network interactions. Plant Physiol 158:1139–1145. https://doi.org/10.1104/pp.112.19323

Ferry-Dumazet H, Gil L, Deborde C, Moing A, Bernillon S, Rolin D, Nikolski M, de Daruvar A, Jacob D (2011) MeRy-B: a web knowledgebase for the storage, visualization, analysis and annotation of plant NMR metabolomic profiles. BMC Plant Biol 11:104. https://doi.org/10.1186/1471-2229-11-104

Filloux A, Ramos JL (2014) Preface. In: Clifton NJ (ed) Pseudomonas methods and protocols, Methods in molecular biology, vol 1149. Humana, Totowa, NJ, p v. https://doi.org/10.1007/978-1-4939-0473-0

Fletcher JS, Rabbani S, Henderson A, Blenkinsopp P, Thompson SP, Lockyer NP, Vickerman JC (2008) A new dynamic in mass spectral imaging of single biological cells. Anal Chem 80:9058–9064. https://doi.org/10.1021/ac8015278

Fukushima A, Takahashi M, Sakurai N (2018) RIKEN Plant Metabolome MetaDatabase: an integrated plant metabolome data repository based on the semantic web. Semantic Web Appl Tools Healthc Life Sci 2018:2–3. https://doi.org/10.6084/m9.figshare.7331036.v1

García-Pérez P, Zhang L, Miras-Moreno B, Lozano-Milo E, Landin M, Lucini L, Gallego PP (2021) The combination of untargeted metabolomics and machine learning predicts the biosynthesis of phenolic compounds in *Bryophyllum* medicinal plants (Genus kalanchoe). Sci Rep 10:19524. https://doi.org/10.3390/plants10112430

Gikas E, Koulakiotis NS, Tsarbopoulos A (2021) Phytochemical differentiation of saffron (*Crocus sativus* L.) by high resolution mass spectrometry metabolomic studies. Molecules 26:2180. https://doi.org/10.3390/molecules26082180

Gogna N, Hamid N, Dorai K (2015) Metabolomic profiling of the phytomedicinal constituents of *Carica papaya* L. leaves and seeds by 1H NMR spectroscopy and multivariate statistical analysis. J Pharm Biomed Anal 115:74–85. https://doi.org/10.1016/j.jpba.2015.06.035

Gonulalan EM, Nemutlu E, Bayazeid O, Koçak E, Yalçın FN, Demirezer LO (2020) Metabolomics and proteomics profiles of some medicinal plants and correlation with BDNF activity. Phytomedicine 74:152920. https://doi.org/10.1016/j.phymed.2019.152920

Govindaraghavan S, Hennell JR, Sucher NJ (2012) From classical taxonomy to genome and metabolome: towards comprehensive quality standards for medicinal herb raw materials and extracts. Fitoterapia 83:979–988. https://doi.org/10.1016/j.fitote.2012.05.001

Gray GR, Heath D (2005) A global reorganization of the metabolome in *Arabidopsis* during cold acclimation is revealed by metabolic fingerprinting. Physiol Plant 124:236–248. https://doi.org/10.1111/j.1399-3054.2005.00507.x

Halabalaki M, Vougogiannopoulou K, Mikros E, Skaltsounis AL (2014) Recent advances and new strategies in the NMR-based identification of natural products. Curr Opin Biotechnol 25:1–7. https://doi.org/10.1016/j.copbio.2013.08.005

Hall RD (2006) Plant metabolomics: from holistic hope, to hype, to hot topic. New Phytol 169:453–468. https://doi.org/10.1111/j.1469-8137.2005.01632.x

Han L, Wang P, Wang Y, Zhao Q, Zheng F, Dou Z, Yang W, Hu L, Liu C (2019) Rapid discovery of the potential toxic compounds in *Polygonum multiflorum* by UHPLC/Q-Orbitrap-MS-based metabolomics and correlation analysis. Front Pharmacol 10:329. https://doi.org/10.3389/fphar.2019.00329

Heyman HM, Meyer JJM (2012) NMR-based metabolomics as a quality control tool for herbal products. S Afr J Bot 82:21–32. https://doi.org/10.1016/j.sajb.2012.04.001

Hill CB, Roessner U (2015) Advances in high-throughput untargeted LC–MS analysis for plant metabolomics. In: Advanced LC-MS applications in metabolomics. pp 58–71. https://doi.org/10.4155/fseb2013.14.54

Hong J, Yang L, Zhang D, Shi J (2016) Plant metabolomics: an indispensable system biology tool for plant science. Int J Mol Sci 17:767. https://doi.org/10.3390/ijms17060767

Horai H, Arita M, Kanaya S, Nihei Y, Ikeda T, Suwa K, Ojima Y, Tanaka K, Tanaka Y, Aoshima K, Oda Y, Kakazu K, Kusano M, Tohge T, Matsuda F, Sawada Y, Hirai MY,

Nakanishi H, Ikeda K, Akimoto N, Maoka T, Takahashi H, Ara T, Sakurai N, Suzuki H, Shibata D, Neumann S, Iida T, Tanaka K, Funatsu K, Matsuura F, Soga T, Taguchi R, Saito K, Nishioka T (2010) MassBank: a public repository for sharing mass spectral data for life sciences. J Mass Spectrom 45:703–714. https://doi.org/10.1002/jms.1777

James JT, Tugizimana F, Steenkamp PA, Dubery IA (2013) Metabolomic analysis of methyl jasmonate-induced triterpenoid production in the medicinal herb *Centella asiatica* (L.) urban. Molecules 18:4267–4281. https://doi.org/10.3390/molecules18044267

Jaroch K, Goryńska PZ, Goryński K, Stefański T, Bojko B (2018) Untargeted screening of phase I metabolism of combretastatin A4 by multi-tool analysis. Talanta 182:22–31. https://doi.org/10.1016/j.talanta.2018.01.051

Johnson CH, Ivanisevic J, Siuzdak G (2016) Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol 17:451–459. https://doi.org/10.1038/nrm.2016.25

Joshi T, Yao Q, Franklin LD, Brechenmacher L, Valliyodan B, Stacey G, Nguyen H, Xu D (2010) SoyMetDB: The Soybean Metabolome Database. Proceedings - 2010 IEEE International Conference on Bioinformatics and Biomedicine BIBM 2010:203–208. https://doi.org/10.1109/BIBM.2010.5706563

Kale NS, Haug K, Conesa P, Jayseelan K, Moreno P, Rocca-Serra P, Nainala VC, Spicer RA, Williams M, Li X, Salek RM, Griffin JL, Steinbeck C (2016) MetaboLights: an open-access database repository for metabolomics data. Curr Protoc Bioinform 53:14.13.1–14.13.18. https://doi.org/10.1002/0471250953.bi1413s53

Kim HK, Choi YH, Verpoorte R (2010) NMR-based metabolomic analysis of plants. Nat Protoc 5:536–549. https://doi.org/10.1038/nprot.2009.237

Kim HK, Choi YH, Verpoorte R (2011) NMR-based plant metabolomics: where do we stand, where do we go? Trends Biotechnol 29:267–275. https://doi.org/10.1016/j.tibtech.2011.02.001

Kiran KR, Swathy PS, Paul B, Shama Prasada K, Radhakrishna Rao M, Joshi MB, Rai PS, Satyamoorthy K, Muthusamy A (2021) Untargeted metabolomics and DNA barcoding for discrimination of *Phyllanthus* species. J Ethnopharmacol 273:113928. https://doi.org/10.1016/j.jep.2021.113928

Klepárník K (2015) Recent advances in combination of capillary electrophoresis with mass spectrometry: methodology and theory. Electrophoresis 36:159–178. https://doi.org/10.1002/elps.201400392

Kopka J, Fernie A, Weckwerth W, Gibon Y, Stitt M (2004) Metabolite profiling in plant biology: platforms and destinations. Genome Biol 5:1–9. https://doi.org/10.1186/gb-2004-5-6-109

Krastanov A (2010) Metabolomics - the state of art. Biotechnol Biotechnol Equip 24:1537–1543. https://doi.org/10.2478/V10133-010-0001-y

Kruk J, Doskocz M, Jodłowska E, Zacharzewska A, Łakomiec J, Czaja K, Kujawski J (2017) NMR techniques in metabolomic studies: a quick overview on examples of utilization. Appl Magn Reson 48:1–21. https://doi.org/10.1007/s00723-016-0846-9

Kumar A, Kumar R, Sharma M, Kumar U, Prasad Gajula MNV, Singh KP (2018) Uttarakhand medicinal plants database (UMPDB): a platform for exploring genomic, chemical, and traditional knowledge. Data 3:7. https://doi.org/10.3390/data3010007

Lamine M, Mliki A (2021) *Citrus sinensis* tissue-specific specialized metabolism elucidated via non-targeted metabolomics strategy. Open J Nutr Food Sci 3:1015

Lee KM, Jeon JY, Lee BJ, Lee H, Choi HK (2017) Application of metabolomics to quality control of natural product derived medicines. Biomol Ther 25:559–568. https://doi.org/10.4062/biomolther.2016.249

Li ZY, Zhi HJ, Zhang FS, Sun HF, Zhang LZ, Jia JP, Xing J, Qin XM (2013) Metabolomic profiling of the antitussive and expectorant plant *Tussilago farfara* L. by nuclear magnetic resonance spectroscopy and multivariate data analysis. J Pharm Biomed Anal 75:158–164. https://doi.org/10.1016/j.jpba.2012.11.023

Li S, Chen Y, Duan Y, Zhao Y, Zhang D, Zang L, Ya H (2021) Widely targeted metabolomics analysis of different parts of *Salsola collina* pall. Molecules 26:1126. https://doi.org/10.3390/molecules26041126

Liang L, Xu J, Zhou WW, Brand E, Chen HB, Zhao ZZ (2018) Integrating targeted and untargeted metabolomics to investigate the processing chemistry of polygoni multiflori radix. Front Pharmacol 9:934. https://doi.org/10.3389/fphar.2018.00934

Liland KH (2011) Multivariate methods in metabolomics - from pre-processing to dimension reduction and statistical analysis. TrAC Trend Anal Chem 30:827–841. https://doi.org/10.1016/j.trac.2011.02.007

Liu NQ, Cao M, Frédérich M, Choi YH, Verpoorte R, van der Kooy F (2010) Metabolomic investigation of the ethnopharmacological use of *Artemisia afra* with NMR spectroscopy and multivariate data analysis. J Ethnopharmacol 128:230–235. https://doi.org/10.1016/j.jep.2010.01.020

Liu W, Song Q, Cao Y, Xie N, Li Z, Jiang Y, Zheng J, Tu P, Song Y, Li J (2019) From 1H NMR-based non-targeted to LC–MS-based targeted metabolomics strategy for in-depth chemome comparisons among four *Cistanche* species. J Pharm Biomed Anal 162:16–27. https://doi.org/10.1016/j.jpba.2018.09.013

Liu FJ, Jiang Y, Li P, Liu YD, Yao ZP, Xin GZ, Li HJ (2020) Untargeted metabolomics coupled with chemometric analysis reveals species-specific steroidal alkaloids for the authentication of medicinal Fritillariae Bulbus and relevant products. J Chromatogr A 1612:460630. https://doi.org/10.1016/j.chroma.2019.460630

Ludwig C, Viant MR (2010) Two-dimensional J-resolved NMR spectroscopy: review of a key methodology in the metabolomics toolbox. Phytochem Anal 21:22–32. https://doi.org/10.1002/pca.1186

Lv M, Chen J, Gao Y, Sun J, Zhang Q, Zhang M, Xu F, Zhang Z (2015) Metabolomics based on liquid chromatography with mass spectrometry reveals the chemical difference in the stems and roots derived from *Ephedra sinica*. J Sep Sci 38:3331–3336. https://doi.org/10.1002/jssc.201500529

Mamedov N (2012) Medicinal plants studies: history, challenges and prospective. Med Aromat Plants 1:8. https://doi.org/10.4172/2167-0412.1000e133

Mazlan RNAR, Rukayadi Y, Maulidiani M, Ismail IS (2018) Solvent extraction and identification of active anticariogenic metabolites in *Piper cubeba* L. through 1H-NMR-based metabolomics approach. Molecules 23:173. https://doi.org/10.3390/molecules23071730

Miettinen K, Dong L, Navrot N, Schneider T, Burlat V, Pollier J, Woittiez L, Van Der Krol S, Lugan R, Ilc T, Verpoorte R, Oksman-Caldentey KM, Martinoia E, Bouwmeester H, Goossens A, Memelink J, Werck-Reichhart D (2014) The seco-iridoid pathway from *Catharanthus roseus*. Nat Commun 5:3606. https://doi.org/10.1038/ncomms4606

Mohanraj K, Karthikeyan BS, Vivek-Ananth RP, Chand RPB, Aparna SR, Mangalapandi P, Samal A (2018) IMPPAT: a curated database of Indian medicinal plants, phytochemistry and therapeutics. Sci Rep 8:1–17. https://doi.org/10.1038/s41598-018-22631-z

Montoro P, Maldini M, Piacente S, Macchia M, Pizza C (2010) Metabolite fingerprinting of *Camptotheca acuminata* and the HPLC-ESI-MS/MS analysis of camptothecin and related alkaloids. J Pharm Biomed Anal 51:405–415. https://doi.org/10.1016/j.jpba.2009.05.013

Mumtaz A, Ashfaq UA, ulQamar MT, Anwar F, Gulzar F, Ali MA, Saari N, Pervez MT (2017) MPD3: a useful medicinal plants database for drug designing. Nat Prod Res 31:1228–1236. https://doi.org/10.1080/14786419.2016.1233409

Nafiu MO, Hamid AA, Muritala HF, Adeyemi SB (2017) Quality control of medicinal plants in Africa. In: Kuete V (ed) Medicinal spices and vegetables from Africa. Elsevier Inc., Amsterdam, pp 171–204. https://doi.org/10.1016/B978-0-12-809286-6/00007-8-

Niedenführ S, Wiechert W, Nöh K (2015) How to measure metabolic fluxes: a taxonomic guide for 13C fluxomics. Curr Opin Biotechnol 34:82–90. https://doi.org/10.1016/j.copbio.2014.12.003

Okada T, Mochamad Afendi F, Altaf-Ul-Amin M, Takahashi H, Nakamura K, Kanaya S (2010) Metabolomics of medicinal plants: the importance of multivariate analysis of analytical chemistry data. Nat Prod Res 6:179–196. https://doi.org/10.2174/157340910791760055

Pandey S, Patel MK, Mishra A, Jha B (2015) Physio-biochemical composition and untargeted metabolomics of cumin (*Cuminum cyminum* L.) make it promising functional food and help in mitigating salinity stress. PLoS One 10:1–25. https://doi.org/10.1371/journal.pone.0144469

Pérez EMS, Iglesias MJ, Ortiz FL, Pérez IS, Galera MM (2010) Study of the suitability of HRMAS NMR for metabolic profiling of tomatoes: application to tissue differentiation and fruit ripening. Food Chem 122:877–887. https://doi.org/10.1016/j.foodchem.2010.03.003

Perini M, Paolini M, Camin F, Appendino G, Vitulo F, De Combarieu E, Sardone N, Martinelli EM, Pace R (2018) Combined use of isotopic fingerprint and metabolomics analysis for the authentication of saw palmetto (*Serenoa repens*) extracts. Fitoterapia 127:15–19. https://doi.org/10.1016/j.fitote.2018.04.011

Plazas E, Casoti RR, Murillo MA, Da Costa FB, Cuca LE (2019) Metabolomic profiling of *Zanthoxylum* species: identification of anti-cholinesterase alkaloids candidates. Phytochemistry 168:112128. https://doi.org/10.1016/j.phytochem.2019.112128

Pope GA, MacKenzie DA, Defernez M, Aroso MAMM, Fuller LJ, Mellon FA, Dunn WB, Brown M, Goodacre R, Kell DB, Marvin ME, Louis EJ, Roberts IN (2007) Metabolic footprinting as a tool for discriminating between brewing yeasts. Yeast 24:667–679. https://doi.org/10.1002/yea.1499

Quansah E, Karikari TK (2016) Potential role of metabolomics in the improvement of research on traditional African medicine. Phytochem Lett 17:270–277. https://doi.org/10.1016/j.phytol.2016.08.004

Rahman A, Choudhary MI (eds) (2015) Drug design and discovery in Alzheimer's disease. Elsevier, Amsterdam, pp 3–26

RaoGajula SN, Nanjappan S (2021) Metabolomics: a recent advanced omics technology in herbal medicine research. In: Aftab T, Hakeem KR (eds) Medicinal and aromatic plants. Elsevier Inc., Amsterdam, pp 97–117. https://doi.org/10.1016/b978-0-12-819590-1.00005-7

Rastogi S, Shah S, Kumar R, Kumar A, Shasany AK (2020) Comparative temporal metabolomics studies to investigate interspecies variation in three *Ocimum* species. Sci Rep 10:1–15. https://doi.org/10.1038/s41598-020-61957-5

Ravi BG, Guardian MGE, Dickman R, Wang ZQ (2020) Profiling and structural analysis of cardenolides in two species of *Digitalis* using liquid chromatography coupled with high-resolution mass spectrometry. J Chromatogr A 1618:460903. https://doi.org/10.1016/j.chroma.2020.460903

Ren JL, Zhang AH, Kong L, Wang XJ (2018) Advances in mass spectrometry-based metabolomics for investigation of metabolites. RSC Adv 8:22335–22350. https://doi.org/10.1039/C8RA01574K

Rezaee R, Hosseinzadeh H (2013) Safranal: from an aromatic natural product to a rewarding pharmacological agent. Iran J Basic Med Sci 16:12–26. https://doi.org/10.22038/ijbms.2013.244

Ribbenstedt A, Ziarrusta H, Benskin JP (2018) Development, characterization and comparisons of targeted and non-targeted metabolomics methods. PLoS One 13:1–18. https://doi.org/10.1371/journal.pone.0207082

Rizzato G, Scalabrin E, Radaelli M, Capodaglio G, Piccolo O (2017) A new exploration of licorice metabolome. Food Chem 221:959–968. https://doi.org/10.1016/j.foodchem.2016.11.068

Roberts LD, Souza AL, Gerszten RE, Clish CB (2012) Targeted metabolomics. Curr Protoc Mol Biol 98:30.2.1–30.2.24. https://doi.org/10.1002/0471142727.mb3002s98

Rodríguez-López CE, Hernández-Brenes C, Díaz De La Garza RI (2015) A targeted metabolomics approach to characterize acetogenin profiles in avocado fruit (*Persea americana* Mill.). RSC Adv 5:106019–106029. https://doi.org/10.1039/c5ra22854a

Roessner U, Beckles DM (2009) Metabolite measurements. In: Schwender J (ed) Plant metabolic networks. Springer, New York. https://doi.org/10.1007/978-0-387-78745-9_3

Saito K, Matsuda F (2010) Metabolomics for functional genomics, systems biology, and biotechnology. Annu Rev Plant Biol 61:463–489. https://doi.org/10.1146/annurev.arplant.043008.092035

Salem MA, De Souza LP, Serag A, Fernie AR, Farag MA, Ezzat SM, Alseekh S (2020a) Metabolomics in the context of plant natural products research: from sample preparation to metabolite analysis. Metabolites 10:1–30. https://doi.org/10.3390/metabo10010037

Salem MA, Radwan RA, Mostafa ES, Alseekh S, Fernie AR, Ezzat SM (2020b) Using an UPLC/MS-based untargeted metabolomics approach for assessing the antioxidant capacity and anti-aging potential of selected herbs. RSC Adv 10:31511–31524. https://doi.org/10.1039/d0ra06047j

Salim V, Wiens B, Masada-Atsumi S, Yu F, De Luca V (2014) 7-Deoxyloganetic acid synthase catalyzes a key 3 step oxidation to form 7-deoxyloganetic acid in *Catharanthus roseus* iridoid biosynthesis. Phytochemistry 101:23–31. https://doi.org/10.1016/j.phytochem.2014.02.009

Sargia B, Singh B, Gupta N, Gahlot LK, Gulati T, Hasija Y (2018) MED-PDB: an online database of medicinal plants. J Adv Pharm Educ Res 7:204–207

Sawada Y, Nakabayashi R, Yamada Y, Suzuki M, Sato M, Sakata A, Akiyama K, Sakurai T, Matsuda F, Aoki T, Hirai MY, Saito K (2012) RIKEN tandem mass spectral database (ReSpect) for phytochemicals: a plant-specific MS/MS based data resource and database. Phytochemistry 82:38–45. https://doi.org/10.1016/j.phytochem.2012.07.007

Schippmann U, Leaman DJ, Cunningham B (2002) Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. In: FAO (ed) Biodiversity and the ecosystem approach in agriculture, forestry and fisheries. Satellite event on the occasion of the ninth regular session of the Commission on Genetic Resources for Food and Agriculture, Rome, 12–13 Oct 2002. Inter-Depart, vol 676, pp 1–21

Schrimpe-Rutledge AC, Codreanu SG, Sherrod SD, Mclean JA (2016) Untargeted metabolomics strategies—challenges and emerging directions. J Am Soc Mass Spectr 27:1897–1905. https://doi.org/10.1007/s13361-016-1469-y

Seeley EH, Caprioli RM (2012) 3D imaging by mass spectrometry: a new frontier. Anal Chem 84:2105–2110. https://doi.org/10.1021/ac2032707

Shafi A, Zahoor I (2021) Metabolomics of medicinal and aromatic plants: goldmines of secondary metabolites for herbal medicine research. In: Aftab T, Hakeem KR (eds) Medicinal and aromatic plants. Elsevier Inc., Amsterdam, pp 261–267. https://doi.org/10.1016/B978-0-12-819590-1/00012-4

Shah SH, Kraus WE, Newgard BC (2012) Metabolomic profiling for identification of novel biomarkers and mechanisms related to common cardiovascular diseases: form and function. Circulation 126:1110–1120. https://doi.org/10.1161/CIRCULATIONAHA.111.060368

Shyur LF, Yang NS (2008) Metabolomics for phytomedicine research and drug development. Curr Opin Chem Biol 12:66–71. https://doi.org/10.1016/j.cbpa.2008.01.032

Smedsgaard J (2007) Analytical tools. In: Villas-Bôas S, Roessner U, Hansen MAE, Smedsgaard J, Nielsen J (eds) Metabolite analysis: an introduction. Wiley, Hoboken, NJ, p 95

Srivastava P, Singh M, Devi G, Chaturvedi R (2014) Herbal medicine and biotechnology for the benefit of human health. In: Verma AS, Singh A (eds) Animal biotechnology: models in discovery and translation. Academic, San Diego, pp 563–575. https://doi.org/10.1016/B978-0-12-416002-6.00030-4

Sugimoto M, Kawakami M, Robert M, Soga T (2012) Bioinformatics tools for mass spectroscopy-based metabolomic data processing and analysis. Curr Bioinformatics 7:96–108. https://doi.org/10.2174/157489312799304431

Sumner LW (2010) Recent advances in plant metabolomics and greener pastures. F1000 Biol Rep 2:5–9. https://doi.org/10.3410/B2-7

Syahdi RR, Iqbal JT, Munim A, Yanuar A (2019) HerbalDB 2.0: optimization of construction of three-dimensional chemical compound structures to update Indonesian medicinal plant database. Pharmacogn J 11:1189–1194. https://doi.org/10.5530/pj.2019.11.184

Syed AH, Khan T (2017) SHPIS: a database of medicinal plants from Saudi Arabia. Int J Adv Comput Sci Appl 8:49–53. https://doi.org/10.14569/IJACSA.2017.080507

Taha GA, Abdel-Farid IB, Elgebaly HA, Mahalel UA, Sheded MG, Bin-Jumah M, Mahmoud AM (2020) Metabolomic profiling and antioxidant, anticancer and antimicrobial activities of *Hyphaene thebaica*. Processes 8:1–13. https://doi.org/10.3390/pr8030266

Tajidin NE, Shaari K, Maulidiani M, Salleh NS, Ketaren BR, Mohamad M (2019) Metabolite profiling of *Andrographis paniculata* (Burm. f.) Nees. young and mature leaves at different harvest ages using 1H NMR-based metabolomics approach. Sci Rep 9:16766. https://doi.org/10.1038/s41598-019-52905-z

Tota K, Rayabarapu N, Moosa S, Talla V, Bhyravbhatla B, Rao S (2013) InDiaMed: a comprehensive database of Indian medicinal plants for diabetes. Bioinformation 9:378. https://doi.org/10.6026/2F97320630009378

Turi EC, Murch SJ (2013) Targeted and untargeted phytochemistry of *Ligusticum canbyi*: indoleamines, phthalides, antioxidant potential, and use of metabolomics as a hypothesis-generating technique for compound discovery. Planta Med 79(14):1370–1379. https://doi.org/10.1055/s-0033-1350618

Udayakumar M, Chandar DP, Arun N, Mathangi J, Hemavathi K, Seenivasagam R (2012) PMDB: plant metabolome database-a metabolomic approach. Med Chem Res 21:47–52. https://doi.org/10.1007/s00044-010-9506-z

Ueno VA, Sawaya ACHF (2019) Influence of environmental factors on the volatile composition of two Brazilian medicinal plants: *Mikania laevigata* and *Mikania glomerata*. Metabolomics 15:1–11. https://doi.org/10.1007/s11306-019-1546-x

Van Moerkercke A, Fabris M, Pollier J, Baart GE, Rombauts S, Hasnain G, Rischer H, Memelink J, Oksman-Caldentey KM, Goossens A (2013) CathaCyc, a metabolic pathway database built from *Catharanthus roseus* RNA-seq data. Plant Cell Physiol 54:673–685. https://doi.org/10.1093/pcp/pct039

Villas-Bôas SG, Noel S, Lane GA, Attwood G, Cookson A (2006) Extracellular metabolomics: a metabolic footprinting approach to assess fiber degradation in complex media. Anal Biochem 349:297–305. https://doi.org/10.1016/j.ab.2005.11.019

Wallace ED, Oberlies NH, Cech NB, Kellogg JJ (2018) Detection of adulteration in *Hydrastis canadensis* (goldenseal) dietary supplements via untargeted mass spectrometry-based metabolomics. Food Chem Toxicol 120:439–447. https://doi.org/10.1016/j.fct.2018.07.033

Wang JH, Byun J, Pennathur S (2010) Analytical approaches to metabolomics and applications to systems biology. Semin Nephrol 30:500–511. https://doi.org/10.1016/j.semnephrol.2010.07.007

Wang T, Zou Q, Guo Q, Yang F, Wu L, Zhang W (2019a) Widely targeted metabolomics analysis reveals the effect of flooding stress on the synthesis of flavonoids in *Chrysanthemum morifolium*. Molecules 24:3695. https://doi.org/10.3390/molecules24203695

Wang X, Bai J, Wang W, Zhang G (2019b) Leaf metabolites profiling between red and green phenotypes of *Suaeda salsa* by widely targeted metabolomics. Funct Plant Biol 46:845–856. https://doi.org/10.1071/FP18182

Wang J, Lou H, Liu Y, Han H, Ma F, Pan W, Chen Z (2022) Profiling alkaloids in *Aconitum pendulum* N. Busch collected from different elevations of Qinghai province using widely targeted metabolomics. Phytochemistry 195:113047. https://doi.org/10.1016/j.phytochem.2021.113047

Wen W, Li D, Li X, Gao Y, Li W, Li H, Liu J, Liu H, Chen W, Luo J, Yan J (2014) Metabolome-based genome-wide association study of maize kernel leads to novel biochemical insights. Nat Commun 5:1–10. https://doi.org/10.1038/ncomms4438

Williamson EM (2001) Synergy and other interactions in phytomedicines. Phytomedicine 8:401–409. https://doi.org/10.1078/0944-7113-00060

Wolfender J, Marti G, Thomas A, Bertrand S (2015) Current approaches and challenges for the metabolite profiling of complex natural extracts. J Chromatogr A 1382:136–164. https://doi.org/10.1016/j.chroma.2014.10.091

Wrzodek C (2012) User's guide for InCroMAP: integrated analysis of microarray data from different platforms. Center for Bioinformatics Tuebingen (ZBIT), Tubingen, Germany

Wrzodek C, Eichner J, Zell A (2012) Pathway-based visualization of cross-platform microarray datasets. Bioinformatics 28:3021–3026. https://doi.org/10.1093/bioinformatics/bts583

Wurtele ES, Chappell J, Daniel Jones A, Celiz MD, Ransom N, Hur M, Rizshsky L, Crispin M, Dixon P, Liu J, Widrlechner MP, Nikolau BJ (2012) Medicinal plants: a public resource for metabolomics and hypothesis development. Metabolites 2:1032–1059. https://doi.org/10.3390/metabo2041031

Xia J, Sinelnikov IV, Han B, Wishart DS (2015) MetaboAnalyst 3.0-making metabolomics more meaningful. Nucleic Acids Res 43:W251–W257. https://doi.org/10.1093/nar/gkv380

Xiang Z, Wang XQ, Caib XJ, Zenga S (2011) Metabolomics study on quality control and discrimination of three *Curcuma* species based on gas chromatograph-mass spectrometry. Phytochem Anal 22:411–418. https://doi.org/10.1002/pca.1296

Xiong A, Yang L, Ji L, Wang Z, Yang X, Chen Y, Wang X, Wang C, Wang Z (2012) UPLC-MS based metabolomics study on *Senecio scandens* and *S. vulgaris*: an approach for the differentiation of two *Senecio* herbs with similar morphology but different toxicity. Metabolomics 8: 614–623. https://doi.org/10.1007/s11306-011-0354-8

Xu M, Wang Y, Wang Q, Guo S, Liu Y, Liu J, Tang Z, Wang Z (2020) Targeted development-dependent metabolomics profiling of bioactive compounds in *Acanthopanax senticosus* by UPLC-ESI-MS. Nat Prod Commun 15:1–11. https://doi.org/10.1177/1934578X20910553

Ya H, Li H, Liu X, Chen Y, Zhang J, Xie Y, Wang M, Xie W, Li S (2022) Profiling of widely targeted metabolomics for the identification of chemical composition in epidermis, xylem and pith of Gleditsiae spina. Biomed chromatogr. https://doi.org/10.1002/bmc.5331

Yang B, Zhang A, Sun H, Dong W, Yan G, Li T, Wang X (2012) Metabolomic study of insomnia and intervention effects of Suanzaoren decoction using ultra-performance liquid-chromatography/electrospray-ionization synapt high-definition mass spectrometry. J Pharm Biomed Anal 58:113–124. https://doi.org/10.1016/j.jpba.2011.09.033

Ye Y, Zhang X, Chen X, Xu Y, Liu J, Tan J, Li W, Tembrock LR, Wu Z, Zhu G (2022) The use of widely targeted metabolomics profiling to quantify differences in medicinally important compounds from five *Curcuma* (Zingiberaceae) species. Ind Crop Prod 175:114289. https://doi.org/10.1016/j.indcrop.2021.114289

Yingchutrakul Y, Sittisaree W, Mahatnirunkul T, Chomtong T, Tulyananda T, Krobthong S (2021) Cosmeceutical potentials of *Grammatophyllum speciosum* extracts: anti-inflammations and anti-collagenase activities with phytochemical profile analysis using an untargeted metabolomics approach. Cosmetics 8:116. https://doi.org/10.3390/cosmetics8040116

Yu C, Luo X, Zhan X, Hao J, Zhang L, Song YBL, Shen C, Dong M (2018) Comparative metabolomics reveals the metabolic variations between two endangered *Taxus* species (*T. fuana* and *T. yunnanensis*) in the Himalayas. BMC Plant Biol 18:197. https://doi.org/10.1186/s12870-018-1412-4

Yuliana ND, Jahangir M, Verpoorte R, Choi YH (2013) Metabolomics for the rapid dereplication of bioactive compounds from natural sources. Phytochem Rev 12:293–304. https://doi.org/10.1007/s11101-013-9297-1

Zamboni N (2011) 13C metabolic flux analysis in complex systems. Curr Opin Biotechnol 22:103–108. https://doi.org/10.1016/j.copbio.2010.08.009

Zhao SS, Zhong X, Tie C, Chen DDY (2012) Capillary electrophoresis-mass spectrometry for analysis of complex samples. Proteomics 12:2991–3012. https://doi.org/10.1002/pmic.201200221

Zhou Y, Shao L, Zhu J, Li H, Duan H (2021) Comparative analysis of tuberous root metabolites between cultivated and wild varieties of *Rehmannia glutinosa* by widely targeted metabolomics. Sci Rep 11:11460. https://doi.org/10.1038/s41598-021-90961-6