

Metabolomics of medicinal plants – a versatile tool for standardization of herbal products and quality evaluation of Ayurvedic formulations

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Secondary metabolites from plants provide lead molecules for drug development. Metabolomics is a modern omic-technique for comprehensive analysis of phytochemicals. Advances in mass spectrometry (MS) based platforms like GC-MS and LC-MS, helped in separation and identification of several metabolites. Such analysis can be a valuable tool for identifying potential biomolecules from medicinal plants. Despite the potential use, metabolomics data of Indian medicinal plants and spices are extremely limited. Similarly, metabolomic studies on Ayurvedic formulations, e.g. Triphala/Trikatu, are lacking. Our review emphasizes the importance of metabolomics of Indian medicinal plants, crucial for quality evaluation and scientific validation of herbal products.

Keywords: Metabolomics, medicinal plants, metabolite profiling, new molecules, quality evaluation.

Medicinal plants

MEDICINAL plants are integral components of alternative healthcare. Medicinal plants have been clinically explored and used as therapeutic sources since ancient times, as they contain diverse secondary metabolites¹. India has rich resources of therapeutically active plants, which are being explored in AYUSH systems (Ayurveda, Yoga, Unani, Siddha and Homeopathy). Despite their popularity, medicinal plants remained as sources of alternative medicine due to lack of reliable information on their phytochemical constituents and scientific validation². The huge secondary metabolite resources of medicinal plants are fascinating with unique chemical and biological features.

Ayurvedic formulations, their uses and limitations

Ayurvedic formulations are widely used for the prevention, therapy, treatment and management of diseases for better health. However, quality control and regulation of Ayurvedic formulations are still a big challenge. Ayur-

vedic drugs have traditionally been prepared as extracts of single or poly herbs, and are termed as ‘crude extracts’. There have been no deliberate attempts to isolate or purify a single chemical entity from the formulation². Although the chemical composition of these ‘crude extracts’ is not completely known, these traditional formulations contain a large number of phytochemicals with different medicinal properties, and have complex therapeutic properties³. Herbal products are a time honoured medicine used since the ancient era for treatment of various ailments in human beings. The therapeutic and phytochemical importance of herbal medicine has been established for advancement and promotion of human health but its widespread application is limited due to the low bioavailability of phytomolecules. The nature of molecule plays a crucial role in enhancing their rate and extent of absorption administered through any route. The therapeutic action of herbal drugs mainly depends on the solubility, whether it is a hydrophobic or hydrophilic compound. Mostly, the problems that arise with poor lipid soluble compounds are due to limited permeability of biomembranes. The efficacy of any bioactive compound mainly depends on the dosage form and delivery system. There are several factors which can influence the bioavailability of compounds, such as solubility, lipophilicity, first pass metabolism, dissociation constant, etc. Development of novel drug delivery system can be an effective approach to solve these problems⁴. Therapeutic effects of the Ayurvedic drugs can be improved through targeted drug delivery system in which the concentration of drugs increased at the site of action². There are several important factors for herbal drug delivery which need to be considered for better therapeutic activity and enhanced bioavailability. The potency and efficacy of phytoconstituents need to be established through several modern approaches including the pharmacokinetic profiles of the molecules for better absorption and bioavailability⁴.

Metabolomics: a component of ‘omics’ system

There is a continuous quest for new molecules from medicinal plants, as the synthetic libraries in pharmaceutical

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industry have not yielded the expected number of candidates. Various modern technologies have been explored for assessment of plant secondary metabolites. Metabolomics is a novel approach which holds tremendous promise in comprehensive profiling of secondary metabolites⁵. Metabolomics has been accepted as an important sector of post-genome science era which deals with all cellular metabolites. It is derived from transcriptomics, genomics and proteomics in providing systematic approaches to the study of biological systems. Metabolomics has become a powerful tool in drug discovery and development by identification and profiling of secondary metabolites in medicinal plants⁶.

Metabolomic study has become a powerful analytical tool in herbal medicine research for assessment of various secondary metabolites present therein⁷. It may provide a systems biology approach for target compound analysis (TCA) in medicinal plants. Multidisciplinary facets of metabolomics have been exploited. It can be explored in different fields including drug discovery and development, high-throughput screening for evaluation of plant drugs and many others⁸. Several analytical techniques and methods are required for metabolite profiling in herbal products, such as sample preparation, instrumental analysis and data processing. Metabolomics has been employed for quality evaluation, TCA and metabolite fingerprinting of Ayurvedic herbs⁹. There are various factors which affect the quality and standards of food and herbal products including their genetic condition, cultivation, collection, storage, milling and processing for final products. Exhaustive metabolomic profiling of herbal medicines is necessary for scientific validation. Metabolomics can readily help in characterization of several metabolites and chemical markers present in herbal products¹⁰.

Metabolomics of medicinal plants

Medicinal plant-based metabolomics study is of prime importance, because plants synthesize a vast number of primary and secondary metabolites and some of them have potential therapeutic importance. Several secondary metabolites ($\geq 200,000$) from plants have been explored¹¹. For example, ~ 5000 secondary metabolites have been derived from *Arabidopsis thaliana* and approximately 1500 and 2500 from microorganisms and animals respectively. Different genomics-based 'phytochemical arrays (genome, transcriptome, proteome and metabolome)' have been established for measurement and analysis of several aspects including metabolite profiling in plants (Figure 1)¹².

Plant secondary metabolites like paclitaxel (taxol), camptothecin (irinotecan, topotecan) and podophylotoxins (etoposide, teniposide), etc. have been reported to possess potential anticancer activity¹³. Hence, medicinal plants or natural products are being considered as alterna-

tive sources of finding new chemical entities (NCEs) for drug discovery and development. Aspirin (a semi-derivative compound from the *Salix alba*) is a wonder drug being used to treat pain and other complications for many years¹⁴. The ethnic uses of herbs (in the form of crude or extract) as traditional medicines are the right pathways for finding/isolating new molecules like morphine from opium and so on. In the early 19th century, this was a way of drug discovery from natural resources. In many cases of drug analysis, few of the plant secondary metabolites (single compounds) are barely detected due to low presence in plants and hence low therapeutic activity. But in case of herbs and their formulations, the biological activities can be produced synergistically due to the presence of several constituents therein. In this context, metabolomics can be used as an effective platform to understand the phytochemical basis of such therapeutically active phytoconstituents¹⁵.

Metabolomic fingerprinting can be very helpful in the field of herbal medicine for drug discovery, systems biology, gene-function analysis and various diagnostic techniques through different modern hyphenated technologies. A study involved in the characterization of a set of defined metabolites is known as 'targeted' metabolomics and usually combine NMR-MS techniques, which is applied for such types of analysis¹⁶. In this targeted metabolomics approaches, the relative concentration of approximately 200 predetermined metabolites is detected in a sample.

In case of 'untargeted' metabolomics study, the undefined and unknown metabolites from plant may be identified through LC-MS and GC-MS analysis. This can be very useful for characterization and identification of metabolites and can be helpful for evaluation of herbal medicine¹⁷. Metabolomics study has diverse fields of

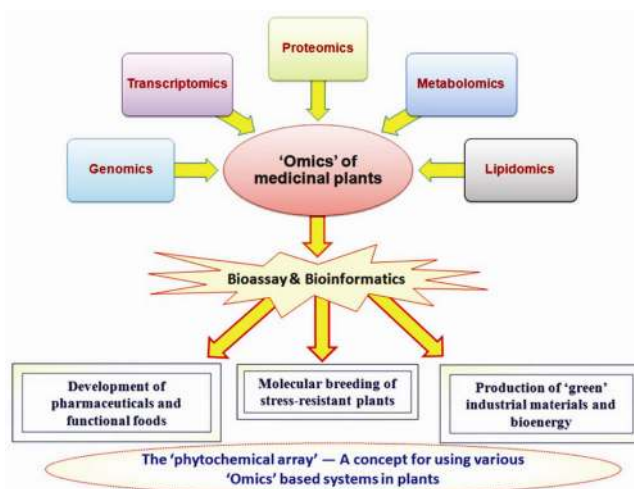


Figure 1. Various phytochemical techniques used in the field of medicinal plant research.

application. It includes metabolite fingerprinting, which can be applied in different aspects like qualitative and quantitative analysis of target compound, identification of a set of compounds, quantification of all metabolites and rapid analysis of metabolites⁸. This study has given rise to special emphasis on phytomedicine research. It can be very useful in shifting the paradigm in drug discovery and development from natural resources.

Metabolomics as a tool for quality evaluation of herbal products

Medicinal plants have been used for primary human needs for a long time in India and other countries. India has the history of one of the oldest civilizations in the world, where many traditional medicines are being practised since ancient times in healthcare management of the community at large¹⁸. The chemical composition of herbal products depends on different factors, i.e. growth of plants, environment, period of collection, drying process and methods for extraction. These factors need to be included in quality evaluation of the Ayurvedic herbs. Modern analytical techniques can be used in quality evaluation and scientific validation of medicinal plants and their formulations to fulfill the market demands. Hence, urgent attention should be given to the purity, quality and several other standardization parameters based on their major biological reference compounds¹⁹. Natural products including medicinal plants have been the focus area for research in multidisciplinary fields for development of templates of new chemical entities (NCEs). Several new lead molecules are being developed through hyphenated technology. The standardization, quality control and biological evaluation of Ayurvedic medicine will be very useful for validating the ancient but effective claims for health care. Consecutively, to improve the accuracy and consistency of herbal/medicinal plant based preparations worldwide, regulatory authorities are mandating research into new analytical methods for stricter standardization of herbal drugs. Such approaches have to be both objective and robust, and should address the reproducibility of the content of the chemical profiles⁸. In order to rationalize the use of herbal products in different forms, more particularly the extracts/marketed product in therapy as is being used nowadays, a need-based and novel concept of chemo-profiling is gaining momentum. Therefore, utmost attention is necessary for promotion and development of HM through international co-ordination and harmonization. Metabolite profiling not only identifies the metabolites relative to distribution of compounds with each other but also compares the nature of compounds.

The profiling of metabolites deals with the output of analytical techniques for qualitative and quantitative estimations of several secondary plant metabolites. These metabolites were sequentially assessed through different

statistical processes and measurements of various spectral and chromatographic peaks. These modern techniques can be utilized for comprehensive analysis of the constituents present in the plant samples²⁰. Plant metabolomics involved the measurement of genotypic and phenotypic changes in the cellular systems. These approaches have been employed for analysis of the upstream changes in genes and proteins and downstream changes in physiological function. It also analyses the complete set of compounds present in plant cell and tissues⁶. For chemical analysis, standardization through the markers and DNA fingerprinting, several fields of phytomics are very useful for assuring consistent quality and efficacy of herbal medicine⁵.

Relevance of metabolomics to Ayurvedic formulations

Metabolite fingerprinting of *Triphala* has been performed by Ponnusankar *et al.*²¹ using HPLC-PDA analysis²¹ (Figure 2). It is a classical Ayurvedic polyherbal preparation which contained dried fruits of *Embolia officinalis* Gaertn. (Euphorbiaceae), *Terminalia bellerica* Roxb. (Combretaceae) and *Terminalia chebula* Retz. (Combretaceae) in equal ratio (1 : 1 : 1 w/w/w). It has been used for gastric disorders and as a rejuvenator of the body because of its potent antioxidant compounds gallic and ellagic acid. Metabolic profile of *Trikatu* has been reported through HPLC-PDA method by Harwansh *et al.*²². It consists of three spices, namely *Piper longum* L. (Piperaceae); *Piper nigrum* L. (Piperaceae) and *Zingiber officinale* Rosc. (Zingiberaceae) in equal ratio (1 : 1 : 1 w/w/w). *Trikatu* is traditionally used to treat stomach disorders and assimilation because of its major chemical constituents piperine and 6-gingerol (Table 1). These bioactive

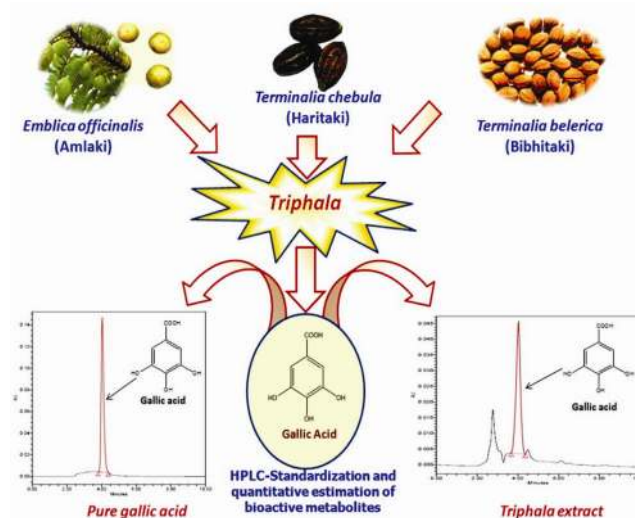


Figure 2. Metabolite profiling and standardization of Ayurvedic formulation – 'Triphala'²¹.

Table 1. Selected cases of metabolite analysis / metabolomics of plants used in the Ayurvedic formulations of 'Triphala' and 'Trikatu'

Latin name	Plant part analysed	Type of metabolite	Method	Reference	Remarks: in relation to metabolomics data
TRIPHALA					
<i>Phyllanthus emblica</i>	Fruit	Tannins and phenolics	HPLC coupled with spectroscopy	29	Fruits of two varieties from China, non-comprehensive, only phenolic compounds
<i>Terminalia chebula</i>	Fruit	Polyphenols and flavonoids	GC-MS with focus on pyrogallol	30	Non-comprehensive work; reports only on compounds like kaempferol-3-O-rutinoside flavonoid and Vitamin E
<i>Terminalia chebula</i> Retz., <i>Terminalia bellerica</i> Roxb., and <i>Phyllanthus emblica</i> L.	Fruit (1 : 1 : 1 mixture of fruit powders)	24 to 27 compounds	GCMS	31	Non-comprehensive work; 28 compounds were reported
TRIKATU					
<i>Piper nigrum</i>	Fruit (independently tested)	Terpenes and others (13 compounds)	Countercurrent chromatography, ESI-MS, NMR	32	Non-comprehensive work focus on bioactive compounds
<i>P. longum</i> L., <i>P. nigrum</i> L., and <i>P. chaba</i> H.	Fruits	Monoterpenes and sesquiterpenes	GCMS	33	Work done with essential oils
<i>Zingiber officinale</i>	Rhizome	Terpene and others (17 compounds)	GCMS	34	Only essential oils

Table 2. Metabolite analysis of some Indian medicinal plants, among the prioritized species by National Medicinal Plants Board (NMPB), India

Latin name	Plant part used in traditional medicine	Metabolites of the plant analysed*	Reference**	Remarks on metabolomics data
<i>Aconitum heterophyllum</i> Wall. ex Royle (Ateesh)	Root	Diterpenoid alkaloids	35	No data on metabolomics
<i>Aegle marmelos</i> (L.) Corr.(Bel)	Leaf, fruit and root bark	Alkaloids	36	Leaves, fruits and root bark are the important parts but metabolomic studies were carried only on fruits
<i>Andrographis paniculata</i> Wall. ex Nees (Kalmegh)	Whole plant	Andrographide (Bicyclic diterpenoid)	37	No data from India, metabolomic studies were carried in China
<i>Bacopa monnieri</i> (L.) Pennell (Brahmi)	Whole plant	Genistein 4-O-glucoside (Isoflavones)	38	One report from India on whole plant
<i>Cassia angustifolia</i> Vahl.(Senna)	Leaf	Sennosides (dianthrone O-glycoside)	39	No data on metabolomics
<i>Commiphora wightii</i> (Arn.) Bhandari (Guggal)	Gum resin	Non-targeted metabolite profiling – primary and secondary metabolites (NMR and GC-MS)	40	Gum resin is the important part but metabolomic studies were carried on leaves, stem, roots, latex and fruits
<i>Embelia ribes</i> Burm f. (Viavidang)	Fruit	Embelin (Benzoquinone)	41	No data on metabolomics
<i>Embelia officinalis</i> Gaertn (Amla)	Fruit	Phenolics	42	Fruit is the important part, but metabolomic studies were carried on leaves
<i>Gymnema sylvestris</i> R. Br. (Gudmar)	Leaf	Gymnemic acid (Pentacyclic triterpenoid)	43	No data on metabolomics
<i>Ocimum sanctum</i> L. (Tulsi)	Leaf	Eugenol by LC-MS (Phenolic acid)	44	Metabolomic studies were carried on Leaf
<i>Piper longum</i> L. (Pippali)	Fruit	Catechin (Phenolic compound)	45	Fruit is the important part, metabolomic studies were carried on fruit, leaf and root
<i>Rauwolfia serpentina</i> Benth. ex Kurz (Sarpagandha)	Root	Reserpine (Alkaloid)	46	Root is the important part, but metabolomic studies were carried on seed
<i>Santalum album</i> L. (Chandan)	Oils from stem	Phenylpropanoids	47	No data from India, metabolomics studies on essential oils of seed was reported from Italy
<i>Withania somnifera</i> (L.) Dunal (Ashwagandha)	Root	Withanolides (Steroidal lactones)	48	Root is the important part, but metabolomics studies were carried on fruit

**Most of the analytical studies are quite limited to either a few molecules or a few classes of compounds. **Where ever available, references from Indian institutes/authors are cited.

compounds have therapeutic potential including anti-inflammatory activity. The metabolic profiling of Ayurvedic formulations helps to make strong scientific evidence which increases their acceptability by the scientific community. Metabolic characterization of several *Panax* species (*Panax ginseng*, *Panax notoginseng* and *Panax japonicas*) have been analysed by UPLC-QTOF-MS. Various secondary metabolites, e.g. chikusetsusaponin IVa, ginsenoside R0, ginsenoside Rc, ginsenoside Rb1, ginsenoside Rb2 and ginsenoside Rg2 have been identified and reported through this technique²³. Phytoconstituents of *Tussilago farfara* L. have been explored by metabolomics approach and have different pharmacological activities (antitussive and expectorant). Chlorogenic acid, 3,5-dicaffeoylquinic acid and rutin have been characterized by ¹H NMR conjugated spectrometric techniques which are responsible for anti-tussive and expectorant activities²⁴.

Gross metabolic profiling in *Urtica* showed that phenolic acids were responsible for potent inhibition of the inflammation response²⁵. Huang-Lian-Jie-Du-Decoction (HLJDD) is an antipyretic and detoxifying product in traditional Chinese medicine (TCM). It contains *Radix scutellariae*, *Fructus gardenia* which exhibit neuroprotective effects. To comprehensively and holistically assess its therapeutic effect on ischemic stroke, a novel integrative metabolomics approach was applied²⁶. In another metabolomics study, efficacy of xiaoyaosan on rat model of chronic unpredictable mild stress was investigated and it is a valuable tool to study the therapeutic efficacy and potential biomarkers of complex prescriptions²⁷. A metabolomics screening of capsules containing *Artemisia afra* and *A. annua* was performed by NMR-PCA analysis. The results showed that artemisinin was confirmed in *A. afra* and not in *A. annua*. In addition, the concentration of artemisinin in the plant material was determined with a sensitive LC-MS method. Metabolite analysis of some Indian medicinal plants, in particular, the species, prioritized by National Medicinal Plants Board (NMPB) is listed Table 2. This analysis indicated that, even if the company has used *A. annua* in their capsules, the dosage of artemisinin will be far too low to be effective²⁸. Considering the large diversity of plants based on their cultivars and uses, there is an urgent need to prepare a metabolite library which would enable the quantitative and qualitative profiling of metabolome more quickly and efficiently.

Conclusions

Plant metabolomics provides a comprehensive understanding of the spectrum of phytochemical constituents of plants. The metabolomics approach is being exploited in a wide range of applications including medical science, synthetic biology, Ayurvedic medicine and predictive

modelling of plant/animal/microbial systems. The working principle of metabolomics deals with sample preparation, separation of compounds, identification, data processing and finally analysis. Due to the development of effective technology for separation and identification of metabolites, the technique of metabolomics is fast becoming a versatile tool for exploitation of medicinal plants, for biomarker-driven drug discovery and development. Measurement and analysis of metabolites can be a precise and potentially valuable technology for identifying biomarkers. Metabolomics offers a promising approach to plant metabolite fingerprinting and such studies are urgently needed for better understanding of Indian medicinal plants.

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