

Spectroscopic Techniques and Their Application in Metabolic Profiling of Wood: A Minireview

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Abstract

GC/LC-MS and NMR spectroscopy have increasingly occupied a central position in the methodologies developed for metabolic analysis. This brief review deals with introduction of terminology used in metabolic analysis. Gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance spectroscopy (NMR) commonly used in metabolic analysis and metabolic profiling of wood and wood based material by spectroscopic techniques. Apart from the description of the different methods, this review will try to direct the reader to the main approaches for analysis of metabolites in wood science.

Keywords: Metabolic profiling, Wood, GC/LC-MS, NMR Spectroscopy.

1. Introduction:

The widespread use of spectroscopic techniques for metabolic studies (metabolomics), along with its exceptional capacity to resolve molecules in complex metabolite mixtures, have made these techniques as preferred technology platform for metabolic analysis.^[1] These techniques have a number of unique advantages in particular, simple, non-biased, easily quantifiable, high throughput, economic and permit the molecular level identification. Metabolic analysis of steroids, acids, and neutral and acidic urinary drug were analysed by chromatography coupled spectrometric technologies in the early 1970's.^[2, 3] Subsequently, the metabolic profiles concepts were used to screen, diagnose, and access health in the 1980's.^[4, 5] However, it was not until the early 1990's used as a diagnostic technique in plant,^[6-8] and after 2000,s to till date, it's also extensively used in plant science.^[9]

Metabolomics is a complicated interdisciplinary science that consists of a diverse set of approaches, including bioscience, analytical chemistry, natural product chemistry, chemometrics, and informatics.^[10, 11] It further classified in different approaches, "Metabolic profiling" is the identification and quantification of metabolites related through their metabolic pathway(s) or similarities in their chemistry while "targeted metabolite profiling" or "metabolite target analysis" is a qualitative and quantitative analysis of one or a few pre-defined metabolites.^[10] The "Metabolite fingerprinting" is a rapid and high-throughput method where metabolite profile is obtained from samples without their identification and quantification.^[11] Metabolomic studies make use mostly of techniques which rely on chromatographic separation of metabolites using either gas chromatography (GC) or liquid chromatography (LC) coupled to mass spectrometry (MS) to analyze complex mixtures of extracted metabolites. While nuclear magnetic resonance spectroscopy (NMR) metabolite fingerprinting approaches provides a valuable metabolite signature of complex plant extracts combined with unbiased quantitative accuracy.^[12]

2. The Process of Metabolomic Analysis

Metabolomic analyses consist of a sequence of steps, including sample preparation, metabolite extraction, derivatization, metabolite, separation, detection, and data treatment (Fig. 1). The detection and data analyses have been essential steps in all reported metabolomics studies. The metabolic analysis mainly includes GC/LC-MS, and NMR spectroscopic techniques for detection and data analysis (Figure 1 and Figure 2).

3. Commonly Used Techniques in Chemical Profiling

3.1 Gas Chromatography-Mass Spectrometry (GC-MS): Gas chromatography (GC) coupled to MS has been extensively used in metabolite analysis because of its high separation efficiency that can resolve very complex metabolite mixtures. In addition, it is the easier, complete and reliable identification of compounds using an automated mass spectral deconvolution and identification system (AMDIS).^[13]

It can be used as a low-cost method to analyze a wide range of volatile compounds, further through chemical derivatization it is also possible to analyze many semi volatile metabolites. More recently, the introduction of GC-TOFMS systems offered an attractive supplement to quadrupole instruments and provided greater mass accuracy. TOF instruments also provide high scan speeds that are compatible with ultrafast GC-MS

and the potential to profile complex mixtures in less time.^[14] Similarly, GC coupled to multistage MS-MS [e.g., MS-MS or MS_n (triple-quadrupole and ion traps)] instruments enabled the acquisition of very detailed fragmentation information, a higher level of molecular specificity, and higher selectivity.^[15]

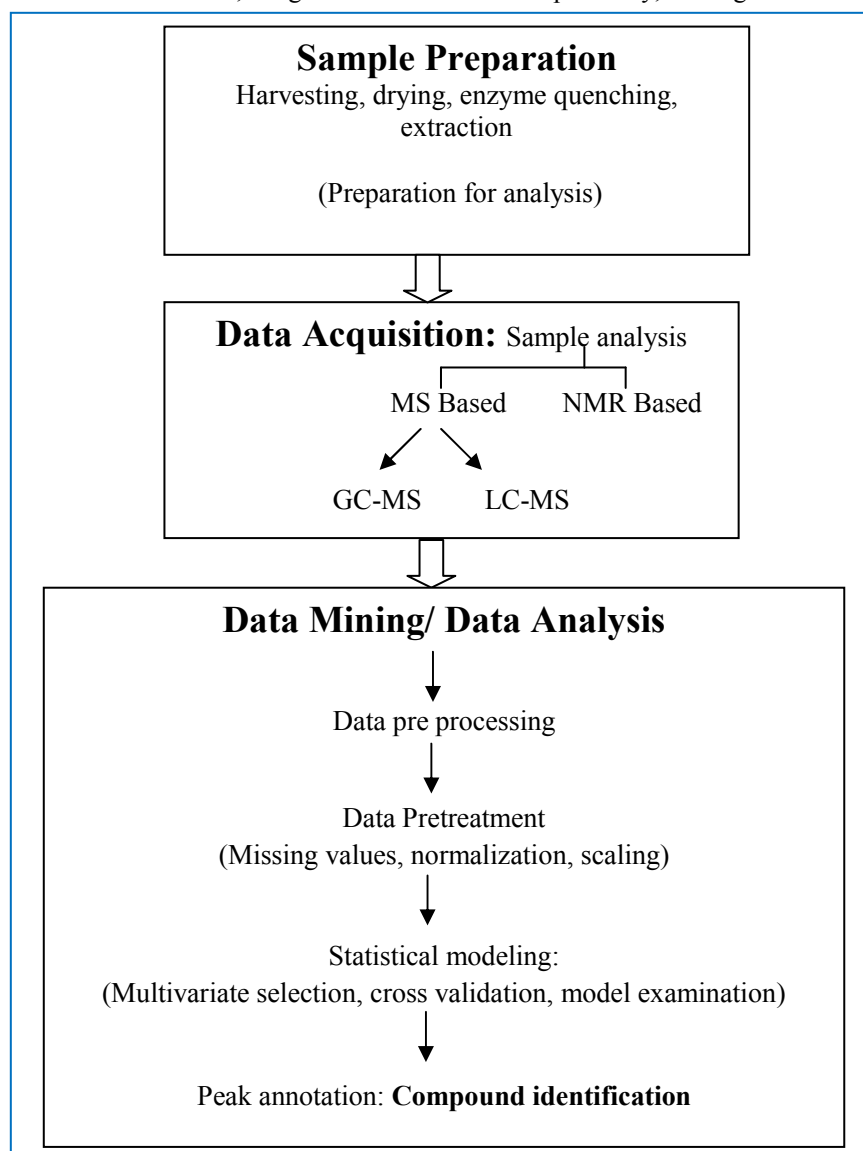


Figure 1. Flowchart for metabolic analysis: main three steps are-(i) sample preparation; (ii) data acquisition and (iii) data mining/data analysis

The method of GC-MS in metabolic analysis application can be divided into two groups: (i) naturally occurring volatile metabolites; and (ii) preparation of non volatile metabolites into volatile through derivatization.^[16] Volatile metabolites are compounds that are usually secreted by the cell/organism and that have a boiling point lower than 300 °C and they can be analyzed by GCMS without any derivatization. The volatiles mainly include ketones, aldehydes, alcohols, esters, furan and pyrrole derivatives, other heterocyclic compounds, isocyanates, isothiocyanates, sulphides, some lipids, and hydrocarbons with 1-12 carbons. The semi volatile and non-volatile metabolites must be chemically modified to volatile prior to GCMS analysis. Derivatization steps involve a decrease of their boiling points, which also increases their stability at high temperatures. Most intracellular metabolites and several extra cellular metabolites like sugars, sugar-phosphates, sugar alcohols, organic acids, amino acids, lipids, peptides, long-chain alcohols, alkaloids, amines, amides, etc. are semi-volatile metabolites.

3.2 Liquid Chromatography-Mass Spectrometry (LC-MS): Liquid chromatography is a fundamental separation technique in the life sciences and related fields of chemistry. Unlike gas chromatography, which is unsuitable for nonvolatile and thermally fragile molecules, liquid chromatography can safely separate a very

wide range of organic compounds, from small-molecule drug metabolites to peptides and proteins.¹¹⁷ Till date, in LC-MS based metabolic analysis, a wide variety of mass spectrometers, e.g. quadrupole-time of flight (Q-TOF), quadrupole-ion trap (Q-Trap), Fourier transform ion cyclotron resonance (FT-ICR) and Orbitrap MS, have been used with electrospray ionization (ESI) source.^{118, 191}

LC-MS is very commonly used in pharmacokinetic studies, drug development of pharmaceuticals and proteomics/metabolomics.¹²⁰⁻²²¹ It is frequently used in different stages of drug development, such as peptide mapping, glycoprotein mapping, natural products dereplication, bioaffinity screening, *in vivo* drug screening, metabolic stability screening, metabolite identification, impurity identification, quantitative bioanalysis, and quality control.^{120, 211} It is most commonly used for proteomic analysis of complex samples where peptide masses may overlap. Samples of complex biological fluids like human serum may be run in a modern LC-MS/MS system and result in over 1000 proteins being identified.¹²²¹

3.3 Nuclear Magnetic Resonance (NMR): NMR spectroscopy measures the resonances of magnetic nuclei such as ^1H , ^{13}C and ^{15}N that interact with an external magnetic field.¹²³¹ It offers non-invasive structural analysis of metabolites in crude extracts, cell suspensions, intact tissues or whole organisms allowing *in vivo* analysis.¹²⁴¹ The NMR spectra are unique and specific for each single metabolite¹²⁵¹ and can be used to identify metabolites of biological origin of which no a-prior knowledge is needed.¹²⁶¹ This method provides simultaneous access to both qualitative and quantitative information requires minimal sample preparation, highly reproducible and high sample throughput.¹²⁷¹

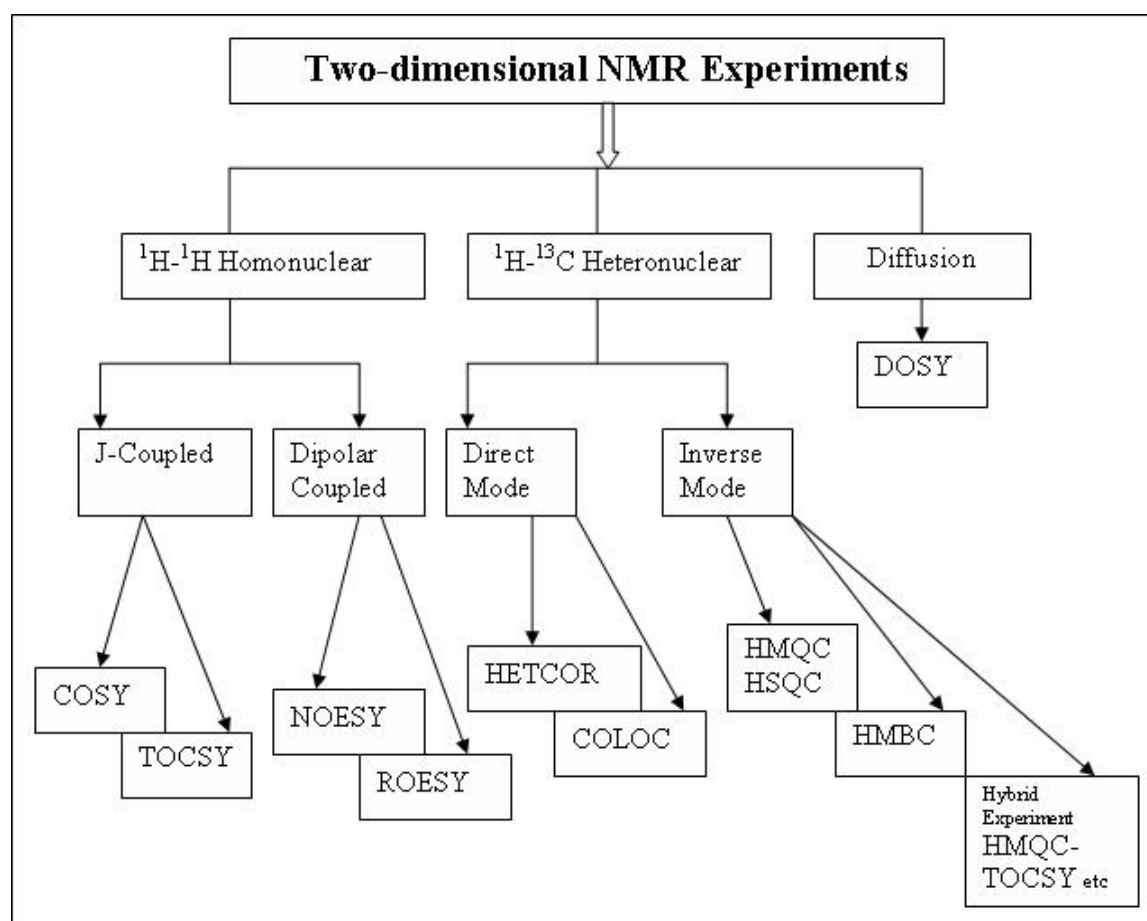


Figure 2. Flowchart for two dimensional NMR experiments

To facilitate the identification of metabolites databases of NMR spectra of common plant metabolites are needed. It contributed to the fast, convenient and effective metabolomics tool, despite the low intrinsic sensitivity. Further to enhance sensitivity, selectivity and spectral resolution two-dimensional NMR is developed which includes correlated spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) spectroscopy, heteronuclear single quantum coherence (HSQC) spectroscopy and heteronuclear multiple bond coherence (HMBC) spectroscopy improve metabolite identification by providing information on the relationship between the signals from two different nuclei.^{126, 271}

Several metabolic analysis/studies have been carried out in wood and wood based material. Here we focus on review of metabolic analysis of wood and wood based materials, including special interest for the economically important timber species of *Pterocarpus* Genus.

4. Metabolic Profiling of Wood and Wood Based Material

Chemical profiling and fingerprinting of plant material including wood by using GC/LC-MS, NMR has been reported as a tool to distinguish closely related species.^[28, 29] G.C. Kite et al. reported a neoflavonoid as chemical marker for identification of Brazilin rosewood by using LC-MS to analyze methanol extracts of xylaria specimens.^[30] Raza Murad Ghalib et al have been reported chemical profiling of the bark and wood of *Sonneratia caseolaris* (L.) by GC-TOFMS.^[31] Overall, thirty-two compounds from the bark and twenty-eight compounds from wood have been detected. Sixteen constituents have been found to be common in both the extracts. Liang Zhu et al., demonstrate that desorption atmospheric pressure chemical ionization mass spectrometry (DAPCI-MS) is a valuable tool for differential analysis of untreated camphor wood products with sufficient sensitivity and high throughput.^[32] Choi YH et al., reported the metabolomic analysis of 11 *Ilex* species, *I. argentina*, *I. brasiliensis*, *I. brevicuspis*, *I. dumosavar. dumosa*, *I. dumosa var. guaranina*, *I. integerrima*, *I. microdonta*, *I. paraguariensis var. paraguariensis*, *I. pseudobuxus*, *I. taubertiana*, and *I. theezans*, by NMR spectroscopy and multivariate data analysis.^[33] The metabolite arbutin was found to be a biomarker for *I. argentina*, *I. brasiliensis*, *I. brevicuspis*, *I. integerrima*, *I. microdonta*, *I. pseudobuxus*, *I. taubertiana*, and *I. theezans*. This reliable method based on the determination of a large number of metabolites makes the chemotaxonomical analysis of *Ilex* species possible.

The past two decades have shown renewed interest on *Pterocarpus* species as highly impressive tree.^[34] *Pterocarpus* species are found to be rich in isoflavonoids, terpenoids, triterpene^[35] and related phenolic compounds, β -sitosterol, lupeol, (-) epicatechin.^[36] Yoganarasimhan et al. also observed pterocarpol, santalins A and B, pterocarptriol, ispterocarpolone, pterocarpo-diolones and kino-tannic acid in the wood of *P. santalinus*.^[37] Arokiyaraj and coworker reported methanol from the leaves of this plant^[38] while ethanol was reported in the extract from stem bark by Kameswara Rao and coworker.^[39]

The general occurrence of pterocarpin, homopterocarpin, and pterostilbene, compounds were reported in the heartwood of *Pterocarpus dalbergioides*, *P. macrocarpus*, *P. soyauxii*, and *P. tinctorius*^[40] were reported the concentration of rare earth elements (REE), thorium and uranium by inductively coupled plasma mass spectrometry (ICP-MS) in the plant species, *Pterocarpus santalinus*, *P. marsupium* and *P. dalbergioides*, along with the soils on which they were growing. They found in all tree species, the concentration of REEs were higher in the heartwood than the leaves. The heartwood of *P. santalinus* accumulated larger quantities of uranium and thorium than the other two species. The heartwood of *P. santalinus* accumulated larger quantities of uranium (concentration of 1.22 ppm) and thorium (mean value of 2.57 ppm) than the other two species.^[41] G. S. Viswanadha et al., investigated the phytochemicals of n-hexane and methanol extract of *P. dalbergioides* heart wood and reported five isoflavonoids.^[42] These isoflavonoids were identified as pterocarpin, homopterocarpin, formononetin, (+)-maackian and santal. The structures of the compounds were unambiguously elucidated by the spectral analysis.

Wood or wood material identification is one of the tools necessary to combat smuggling, illegal logging and trade.^[43] Wood anatomy has been used successfully to identify most of the wood specimens up to species level.^[44] But anatomical tool has limitations in providing a species level identification for few of the economically important timber species.^[45, 46] Although efforts have been made to identify wood using non-anatomical characteristics such as near infrared spectroscopy,^[47] DNA identification of Currently Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) taxa,^[48, 49] identification of extractives,^[50] but these are either not practicable or not satisfactory for full proof detail up to species level for all species. So the spectroscopic techniques (using GC/LC-MS and high field NMR spectroscopy) and statistical analysis may be a supporting tool for identification of wood and wood based material.

5. Conclusion

Metabolic analysis by GC/LC-MS and NMR spectroscopy has shown to be an important tool for distinguish closely related species, chemical analysis of wood extractives; and identification of adulterant in wood based products. Recent studies suggest that the potential of metabolic analysis can be expanded as the tool to combat smuggling, illegal logging and trade wood and wood based material through their identification. However, the development of a wood based metabolome database is needed in order to facilitate compound identification and the development of informative metabolomics which can be the potential of discriminative, predictive, and informative analyses to solve the important problems of the forest department, custom and revenue department, and wood based industries.

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