



Nuclear magnetic resonance (NMR) for plant profiling and disease metabolomics-fast tracking plant based drug discovery from northern India

Mohammad Faizan Bhat, Reyaz Hassan, * Mubashir H Masoodi

Natural Products Research Lab, Department of Pharmaceutical Sciences, Srinagar, Jammu and Kashmir, India

Abstract

Northern India is home to thousands of plant species and sadly, only a little percentage of them have been explored for medicinal properties. This is mostly because the metabolites present in these plants have not been scientifically investigated, mainly because of the prolonged and cumbersome nature of such analysis. Furthermore, the effect of these plant metabolites on specific diseases faces the same limitations of complexity and agility. Nuclear Magnetic Resonance (NMR) is not only an invaluable tool in chemistry but with proper modifications this powerful tool can also be rendered useful for Plant metabolite profiling and disease metabolomics; with high fidelity and speed. Metabolite Fingerprinting of plants through NMR is fast, convenient, and an effective tool for discriminating between groups of related samples and it also identifies the most important regions of the spectrum for further analysis. After rapid metabolite profiling of plants, the most prominent constituents can be further evaluated and isolated with more efficient techniques such as HPLC and LC-MS and then these isolated metabolites can be tested for their effect on diseases through NMR disease metabolomics. This technique has substantial advantage over other metabolomics techniques, such as; no prior purification is needed, both hydrophilic and lipophilic metabolites can be detected, quantitative analysis is viable, very fast, requires small sample amount, is non-invasive/non-destructive and most importantly, reproducible. Overall, NMR is an indispensable tool and is crucially needed to tap into enormous plant based drug discovery potential of medicinally bio-diverse Northern India.

Keywords: NMR, nuclear magnetic resonance, plant based drug discovery, metabolomics, plant profiling

Introduction

Plants and herbs have been used for the treatment and prevention of disease since centuries. WHO reported that the global use of plant based drugs is still significant, especially in rural areas where about 80% of treatments are carried out through herbs and their derived products [1]. Out of thousands of plant species present in the world, only about 20,000 have been screened for their medicinal properties. Plants and their derived products are used as medicines per se or the chemical metabolites present in them are used for primary scaffold and then developed through medicinal chemistry based drug discovery approaches for a more precise and reproducible treatment [2, 3]. However, most of the activities of plants till date are because of only a few classes of chemical compounds present in a plant, these include; glycosides, alkaloids, flavonoids, sterols and tannins [4, 5]. Therefore, a need for isolation, characterization and sometimes just only qualitative analysis for the presence of these major metabolites becomes extremely necessary to fast track proper profiling of plants and then to eventually promote them as leads in plant-based drug discovery. Metabolomics is a term used interchangeably with metabolic profiling or metabolic fingerprinting, all unambiguously meaning the same "identifying and quantifying metabolites in reference to standard known molecules" [6]. While chromatographic techniques like HPLC coupled with MS form the basis of most medicinal profiling techniques, a shift to NMR for fast and accurate metabolomics is severely needed to tap into enormous medicinal potential of

Northern-India, especially Himalayan invasive species; where a lot of potent medicinal metabolites have a high tendency to be naturally synthesized.

Plant Metabolomics by NMR

NMR has significant advantages over other techniques for metabolite fingerprinting. Firstly, it is non-destructive and the samples can be analyzed from tissues, cell-suspension, whole plant and even purified metabolites and extracts [7, 8]. Secondly, depending on the type of metabolic problem at hand, NMR offers an array of detection schemes that can be precisely modified to meet the needs of sample and problem in question [9]. Third, labeling of isotopes of low natural abundance allows for detection of these atoms, which eventually allows for exploration of metabolic pathways [7, 10, 11]. NMR also provides a broader and macroscopic view of plant metabolome without the debilitating sample preparation limitations [12]. Since, almost all the biologically important metabolites contain protons, ¹H NMR serves as an obvious choice for most of metabolomics studies. NMR can not only be used alone for profiling but also combined with MS, Chromatography and data analysis software techniques like MVDA to make plant metabolomics more agile and precise.

Disease Metabolomics by NMR

Once a plant has been properly metabolite profiled, the compounds can be isolated and further characterized. After, characterization the isolated compounds can be tested on

animals or *in vitro* parameters. When NMR is used for rapid detection of metabolites in animals to study mechanisms and magnitude of positive effect, the process is called NMR disease metabolomics. Quite a few NMR techniques can be used for disease metabolomics studies, the easiest being one-dimensional ^1H NMR that is able to detect compounds above micro-molar concentrations. Quantitative analysis is also viable through this technique by employing area per proton analysis, LCMoDel or OPLSA-DA strategy ^[13, 14]. One particular and most important advantage of this method is that the sample required for analysis is very small, a factor that is very favorable for disease metabolomics because of the limited sample volume ^[15]. Other more complicated but more precise disease metabolomics studies include Two-Dimensional techniques, Solid-State NMR (ssNMR), and ^{19}F NMR ^[16].

Conclusion

Nuclear Magnetic Resonance is clearly an indispensable tool for plant-based drug discovery because of the accuracy, precision, and speed of metabolic profiling of the plants and then the screening of the metabolite's effects on disease through the same technique with slight modifications.

References

- Gaur R, Tiwari J. Some little known medicinal plants of Garhwal Himalaya: An ethnobotanical *study*. Medicinal and Poisonous Plants of the Tropics (ed. Leewenberg). Netherlands. 1987, 139-192.
- Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine. 2006; 27(1):1-93.
- Dolabela MF. *et al.* Plant-derived antimalarial agents: new leads and efficient phythomedicines. Part I. Alkaloids. 2009.
- Khazir J, *et al.* Role of plants in anticancer drug discovery. Phytochemistry Letters. 2014; 7:173-181.
- Roy SK. *et al.* Determination of negundoside and agnuside in *Vitex negundo* by qNMR and method validation. Journal of analytical chemistry. 2015; 70(5):639-646.
- Goodacre R, Anklam E. Fourier transform infrared spectroscopy and chemometrics as a tool for the rapid detection of other vegetable fats mixed in cocoa butter. Journal of the American Oil Chemists' Society. 2001; 78(10):993-1000.
- Kruger NJ, Ratcliffe GR, Metabolic organization in plants: a challenge for the metabolic engineer. Advances in plant biochemistry and molecular biology. 2008; 1:1-27.
- Ratcliffe R, Shachar-Hill Y. *Revealing metabolic phenotypes in plants: inputs from NMR analysis*. Biological Reviews. 2005; 80(1):27-43.
- Ratcliffe RG, Roscher A, Shachar-Hill Y. Plant NMR spectroscopy. Progress in Nuclear Magnetic Resonance Spectroscopy. 2001; 39(4):267-300.
- Bacher A. *et al.* Elucidation of novel biosynthetic pathways and metabolite flux patterns by retrobiosynthetic NMR analysis. FEMS Microbiology reviews. 1998; 22(5):567-598.
- Roberts JK. NMR adventures in the metabolic labyrinth within plants. Trends in plant science. 2000; 5(1):30-34.
- Ward JL, Baker JM, Beale MH. Recent applications of NMR spectroscopy in plant metabolomics. The FEBS journal. 2007; 274(5):1126-1131.
- Wishart DS. *Quantitative metabolomics using NMR*. TrAC Trends in Analytical Chemistry. 2008; 27(3):228-237.
- Schicho R. *et al.* Quantitative metabolomic profiling of serum, plasma, and urine by ^1H NMR spectroscopy discriminates between patients with inflammatory bowel disease and healthy individuals. Journal of proteome research. 2012; 11(6):3344-3357.
- Ferrara M. Future perspectives for metabolomics in nutrition research: a nutritionist's view, in Metabolomics as a Tool in Nutrition Research. 2014, 231-236.
- Kruk J. *et al.* NMR techniques in metabolomic studies: A quick overview on examples of utilization. Applied magnetic resonance. 2017; 48(1):1-21.