Can NMR metabolomics play a role in nutritional healthcare?

Somenath Ghatak[1], Prashant K Rai[1], Sakshi Sharma[1], Gaurav Sharma[1], and Rama Jayasundar[1]

[1]*Department of NMR, All India Institute of Medical Sciences, New Delhi, Delhi, India*

Introduction

According to WHO, more than 100 million people will suffer from diabetes by the end of this century and 90% of them with the type strongly associated with lifestyle factors such as inappropriate diet[1]. In healthcare, nutrition is hence recognised to be as important as therapeutics and is gaining attention as a scientific discipline. In this context, plants as natural repository of nutrients are increasingly looked upon from the perspective of functional foods and nutraceuticals, and phytochemical characterisation of nutraceutically and therapeutically important foods using spectroscopic techniques is beginning to play an important role. The aim of this study is to do a multitechnique phytochemical analysis of functional foods and also assess their antioxidant potential, which has both nutraceutical and therapeutic roles.

Materials and Methods

Twelve known nutraceuticals were studied : *Annona squamosa* (fruit), *Asparagus racemosus* (stem), *Cinnamomum tamala* (leaf), *Cuminum cyminum* (seed), *Emblica officinalis* (fruit), *Glycyrrhiza glabra* (root), *Holostemma ada kodien* (fruit), *Musa paradisiaca* (fruit), *Piper nigrum* (seed), *Punica granatum* (fruit), *Vitis vinifera* (fruit) and *Zingiber officinale* (rhizome). The samples were prepared as 10% aqueous solution for all investigations.

NMR: Water suppressed 1D proton NMR spectra were recorded in a 700 MHz spectrometer (Agilent, USA) using a 14 sec relaxation delay, 32 scans, 12 ppm spectral width and 32K data points. Deuterated TSP in a coaxial insert was used as an external reference. Peak assignments were carried out using 2D NMR. Principle Component Analysis (PCA) was performed on the spectral data, which were binned and bucketed at 0.04 ppm intervals using MestReC and Unscrambler X10.2.

Fourier Transformed Infra-Red : FTIR 660 (Agilent, USA) was used to scan the sample in the 750-10,000 nm range in the Attenuated Total Reflection (ATR) mode.

Laser induced breakdown spectroscopy (LIBS) : Elemental analysis was carried out using a 4-channel spectrometer (Ocean optics LIBS 2000+) in the 200-1100 nm wavelength range. The laser energy employed was 175 mJ and repetition rate was of 2 Hz[2].

Antioxidant assay: DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay was carried out to assess the antioxidant potential of the nutraceuticals and IC_{50} value was calculated.

Results and Discussion

Antioxidant activity: All the plant parts showed antioxidant potential with the maximum and minimum activities shown by *Emblica officinalis* (IC_{50} - 1.92 mg/ml) and *Cuminum cyminum* (IC_{50} - 13.11mg/ml), respectively.

NMR: Figures 1a-1c show representative data respectively from proton 1D & 2D NMR and LIBS. Resonances from primary metabolites such as carbohydrates (α and β-glucose) and amino acids (leucine, isoleucine, valine, methionine, proline) and secondary metabolites such as flavonoids, flavonol glycosides, polyphenols were observed in most of the nutraceuticals although with varying intensities. These phytochemicals, either alone or in combination, have both nutraceutical (antioxidants) and therapeutic (reducing oxidative stress and providing antidiabetic phytochemicals) roles. PCA performed on the NMR data revealed two groups based on the IC_{50} values. Nutraceuticals such as *Vitis vinifera, Punica granatum, Musa paradisiaca* and *Annona squamosa,* all of which exhibited high antioxidant potential mapped close to each other and the rest were distanced from this group.

FTIR: The functional groups observed are: OH (1300-1420 cm^{-1}) and C=O (1661-1760 cm^{-1}) [carbohydrates], R-COOR (1773 cm^{-1}), R-NH$_2^+$ (2320-2380 cm^{-1}) [amino acids]; OH (3594-3620 cm^{-1}) [alcohols/phenols]. Many of these groups, also observed in the NMR spectra, are abundantly present in secondary metabolites, known to be associated with high antioxidant activity[2]. The information from NMR and FTIR on amino acids, carbohydrates and polyphenol, hence complement each other.

LIBS: Elements such as Mg, Ca, H, O, C and N were observed in the spectral range from 200-900 nm in all plants with varying amounts (Fig. 1c). Interestingly, high Mg and Ca peaks were seen in nutraceuticals with more antioxidant potential[2].
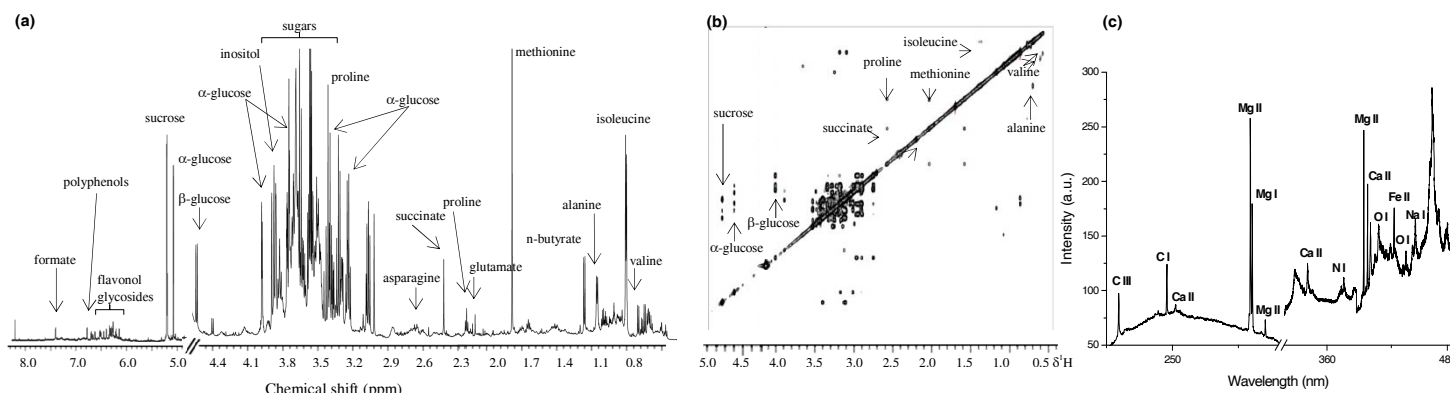

Figure 1: Spectroscopic profiling of *Zingiber officinale*: (a) 1D ^{1}H NMR (b) 2D NMR plot (c) LIBS

Conclusion

Fueled by growing recognition of the association between nutrition and disease prevention, scientific research on functional foods and nutraceuticals is gaining importance. This study presents a comprehensive spectroscopic phytochemical metabolomics of nutraceutical plants using three different techniques each one complementing the other. Spectroscopic profiling combined with multivariate analysis can provide quick information on the nutritional and therapeutic properties of functional foods. Further indepth studies are underway and in a larger sample size.

References

(1) WHO, Geneva, http://www.who.int/mediacentre/factsheets/fs312/en/index.html.
(2) Mehta M, Rai PK, Rai NK, et al. LIBS-based detection of antioxidant elements in seeds of *Emblica officinalis*. Food Biophy. 2010; 5(3): 186-192.