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Dietary Supplements: Towards the Development of a Quality and 'Fingerprinting' Identification Tool Using Nuclear Magnetic Resonance (NMR)

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Introduction

Efforts are underway to establish a non-targeted quality control screen for plant extracts used as dietary supplements. Such a screen will improve the safety and efficacy to the consumer and meet the 2007 FDA ruling requiring cGMP compliance to ensure the quality of dietary supplements. Raw material from plant vary widely due to agricultural, harvesting, or processing methods. Intentional adulteration or non-intentional adulteration may also occur in the commercial product. To add further confusion, plant material used in dietary supplements is often identified generally by the common name (i.e. ginseng) and is not distinguished according to the species (i.e. *Panax ginseng* and *Panax quinquefolius*) or plant part (i.e. leaf, fruit, root, etc.) when used in the commercial product. The large variation in potential components, composition and adulterants suggests that a non-targeted 'fingerprinting' method is needed to characterize a dietary supplement. NMR spectroscopy offers such capabilities and therefore is studied here as a potential screening tool.

In this work we present our progress towards the development of a NMR based quality and fingerprinting screen to provide information such as:

- relative or absolute quantity of key components present in the crude extract
- origin
- plant part
- information on possible adulterants

Methods

Ethanol-water extracts were processed from pine bark, grape seed, blueberry leaves, skullcap (above ground parts) and germander (above ground parts). Methanol extracts were processed from ginseng (root). The extracts were filtered and dried. 25 mg* of each crude extract was dissolved in 0.6 mL of DMSO-d_6. NMR spectra recorded using a 600 MHz Bruker AVANCE III Spectrometer equipped with a 5 mm TCI CryoProbe. NMR data was acquired on all extracts using a noesy1d pulse program (acquisition time per spectrum was 3 minutes). For some extracts a HSQC experiment was also acquired (58 minutes per spectrum).

*Note: much less sample could be used for this analysis. Sample amount was chosen because samples were plentiful and efforts were made to gain information on very low level components for future evaluation.

NMR 'Fingerprinting

The NMR spectrum of each sample type was distinctive and characteristic. Information available in the spectra were enhanced through use of a solvent (DMSO) that retains the exchangeable protons.

Advantages to using NMR include:

- Information rich (ability to view hundreds of molecules per spectrum)
- Fully reproducible and fully quantitative

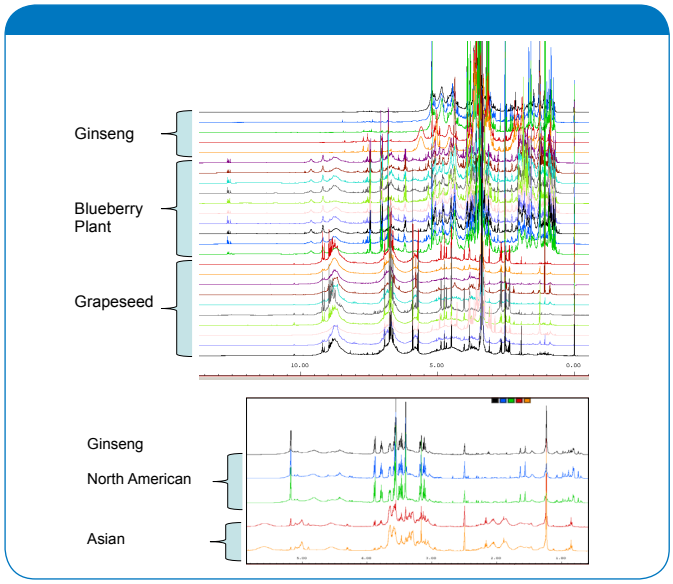

Fig. 1: (a) NMR spectrum of various samples of Ginseng, Blueberry plant and Grape Seed extract showing the distinctive spectrum for each type of plant. The chemical shifts of the exchangeable protons showed high consistency between samples of a similar class and therefore provide a useful fingerprint of the phenolic region of the NMR spectrum. (b) 1D NMR spectra of *Panax ginseng* (North American Ginseng) and *Panax quinguefolius*.

Ginseng Preliminary Screen

A Principal Component Analysis (PCA) of the NMR spectra from the extracts (Fig. 2) demonstrated that the NMR methods were suitable to distinguish various extract types. The view below of the first three PC's shows clear separation of North American and Asian ginseng. Although results were only from a small sample set (5 samples) the details seen in the 1D spectra suggest that the samples from the two continents differ greatly and are readily distinguished (Fig. 1b). Additional samples of ginseng are currently been processed to more accurately define the sample variation for each region.

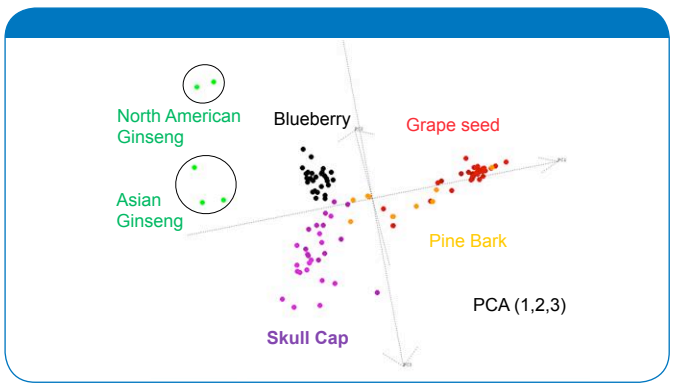

Fig. 2: PCA (1,2,3) of crude natural product extracts generated from NOESY 1D NMR spectra using a bucket size of 0.01 ppm over a 15.8 to -2.5 ppm region and exclusion regions of ethanol peaks (3.52-3.42, 1.12-1.04) and DSS (0.02 to -0.02 ppm). Pareto scaling was used.

Grape Seed Vendor Analysis

The grape seed extract came from 18 different vendors. A PCA of the grape seed extracts by vendor showed that samples from 17 of the vendors grouped together whereas one vendor's samples clearly differed from all other samples. Follow-up analysis revealed that the samples from vendor X were 'beverage grade' samples and were of lower purity than samples from other vendors.

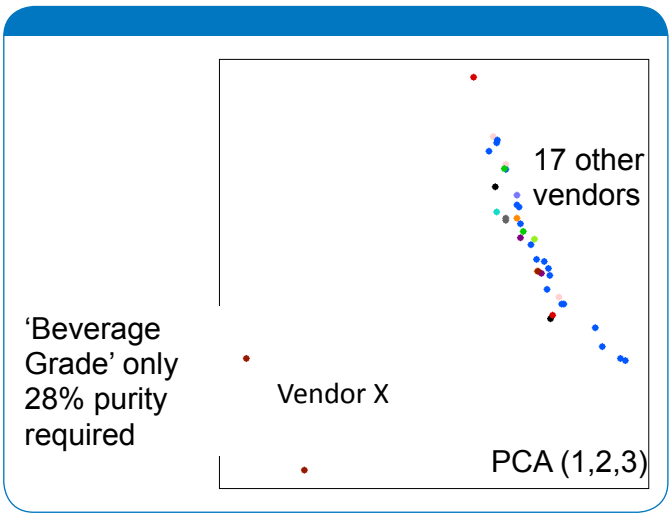

Fig 3: PCA (1 and 2) of grape seed extract colored by vendor.

Adulterated Pine Bark Extract?

The PCA of Pine Bark Extract (PBE) and Grape Seed Extract (GSE) showed that one sample of PBE (PBE_3) was more similar to GSE than to PBE. Further analysis of the 1D spectrum of this sample suggested that this sample was in fact not GSE however it was also very unlike other PBE samples raising the question as to whether or not it had been adulterated or whether it was from a non-traditional pine bark extract source.

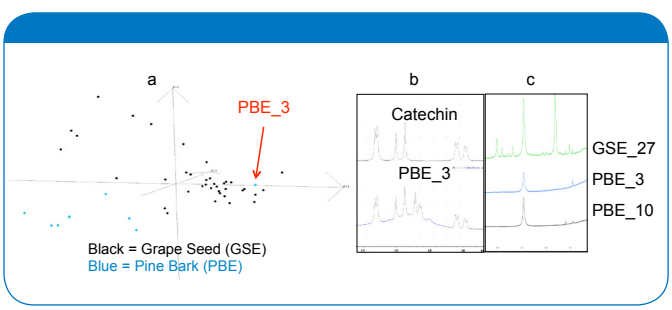

Fig. 4: (a) PCA (1,2,3) of PBE and GSE, (b) 1D NMR spectrum showed that PBE_3 had a very high concentration of catechin as is expected of GSE, however the flavonoid region (c) for the exchangeable protons showed that PBE_3 was not a GSE and was similar to PBE in flavonoid type and concentration.

Quantification of Skullcap and Germander Components

Quantification of key components was performed using a calibrated, simulated NMR signal. This signal was applied to the noesy1d spectra to measure, through integration, the quantities of baicalin, baicalein and scutellarin. The concentration of these metabolites varied considerably over the species studied. Of particular interest, baicalin and scutellarin derivatives were found in a sample labeled as 'germander' (*Teucrium canadense*, SKC_32). This was confirmed with a HSQC spectrum. Germander (*Teucrium*) is not known to contain baicalin and therefore, the sample is suspected of being misidentified. Scutellarin and baicalin are marker compounds which are characteristic of many Scutellaria species.

Table 1: Results from quantification of key metabolites from the 1D NMR of the crude extracts.

Extract Origin	Baicalein (mM)	Baicalin (mM)	Scutellarin (mM)	Sample
S. lateriflora	nd	98.1	10.0	SKC_25
S. incana	42.1	29.6	32.8	SKC_8
S. alpina	4.6	4.5	9.8	SKC_7
S. baicalensis	87.5	7.9	nd	SKC_4
T. canadense	nd	107.5*	10.3*	SKC_32
T. chamaedrys	na	nd	6.2*	SKC_31
nd = not detected, *derivatives of Baicalin and Scutellarin found				

Identification of Components

Individual components within the skullcap and germander samples were identified using a NMR Spectral Database (SBASE) match routine with AMIX Software (Bruker BioSpin). This match routine used signal position and lineshape information to identify likely components in the crude mixture. Where possible, the presence of the suspected component was confirmed using a 2D MATCH with HSQC spectra.

The fingerprint of the components from the 1D NMR in the crude spectra allowed prediction of the species of origin. Confirmation is in progress.

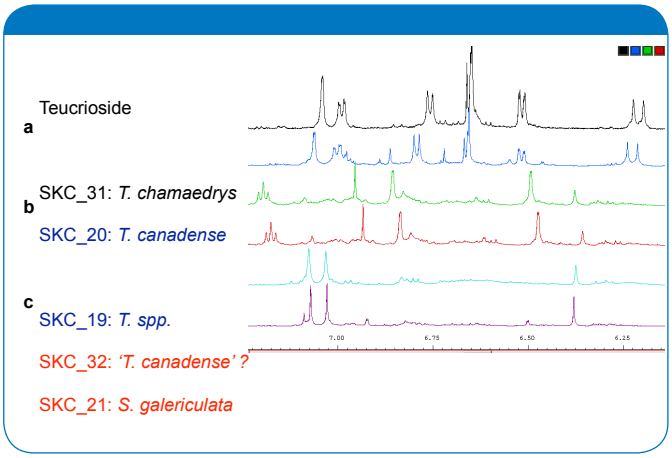

Figure 5: (a) Teucrioside, an identifying compound for germander, was found in the *T. chamaedrys* sample using a AMIX spectral base match (not shown is the match of baicalin, baicalein and scutellarin in skullcap samples using a similar procedure), (b) the unidentified germander sample, SKC_19, is suspected to be from *T. canadense* based on similarity in NMR spectrum with one of this species (SKC_20), (c) the unidentified 'germander' sample, SKC_32 is suspected to be *S. galericulata* based on the similarity of the NMR spectrum with one of this species (SKC_21).

Conclusions

These preliminary data suggest NMR is a powerful tool to evaluate crude natural product extracts for information related to quality control, species identification, identification of components, and quantification of components. This non-targeted approach provides a quick assessment of samples that are not 'typical' and therefore suspect of poor quality, adulteration or mislabeling.

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