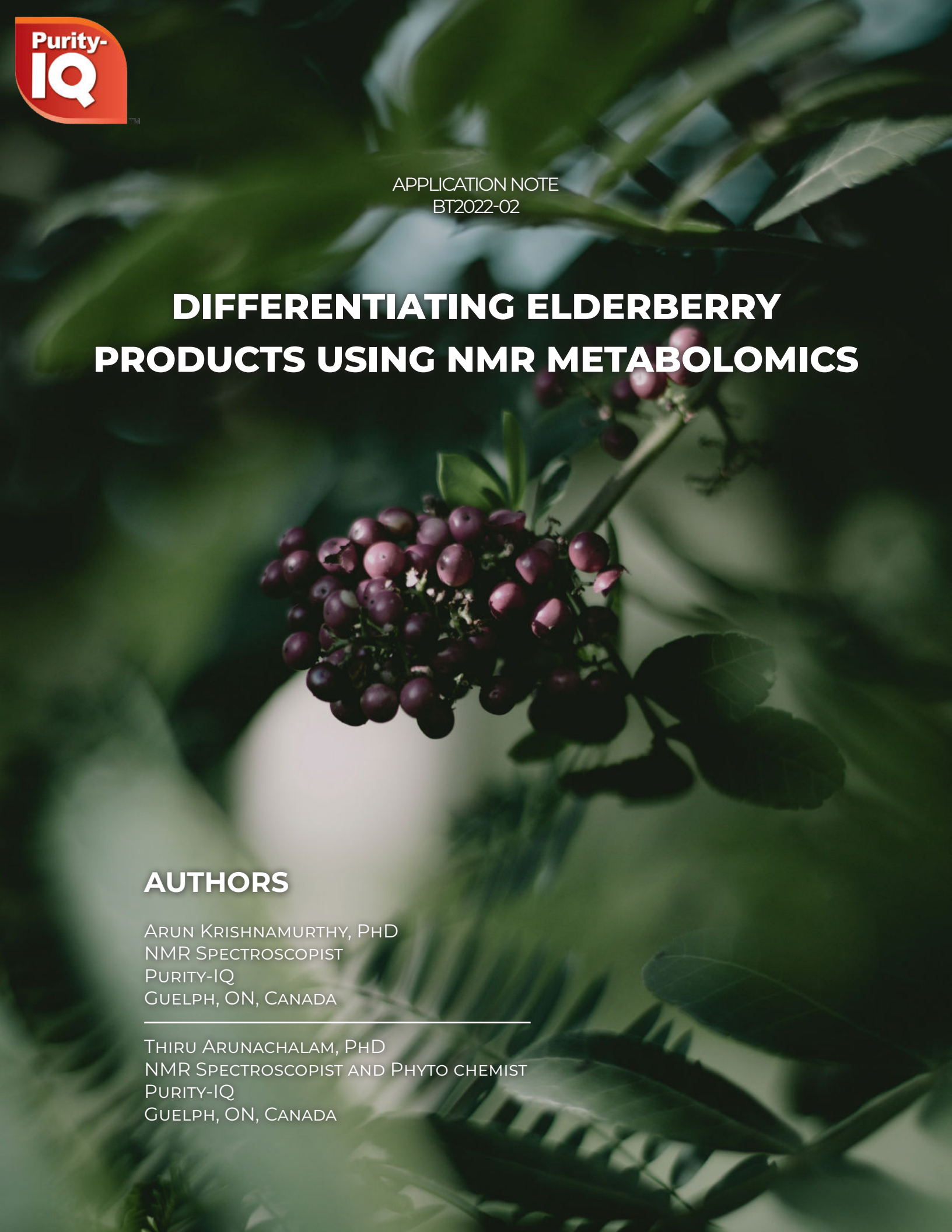
APPLICATION NOTE
BT2022-02

# DIFFERENTIATING ELDERBERRY PRODUCTS USING NMR METABOLOMICS



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## Abstract

In this contribution to our series demonstrating the capabilities of NMR spectroscopy-based metabolomics in determining species identity, authenticating botanical products, and establishing batch-to-batch product consistency, we differentiate elderberry products based on their sources. This will further highlight the advantages of NMR metabolomics, in conjunction with multivariate statistical analysis, as a product/raw material classification tool in the botanical sector.

## Introduction

The American (Sambucus nigra) and European elderberry (Sambucus canadensis) fruits have been used for millennia in traditional medicine as analgesics, antivirals, hemostatics, antiparasitics, and anti-inflammatories, to treat several health ailments, and as dietary supplements to boost immunity. These positive health benefits of the fruit are attributed to a specific class of phenolic molecules called anthocyanins. In addition to their therapeutic properties, anthocyanins confer the colour to the elderberry fruit. For this reason, they are also used as colorants in the food and textile industries. Readers are encouraged to read the review by Gafner et al. published by the American Botanical Council for a detailed understanding of the botany, taxonomy, history of use, cultivars and chemistry of elderberry and other subspecies [1]. The demand for elderberry supplements has grown over the years and experienced an exponential increase during the Covid-19 pandemic due to its popularity as an immune booster and its efficacy in treating flu, cold, and fever symptoms. Market research by ReportLinker forecasts the elderberry market to grow by US$214.88M during 2021-2025, with a compound annual growth rate of 6.52%, which underscores the popularity of the elderberry products [2]. Another report by technavio lists that the elderberry market share in the US is expected to increase by US$109.76M between 2021 and 2026 [3]. The demand-supply imbalance brought about by the drastic reduction in the global supply due to the pandemic and supply-chain issues has led to the severe adulteration of elderberry products; the common adulterants being black rice extract and wild rice extract as colorants, starch as a filler, and grape and other berry extracts as substitutes. The technavio report [3] also lists that the substitution of elderberry products with other berries such as cranberry, blueberry, and acai is gaining popularity amidst the dwindling supply of elderberry, which poses a great threat to the very existence and subsistence of the elderberry market.

NMR-based metabolomics is an elegant approach and an exceptional analytical tool to ensure the quality and authentication of the product, as well as to safeguard elderberry market interests. The analytical technique is accurate, user friendly, employs simple sample preparation methods and offers reproducible results, making it a highly sought-out analytical technique for plant metabolomics. The metabolites constituting plant extracts are unique to each species and yield distinctive NMR spectroscopic profiles, making metabolomics by NMR an attractive and traceable approach for botanical identification. The metabolite profile of a plant product is also sensitive to product processing and manufacturing conditions, cultivars, growth conditions, and the geography of cultivation, which further amplifies the reach of NMR across different product formats. This makes the technique highly resourceful for elderberry product manufacturers and suppliers as the raw material/product can be authenticated and checked for adulteration and substitution. Furthermore, the technique can be employed as a fingerprinting tool to evaluate the consistency between samples from different batches (i.e., batch-to-batch consistency) in the manufacturing sector. In this report, we demonstrate the utility of NMR metabolomics and multivariate statistical analysis in differentiating elderberry products and their common adulterants.

## Materials and Methods

Different formats of elderberry products and adulterants were sourced both commercially and from various raw-material suppliers. Metabolites from these plant products were extracted using different solvents such as water, methanol, chloroform, acetone, ethanol, hexane, and their mixtures. The solvent system that rendered the highest concentration of both polar and non-polar metabolites was adopted for this study. One-dimensional 1H NMR spectra were collected on a 400 MHz Bruker Avance III spectrometer with tetramethyl silane (TMS, 0 ppm) as an internal chemical shift reference. The collected spectra were processed, baseline and phase corrected, and binned using Bruker Amix program for multivariate statistical analysis, performed on the R platform.

## Results and Discussion

The $^{1}$H NMR spectra of different elderberry sample types are plotted in ***Figure 1***. These are quite distinctive given the differences of their spectral profile, whether it is the position of the peaks or their intensities, representing the variance in their metabolic makeup. In addition to identifying the sample sources, the spectral profile of different product types serves as a chemical fingerprint which can be used as an authenticating tool. Multivariate statistical analysis (Principal Component Analysis, PCA) of the NMR data amplifies the observed variance in the spectral profile, the result of which is shown in ***Figure 2***. A total of 232 samples were extracted and analyzed by NMR for this study and each product class includes a rich number of test samples; therefore, the developed model can be deemed accurate. The data points in the plot represent test samples and as seen, they cluster nicely with large variations observed between the different clusters. The data points representing berry extract (Berry EXT 01, Berry EXT 02, Organic & berry conc.) and 14% anthocyanin extract (14% Anthocyanins-1, and 14% Anthocyanins-2) samples aggregate into three and two clusters, respectively, on the PCA plot and are well-differentiated. This suggests variability in their metabolite profiles due to different sources, processing conditions, growing conditions, geographical origins, and other factors.

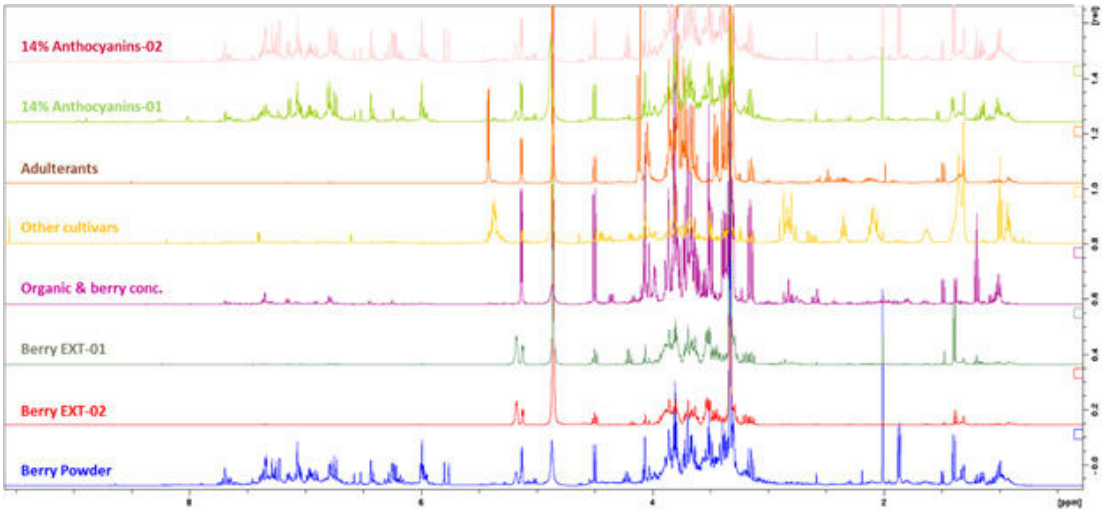


***Figure 1***. $^{1}$H NMR spectra of different botanical products.

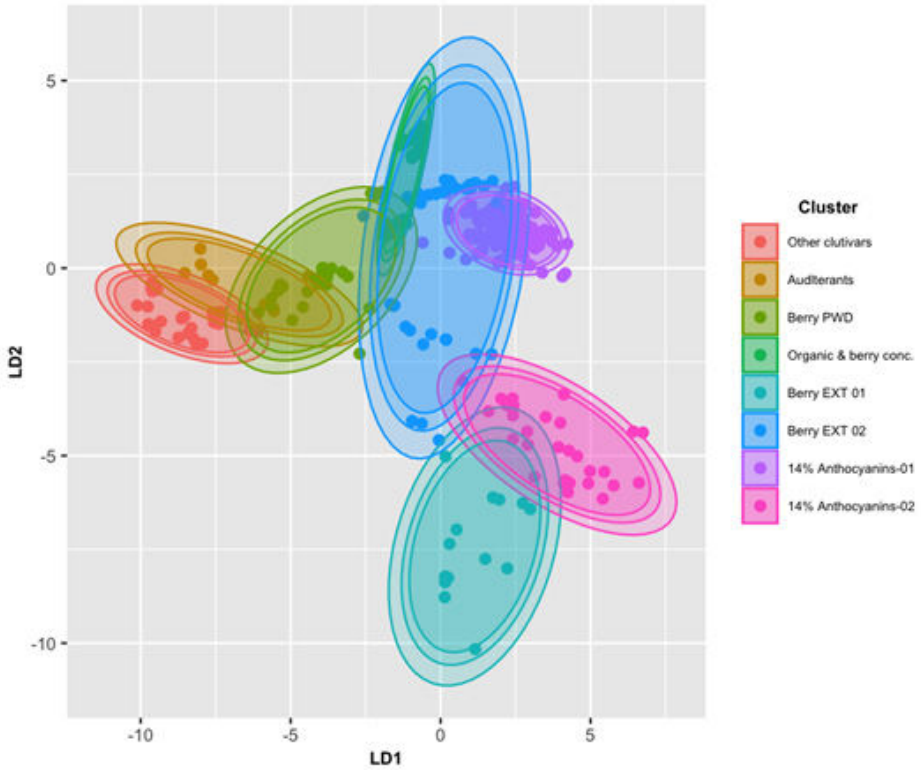


***Figure 2***. Clustering of samples with similar metabolite/spectral profile into different regions in a Principal Component Analysis plot.

## References


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