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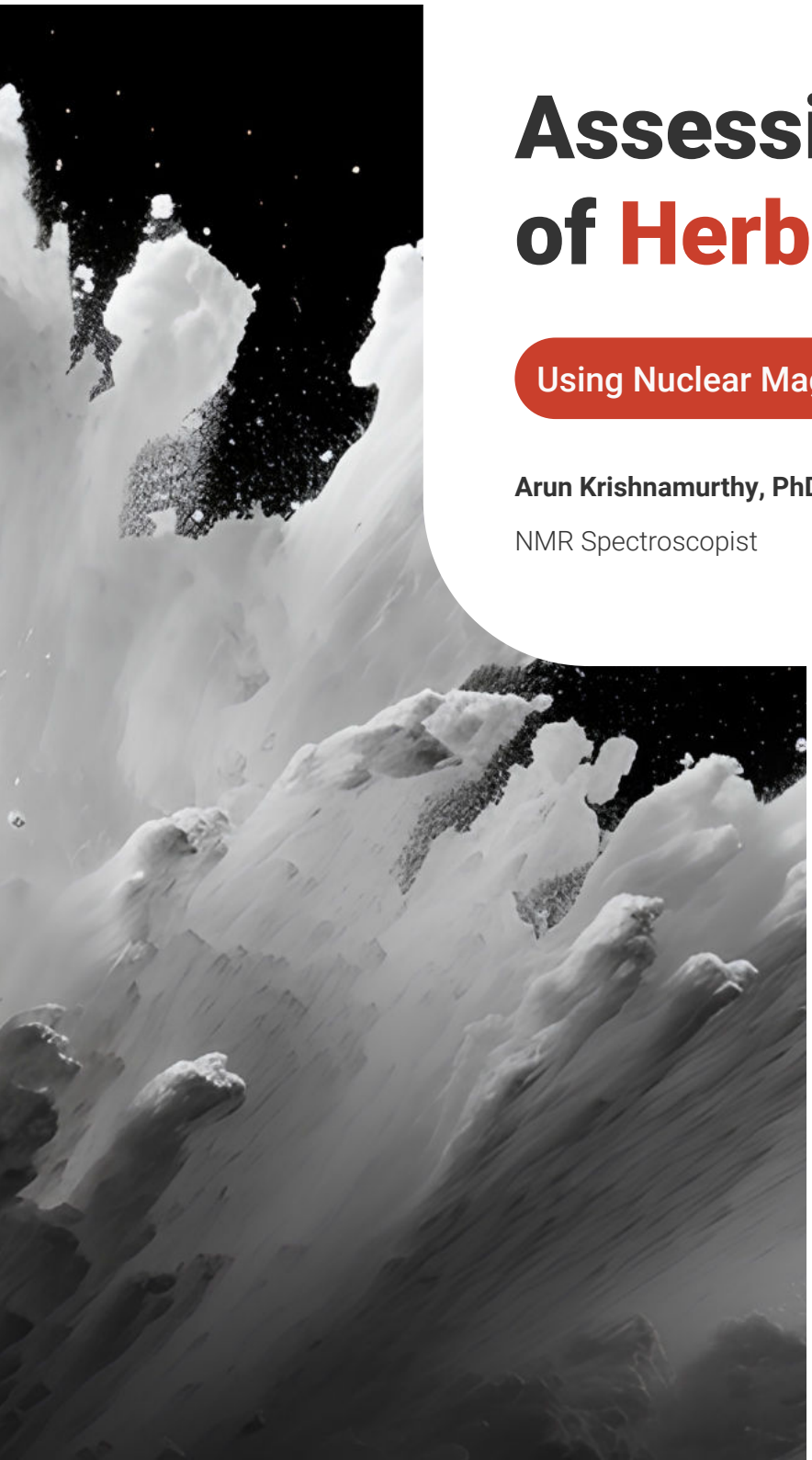

Assessing the Dilution of Herbal Extracts

Using Nuclear Magnetic Resonance

Arun Krishnamurthy, PhD

NMR Spectroscopist

150 Research Lane, Suite 102
Guelph ON, N1G4T2, Canada

info@purity-iq.com

www.purity-iq.com

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Introduction

Maltodextrin, a known carrier and drying aid is prolifically used in the botanical space to manufacture botanical extracts and powders due to its beneficial role as a carrier agent and protectant for bioactives which are susceptible to heat and other processing conditions in the spray drying process. While spray drying is a common industry process, it can change the botanical-to-maltodextrin ratio in the final product due to the volatilization of solvents (water, ethanol, and others) used in the botanical extraction, leading to maltodextrin fractions higher than intended. Moreover, the fraudulent practice of intentional dilution of botanical products with maltodextrin or other carriers to increase the volume of a high-demand product in acute shortage situations, or in some cases to reduce manufacturing costs, is gaining traction. The presence of maltodextrin often complicates product labelling as the label information may not adequately represent the actual chemical composition of the product. For example, a blend of 80% 5:1 concentrate, and 20% carrier yields a 4:1 product, however the blend may not be chemically similar to a true 4:1 extract.

Whether intentional or unintentional, these practices may lead to the manipulation and mislabeling of the concentration information of extracts and ultimately create inferior products, significantly impacting their market value, claimed health benefits and compromising consumer trust and satisfaction. Several conventional analytical techniques employed in species-level authentication of botanical-origin health products require the presence of key biomarkers in the product in appreciable concentrations, making these technologies targeted in nature. One of the prominent factors contributing to biomarker irregularities is the very issue of product dilution with excipients or carriers, either declared or undeclared, which may skew the analytics and prove them ineffective in clearly understanding the true identity and exact chemical nature of the product. Therefore, analytical techniques that are non-targeted in nature and can offer accurate product information including the species authenticity, detection of dilution and extract fraction are warranted.

Nuclear Magnetic Resonance (NMR) spectroscopy with its non-targeted metabolomic profiling capability and inherent quantitative nature proves to be an ideal technique for such applications. The spectral profile can either be assessed for the presence of biomolecules of interest and their concentrations, an aspect that can be considered targeted, or the overall profile (non-targeted) which functions as a chemical fingerprint of the product and can be compared with authentic fingerprints for irregularities. The analytical freedom NMR spectroscopic data offers, including other favourable

attributes like user-friendly sample preparation methods, high reproducibility, retrospective data analysis, and data availability for multivariate statistical analysis, underscores the utility of NMR in addressing and effectively screening the dilution of natural health products. The following analytical concerns are addressed in this note:

- Can NMR determine species authenticity of diluted products?
- Can NMR identify product dilution and assess the fractions of extract and diluent in a product?
- Can NMR identify the diluent type present?
- Can NMR assess the drug extract ratios of products?
- Can NMR methods simultaneously deliver targeted and non-targeted screening?

Four authentic extracts of species *Silybum marianum*, *Panax ginseng*, *Ginkgo biloba*, and *Cordyceps militaris* were blended with different fractions of excipients, Maltodextrin (carrier), Inulin, and Xylooligosaccharide (dietary fibers) (**Table 1**) to demonstrate the above-mentioned capabilities and extracted in triplicate using proprietary Purity-IQ Inc. methods. The spectral data were analyzed visually and compared with authentic sample chemical fingerprints in the Purity-IQ Global Registry for species authentication using multivariate statistical methods.

Sample	Description
10%-CM-ext-10:1 + 90%-MDX	10% *Cordyceps militaris* 10:1 extract + 90% Maltodextrin
40%-CM-ext-10:1 + 60%-MDX	40% *Cordyceps militaris* 10:1 extract + 60% Maltodextrin
80%-CM-ext-10:1 + 20%-MDX	80% *Cordyceps militaris* 10:1 extract + 20% Maltodextrin
90%-CM-ext-10:1 + 10%-MDX	90% *Cordyceps militaris* 10:1 extract + 10% Maltodextrin
10%-GB + 90%-MDX	10% *Ginkgo biloba* extract + 90% Maltodextrin
40%-GB + 60%-MDX	40% *Ginkgo biloba* extract + 60% Maltodextrin
90%-GB + 10%-MDX	90% *Ginkgo biloba* extract + 10% Maltodextrin
10%-PG + 90%-MDX	10% *Panax ginseng* extract + 90% Maltodextrin
40%-PG + 60%-MDX	40% *Panax ginseng* extract + 60% Maltodextrin
90%-PG + 10%-MDX	90% *Panax ginseng* extract + 10% Maltodextrin
10%-SM + 90%-MDX	10% *Silybum marianum* extract + 90% Maltodextrin
40%-SM + 60%-MDX	40% *Silybum marianum* extract + 60% Maltodextrin
90%-SM + 10%-MDX	90% *Silybum marianum* extract + 10% Maltodextrin
10%-SM + 90%-INU	10% *Silybum marianum* extract + 90% Inulin
40%-SM + 60%-INU	40% *Silybum marianum* extract + 60% Inulin
90%-SM + 10%-INU	90% *Silybum marianum* extract + 10% Inulin
10%-SM + 90%-XOS	10% *Silybum marianum* extract + 90% Xylooligosaccharide
40%-SM + 60%-XOS	40% *Silybum marianum* extract + 60% Xylooligosaccharide
90%-SM + 10%-XOS	90% *Silybum marianum* extract + 10% Xylooligosaccharide

Table 1: Blends prepared in-house from authentic botanical extracts.

Can NMR Determine Species Authenticity of Diluted Products?

Even though a product is blended with carriers and excipients, the botanical part of the product can be adequately extracted using customized extraction methods. A salient feature of non-targeted analysis is the reliance on the total spectral profile, the chemical fingerprint of the product/species, rather than on key biomarkers, therefore, the analytics can accurately deliver species authenticity even when the biomarker presence/concentration is compromised because of dilution, a huge drawback with conventional targeted methods. Provided that the extraction method followed can reproduce the expected chemical fingerprint, the spectral data can be confidently used to determine species identity, rendered through visual assessment of the data by comparing the profiles to authentic products as well as multivariate statistical analysis.

- **Visual spectral analysis:** The spectral profiles of pure *Ginkgo biloba* extract, maltodextrin, and extract-maltodextrin blends are plotted in **Figure 1**. As seen, the extraction methods selectively capture the *Ginkgo biloba* extract profile from the blends, which align with that of pure extract confirming the species identity. Notably, the blend with just 10% of *Ginkgo biloba* extract could also be authenticated through this method.
- **Multivariate statistical analysis:** The ^{1}H NMR spectra of the blends along with a subset of species-specific NMR data from the Purity-IQ Global Registry were subjected to distance matrix calculation and Hierarchical Clustering Analysis (HCA), the output of which is plotted as a circular dendrogram in **Figure 2**. All the blends containing maltodextrin ranging from 10% to 90% (w/w) align with authentic samples of respective species confirming their identity. Additionally, the NMR data of blends are subjected to the Linear Discriminant Analysis (LDA) and their species identity is predicted using a model built using authentic samples, and the predictions are listed in **Table 2**. A strong correlation is observed between the output of the two methods (HCA and LDA), demonstrating the utility of multivariate statistical analysis in a non-targeted analytical setting to discern the product identity.

Blends	*Cordyceps militaris* (CM)	*Ginkgo biloba* (GB)	*Panax ginseng* (PG)	*Silybum marianum* (SM)	Predicted species
Prediction Probability					
10%-CM-ext-10:1 + 90%-MDX	**1**	0	0	0	*Cordyceps militaris*
40%-CM-ext-10:1 + 60%-MDX	**1**	0	0	0	*Cordyceps militaris*
80%-CM-ext-10:1 + 20%-MDX	**1**	0	0	0	*Cordyceps militaris*
90%-CM-ext-10:1 + 10%-MDX	**1**	0	0	0	*Cordyceps militaris*
10%-GB + 90%-MDX	0	**1**	0	0	*Ginkgo biloba*
40%-GB + 60%-MDX	0	**1**	0	0	*Ginkgo biloba*
90%-GB + 10%-MDX	0	**1**	0	0	*Ginkgo biloba*
10%-PG + 90%-MDX	0	0	**1**	0	*Panax ginseng*
40%-PG + 60%-MDX	0	0	**1**	0	*Panax ginseng*
90%-PG + 10%-MDX	0	0	**1**	0	*Panax ginseng*
10%-SM + 90%-MDX	0	0	0	**1**	*Silybum marianum*
40%-SM + 60%-MDX	0	0	0	**1**	*Silybum marianum*
90%-SM + 10%-MDX	0	0	0	**1**	*Silybum marianum*

1 – True; 0 - False

Table 2: Linear Discriminant Analysis predictions for blend samples.

Can NMR Identify Product Dilution and Assess the Fractions of Extract and Diluent in a Product?

The customized extraction methods effectively capture the spectral profile of the botanical component of the blend, as well as the total intensity counts from each spectrum. As NMR is inherently quantitative, that is, the acquired data has molecular identity and relative concentration information, the spectra can simultaneously be used to determine species identity as well as the extract fraction in the blend, all in one analysis. In spectral profiles of *Ginkgo biloba* with 90%, 40% and 10% maltodextrin, a linear trend is observed in the total spectral intensity, with 10%-GB+90%-MDX and 100%-GB yielding the lowest and highest intensities, respectively (**Figure 1**). A similar observation is made for blends of other botanical and fungal species extracts as well (**Figure 3**) wherein, the ^{1}H NMR spectral profiles of the blends match with the pure extracts with high confidence and in conjunction with statistical methods yield accurate species information (**Figure 2** and **Table 2**). Moreover, similar linear trends in total intensity counts are also evident in these, underscoring the applicability of these methods to universally test all botanical and fungal extracts for possible dilution. In principle, this approach can be extended to any type of botanical product and dietary supplement where authentication to prove compliance with label claims and assessment of individual components (presence and concentration) are required.

Can NMR Identify the Diluent Present?

NMR spectroscopic analysis employing sample preparation methods that can preferentially extract botanical components from a blend can be extended to products containing excipients other than maltodextrin as well. An example is demonstrated using *Silybum marianum* extract blended with different fractions of Inulin (INU) and Xylooligosaccharide (XOS) (**Figure 4**), the excipients being ubiquitously used in dietary supplements and popular as prebiotic dietary fibers. These types of blends represent both types: cases where the extract is diluted intentionally or where a blend is marketed as a prebiotic dietary fiber product containing the botanical extract. Nevertheless, species authenticity is a criterion for the regulatory compliance of these products and NMR spectroscopy can accurately identify the species of botanical extract, determine the fraction of the extract in the product, and identify the excipient/diluent present. The ^{1}H spectral signature of polysaccharide excipients (MDX, INU, XOS) are distinct as these vary in their composition (different types of sugar residues) and molecular configuration (chain length, chain structure - linear vs. branched, homo- and hetero-polysaccharide nature, and bonding variations (α/β and positions) between different sugar residues). These structural variations render different spectral profiles for the excipients and function as respective chemical fingerprints (MDX, INU, and XOS spectra in **Figure 4**). While the effect of maltodextrin on the blend spectra are minimal, INU and XOS

characteristic resonances are observed in the blend spectra alongside the fingerprint of *Silybum marianum* due to their easily soluble nature. While many targeted analytical methods may suffer from interference from soluble excipients, the NMR profile which is cumulative of the excipient and extract can be easily assessed to accurately determine the total chemistry of the product.

Can NMR Methods Simultaneously Deliver Targeted and Non-Targeted Screening?

The NMR spectral profiles can be used either in a targeted or a non-targeted fashion to answer different analytical questions. While the preceding sections nicely summarize the analytical capabilities of NMR rendered through a non-targeted approach and visual data screening, the same spectral data can further be used to investigate the presence of biomolecules of interest and their concentration. As an example, flavonolignans present in the *Silybum marianum* extract and their concentration in pure extract and blends are identified (**Figure 5**) and quantified (**Figure 6**). The concentrations of these molecules increase linearly as the extract fraction in the blend increases, demonstrating the inherent quantitative nature of NMR. It is worth noting that a single spectroscopic datum of a sample addresses all the above-discussed aspects of a product making NMR spectroscopy a fit-for-all approach and a robust analytical tool.

Examples of Non-Compliant Samples With Maltodextrin Tested in the Purity-IQ Laboratory:

- The ^{1}H NMR spectra of products diluted with maltodextrin are plotted in **Figure 7**, which were tested employing proprietary Purity-IQ methods and comprehensive metabolomic ^{1}H NMR spectral library.
- All these products were labelled as pure extracts at the time of submission for authenticity testing, and the presence of maltodextrin was undisclosed except for the tart cherry fruit powder wherein the maltodextrin was listed as 8% wt/wt.
- Analysis revealed that these samples primarily contained maltodextrin and the spectra lacked peaks/resonances characteristic of biomolecules specific to each species, hence the samples were reported as negative for authenticity.
- Although maltodextrin was listed as a component in the tart cherry fruit powder sample, the analyzed fraction of maltodextrin was above the percentage claimed, and therefore the sample was reported to be out-of-specification.

Highlights

a. Targeted and non-targeted analytical nature delivering total product chemistry

b. Identification and quantification of biomolecules of interest

c. User-friendly sample preparation and extraction methods

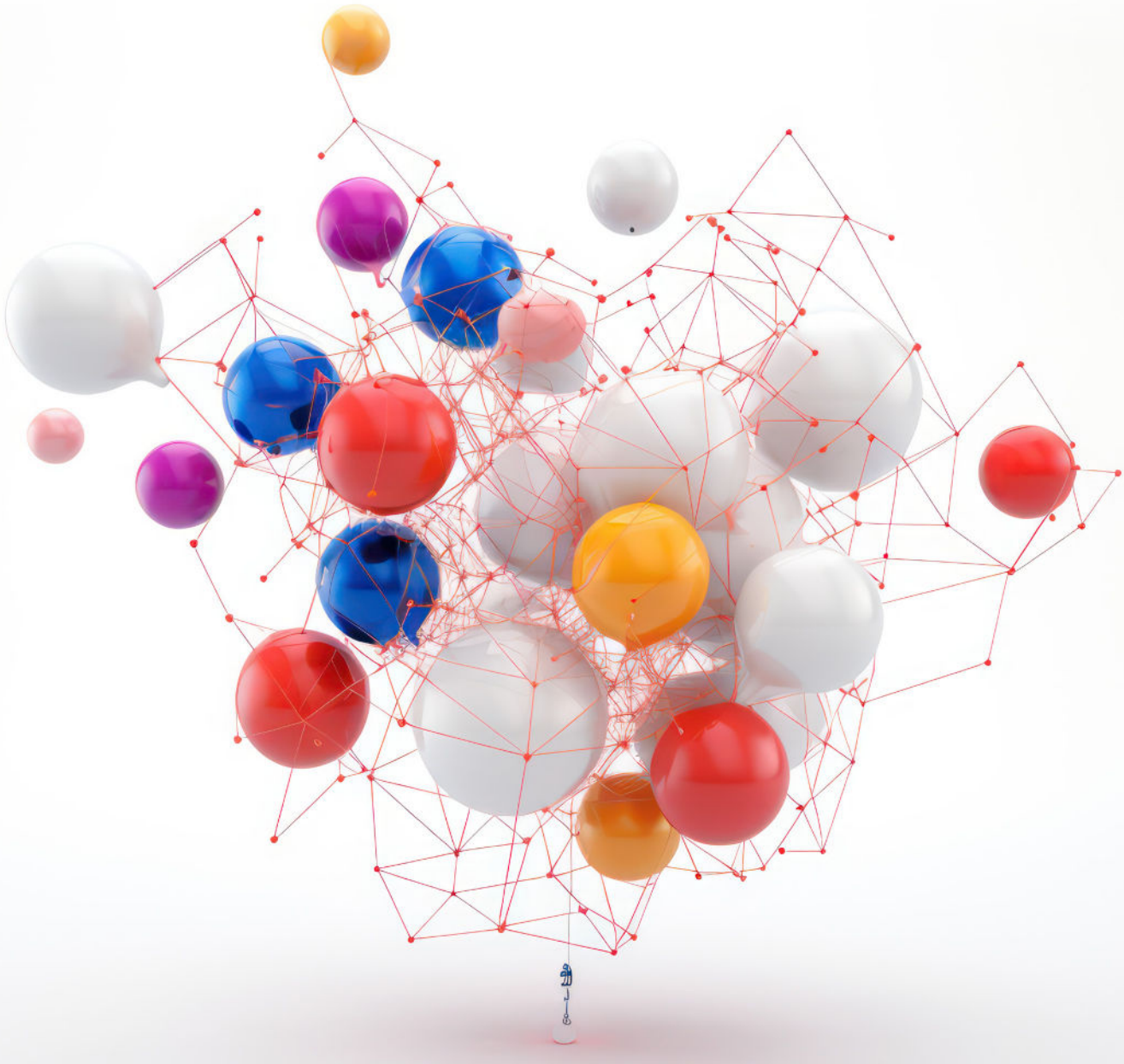
d. High data reproducibility

e. Retrospective data analysis

f. Multivariate statistical analysis of spectral data

g. Comprehensive spectral data library building for screening authenticity and product quality

h. Capability to assess batch-to-batch consistency



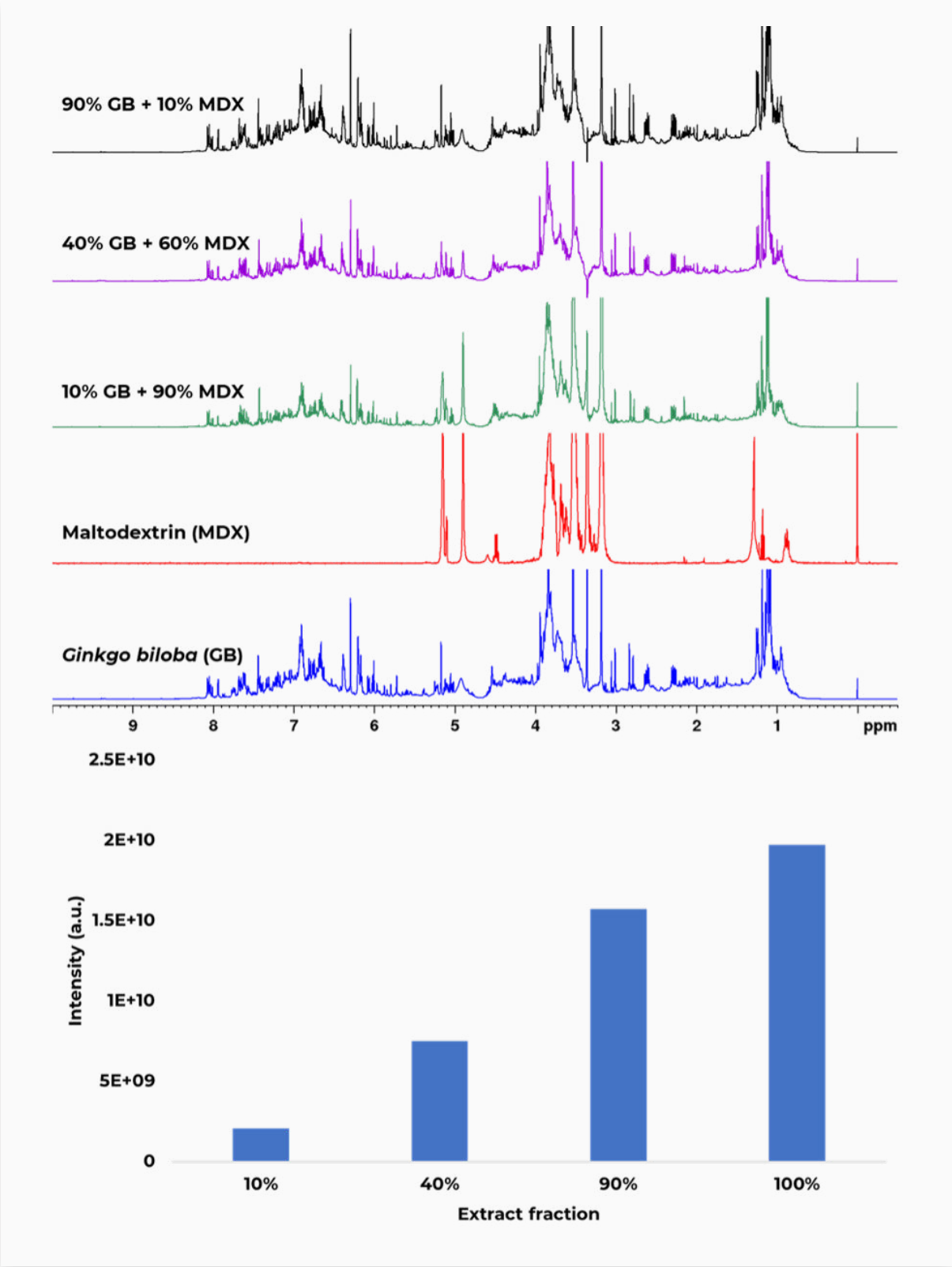


Figure 1: ^{1}H NMR spectral profiles of *Ginkgo biloba* pure extract, maltodextrin, and extract-maltodextrin blends (top); Total spectral intensity of the blends (bottom).

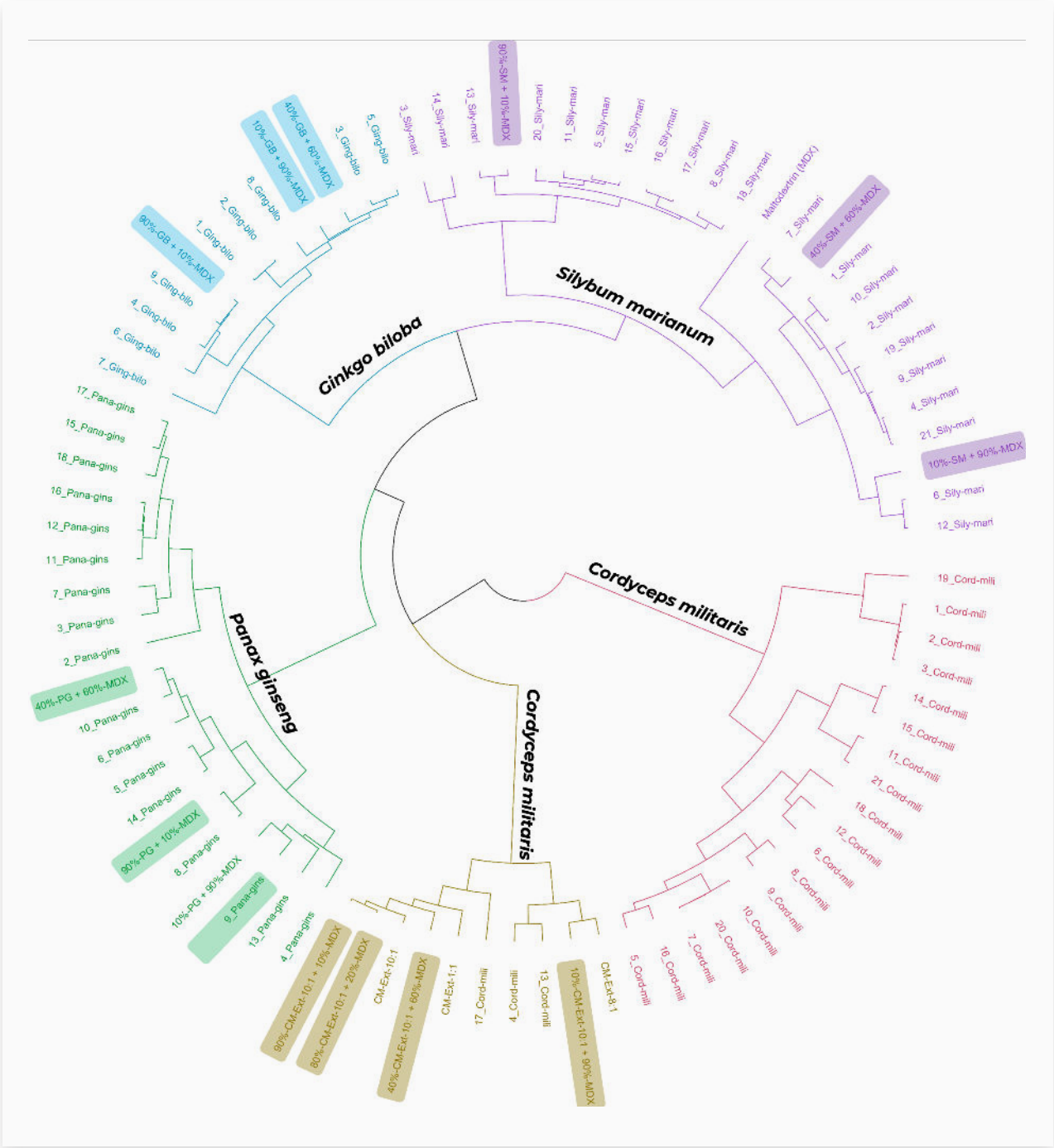


Figure 2: Circular dendrogram from Hierarchical Clustering Analysis showing species assignments for the blended products, which align with respective authentic species samples confirming their species identity. Blend samples of *Silybum marianum* (Sily-mari), *Ginkgo biloba* (Gink-bilo), *Panax ginseng* (Pana-gins), and *Cordyceps militaris* (Cord-mili) are highlighted in purple, blue, green, and gold, respectively.

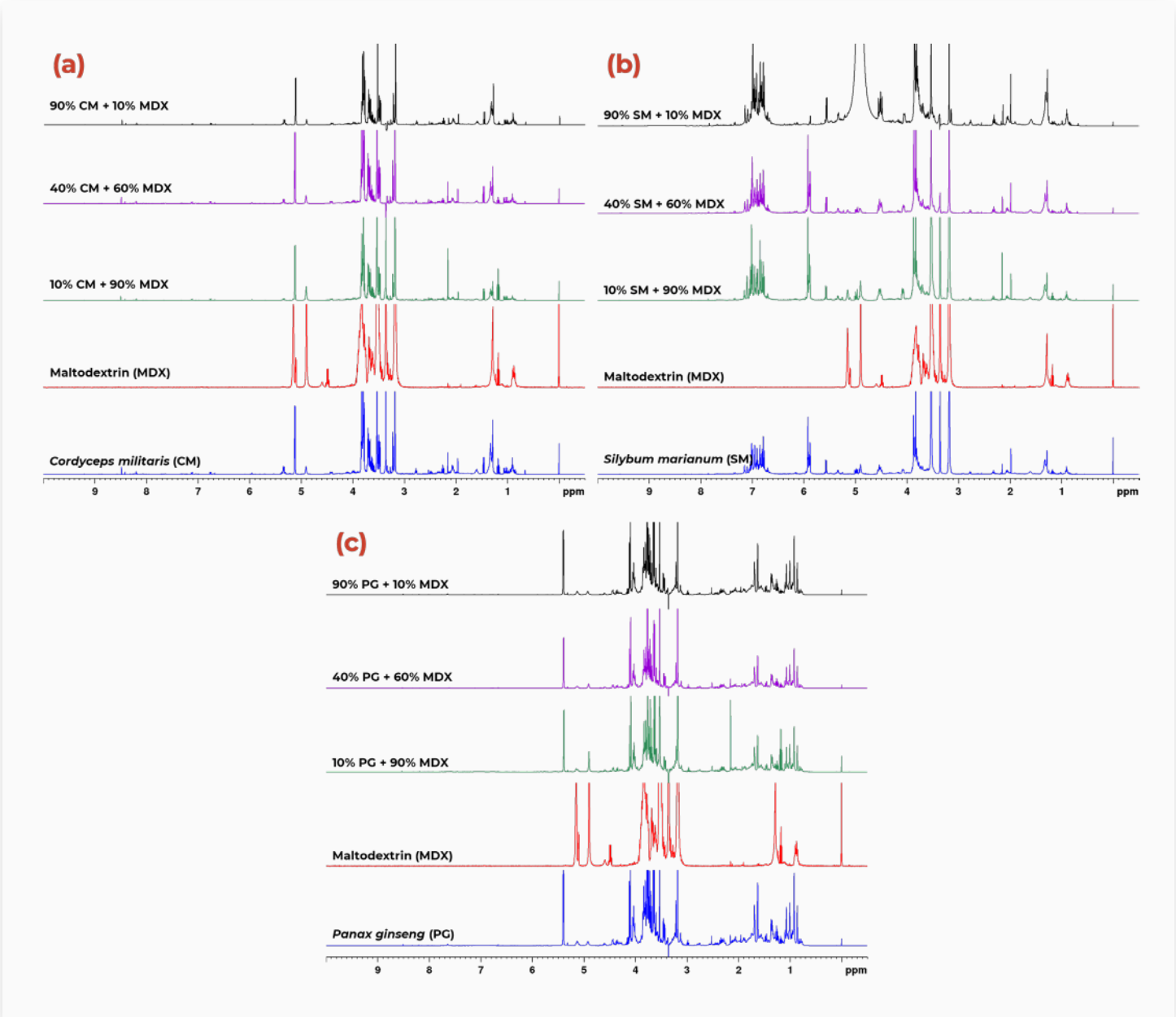


Figure 3: ^{1}H NMR spectra of pure extracts and extract-maltodextrin blends: (a) *Cordyceps militaris*; (b) *Silybum marianum*; (c) *Panax ginseng*

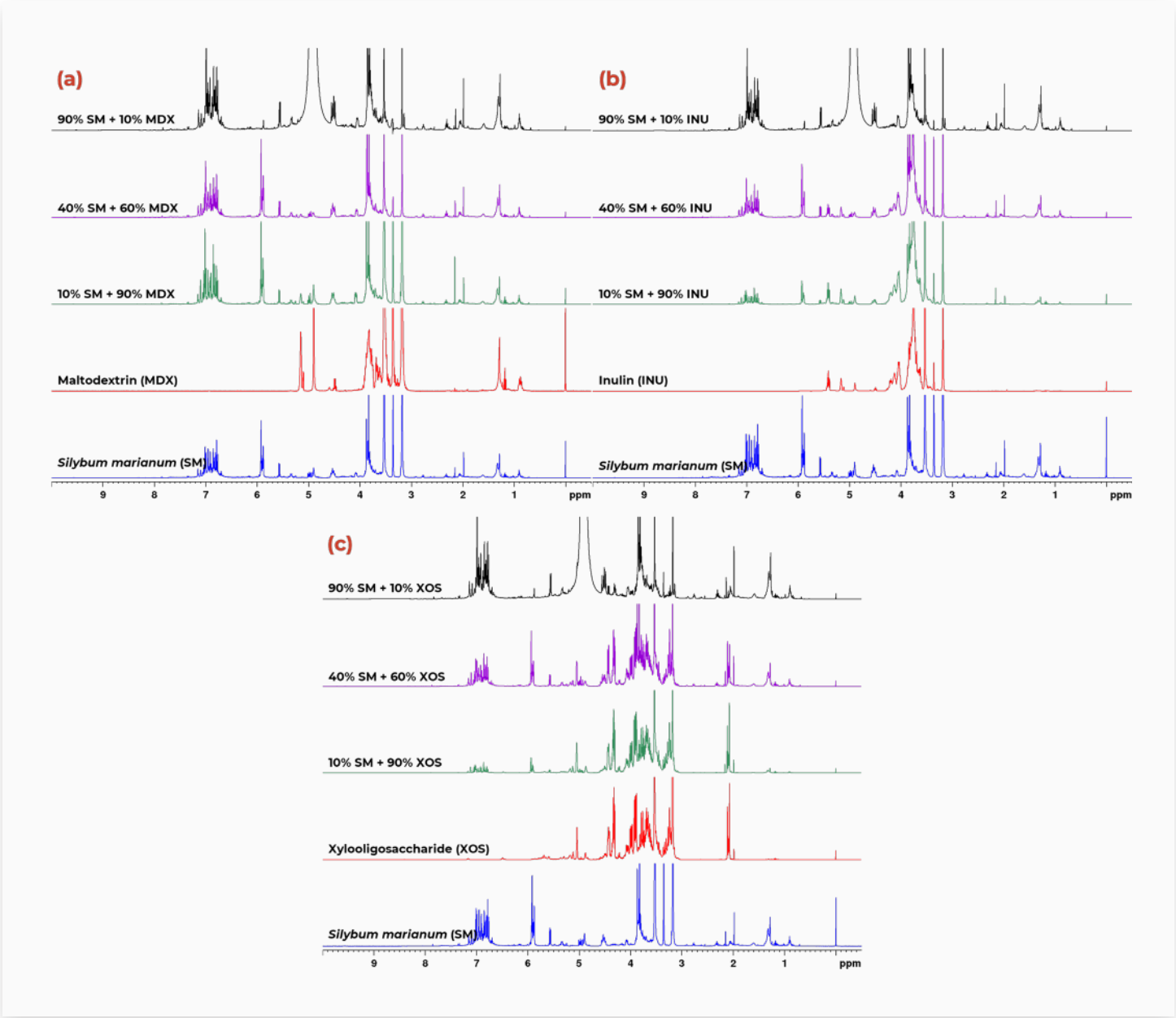


Figure 4: ^{1}H NMR spectral profiles of pure *Silybum marianum* extract and extract-excipient blends: (a) extract-Maltodextrin blends; (b) extract-Inulin blends; (c) extract-Xylooligosaccharide blends.

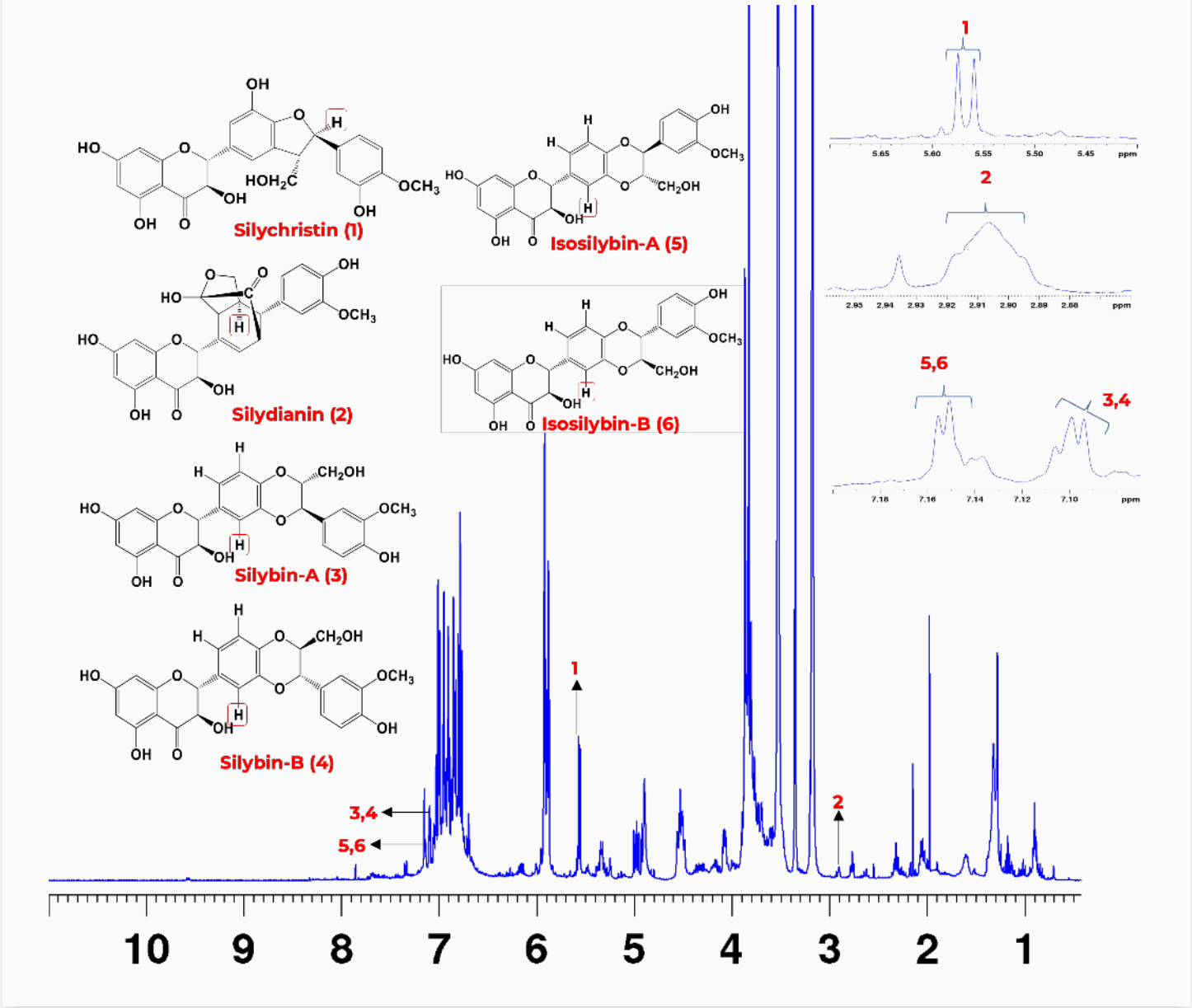


Figure 5: ^{1}H NMR spectrum of *Silybum marianum* extract with peak assignments for different flavonolignans (Journal of agricultural and food chemistry. 2016 Feb 24;64(7):1618-26). The protons yielding identified peaks in the spectrum are marked with red squares in the structures.

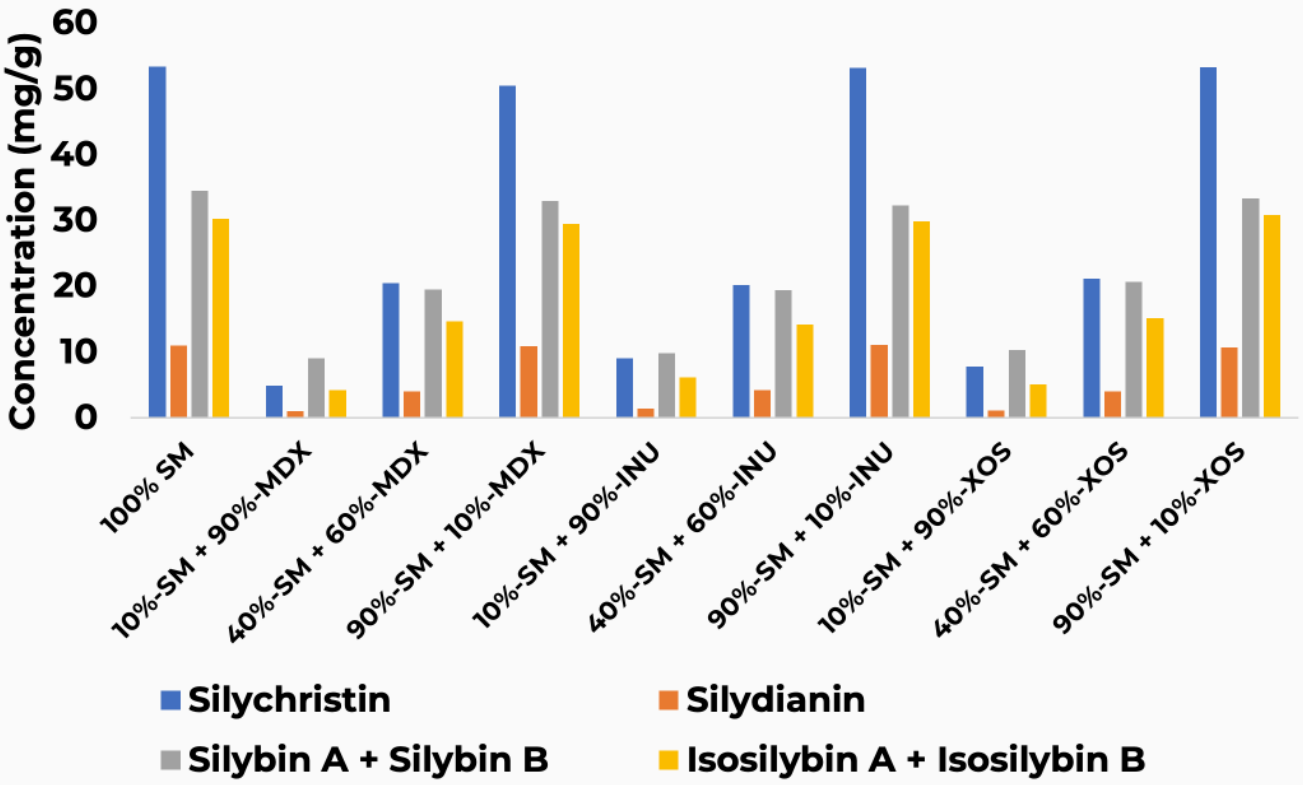


Figure 6: Concentrations of flavonolignans in different Silybum marianum extract and diluent blends.

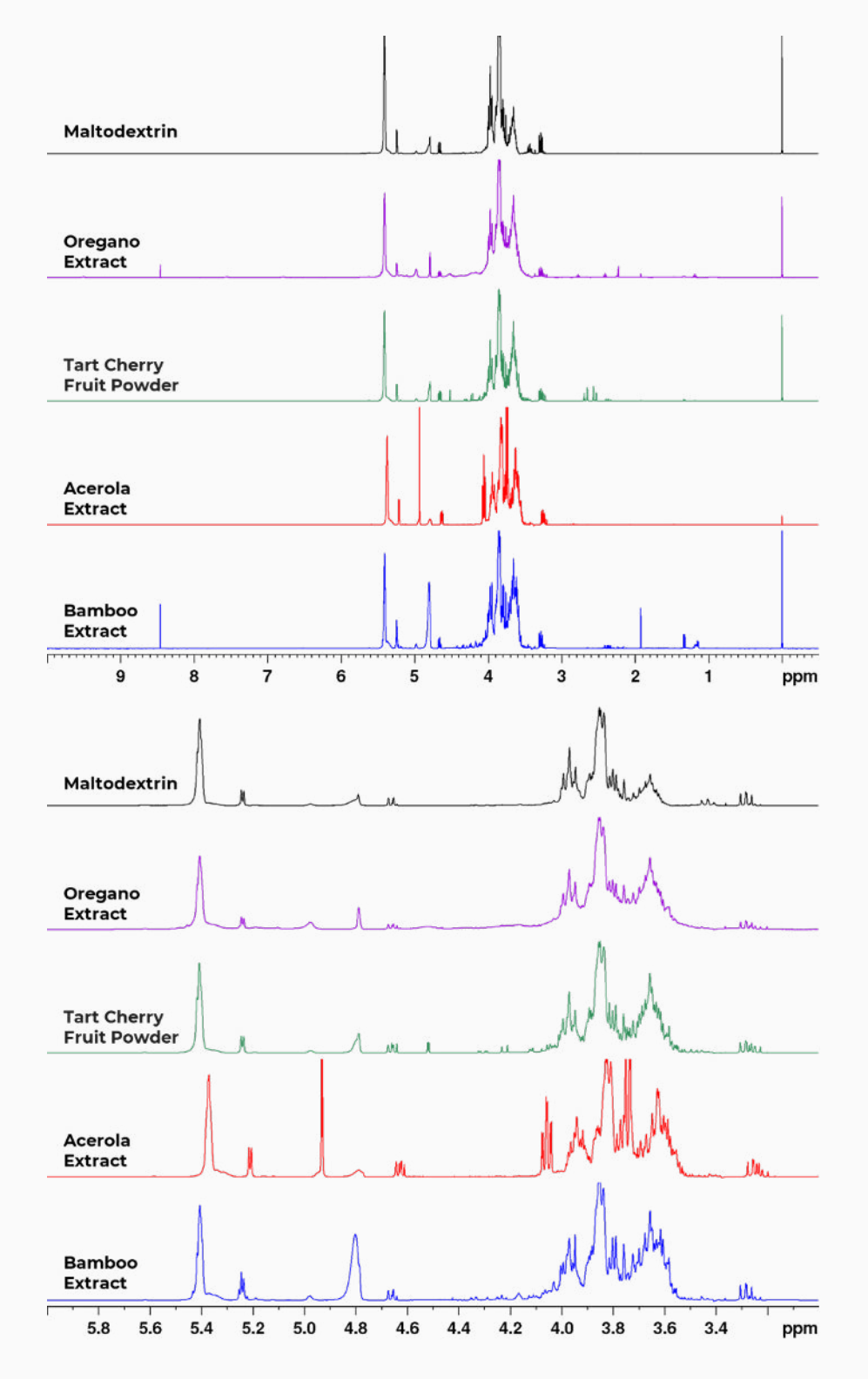


Figure 7: ^{1}H NMR spectra of botanical products purported to be pure and the presence of maltodextrin undisclosed. Full ^{1}H spectra of the products (top) and the spectral region with maltodextrin peaks (bottom).