Report on Analysis of Hericium Mushroom Powder for Mushrooms by the Sea.

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Background

Mushrooms by the Sea grow a NZ species of the Hericium mushroom They have requested some analysis be performed on the mushroom product (mixed fruiting and mycelial tissue) for comparison with overseas grown varieties and products.

Contracted Work.

Samples of NZ mushroom products (up to 3) will be provided for analysis. Comparison products will also be provided (up to 2). Products will be extracted and subjected to chemical analysis to determine how the levels of certain metabolites compare.
Specifically:

- Samples will be compared for beta glucan content (% beta glucan)
- Samples will be analysed using LCMS for the presence of the known metabolites- hericenones and erinacines (this work will refer to previous published work on the NZ species, only ericinones identified). Comparison will be made with the commercial overseas products provided.

Three samples of mushroom powder were provided.
Two of these were US grown products;

- Host Defense Mushrooms Lion's Mane Mushroom mycelium powder. Lot # 072121CM with 55% mushroom powder and 45% dried myceliated brown rice.
- Wilderness Poet's Lion Mane mushroom powder. Batch #03222383 (no additive)

The third sample was the NZ Lion's Main powder provided by the client (no additive).

Results.

1. Glucan Analysis.

Samples of the three powders were analysed for glucan content using a Megazyme assay kit. This method involves calculation of the total glucan followed by determination of the α-glucan content. The β-glucan content is the difference between the total glucan and the α-glucan.

Table 1. Results from Glucan Assay.

Sample name	Total glucan in %	α-glucan in %	β-glucan in %
Wilderness Poets	42.25 ± 2.30	26.39 ± 0.52*	15.85 ± 2.25**
Host Defense	48.57 ± 1.30	24.96 ± 1.85*	23.61 ± 2.36**
NZ Lion's Mane	9.19 ± 0.60	0.76 ± 0.04	8.41 ± 0.57

- *these numbers are based on the initial analysis using the undiluted sample. However, the absorption was very high (1.9) and therefore out of range*

** *these numbers are calculated using the initial result for α-glucans.*

Since the results for the α-glucan determination on the two US samples were well out of range, this part of the analysis was repeated with diluted samples with the following results (Table 2).

Table 2. Alpha-Glucan Results for US samples (repeat analysis).

Sample name	α-glucan in % (1st repeat)	α-glucan in % (2nd repeat)
Wilderness Poets	78.26 ± 2.48	61.46 ± 4.76
Host Defense	80.29 ± 0.89	78.18 ± 1.36

The determination of the total glucans was also repeated yielding similar results to the original experiment.

The results obtained for the NZ Lion's Mane sample appear to be lower than the literature reports for total glucan in *Hericium erinaceus* species of around 38% and an α-glucan content of around 3 %; The total glucan content in the US samples appears similar to the reported values, however the α-glucan is much higher in these samples.
We believe that something in the US samples is interfering with the α-glucan measurement.

The ratio of α- and β-glucan in the NZ sample is similar to literature so this sample does not show the same interference.

2. Metabolite Analysis.

Hericium mushroom species are known to contain a series of metabolites with potential bioactivity. These include the hericerins, erinacines and hericenones. In 2020, Chen *et al.* published a report showing the presence of hericenone C in the New Zealand Heracium (*H. novae-zealandiae).*

We performed an initial analysis of ethanolic extracts of the three mushrooms provided. Using LCMS with UV and low-resolution mass spectrometry we were unable to definitively locate any of the known metabolites in the mushroom extracts. We do not have access to any standards for these compounds which made the identification more difficult.

A further study on the samples was performed using high-resolution LCMS. In this experiment we searched for specific exact masses which match those of the known Hericium metabolites. The molecular structures are also loaded into the data processor so that any ions which might be derived from breakdown of the metabolites can be picked up. The data is sorted so that only peaks showing exact masses within a tight window are selected as "hits". The data is then sense checked based on retention times (compare with literature) and ion types seen. This is a powerful technique which is able to find compounds of interest that would normally be hidden under other metabolites.
Using this method, we were able to tentatively identify the presence of several metabolites of interest in the NZ mushroom powder extract. Figure 1 shows chromatograms of 5 compounds identified in the NZ extract, erinacines A and G, and Hericenones A and J. Note that we are seeing two peaks for Hericenone J, possibly one of these peaks is Erinacerin B which is an isomer of Hericenone J. Figure 2 shows the same chromatograms but all at the same scale to give an indication of the relative amounts of these components.

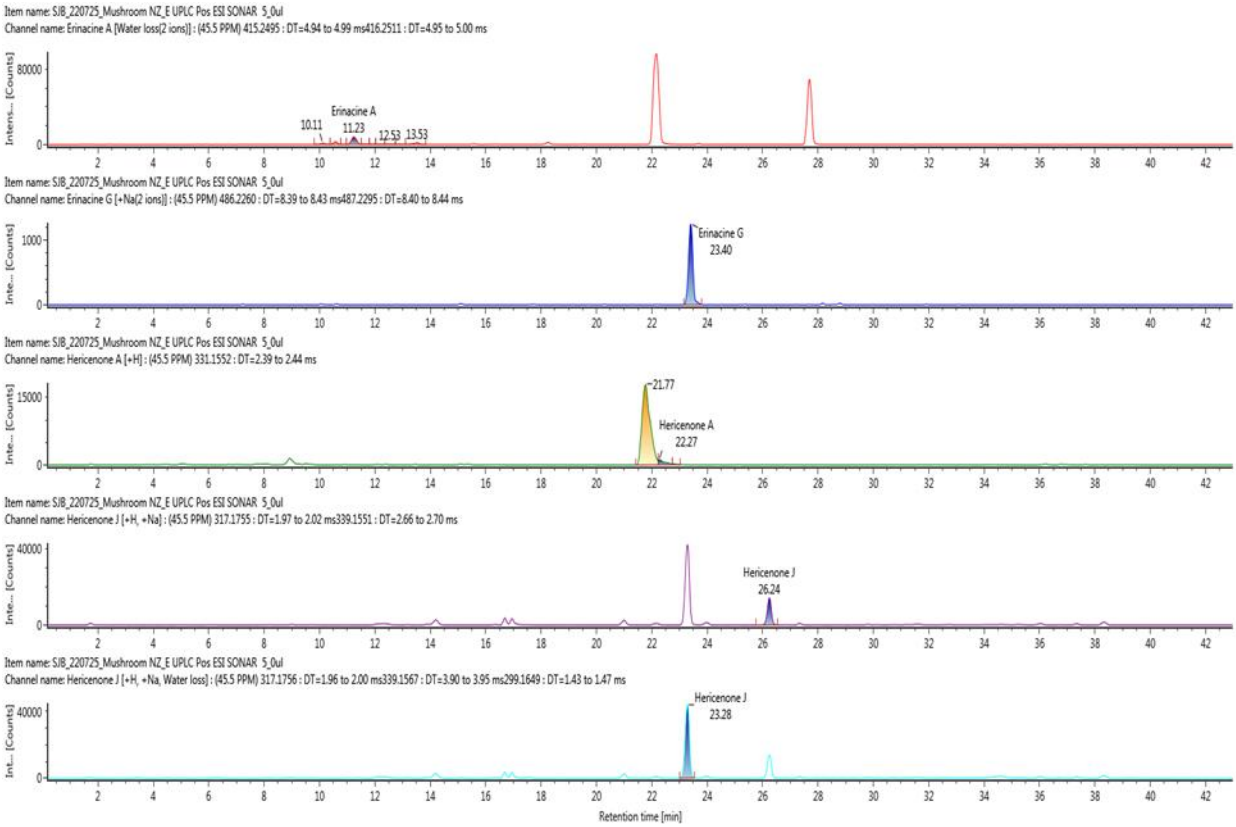

Figure 1. Metabolite peaks for identified components in the NZ Mushroom.

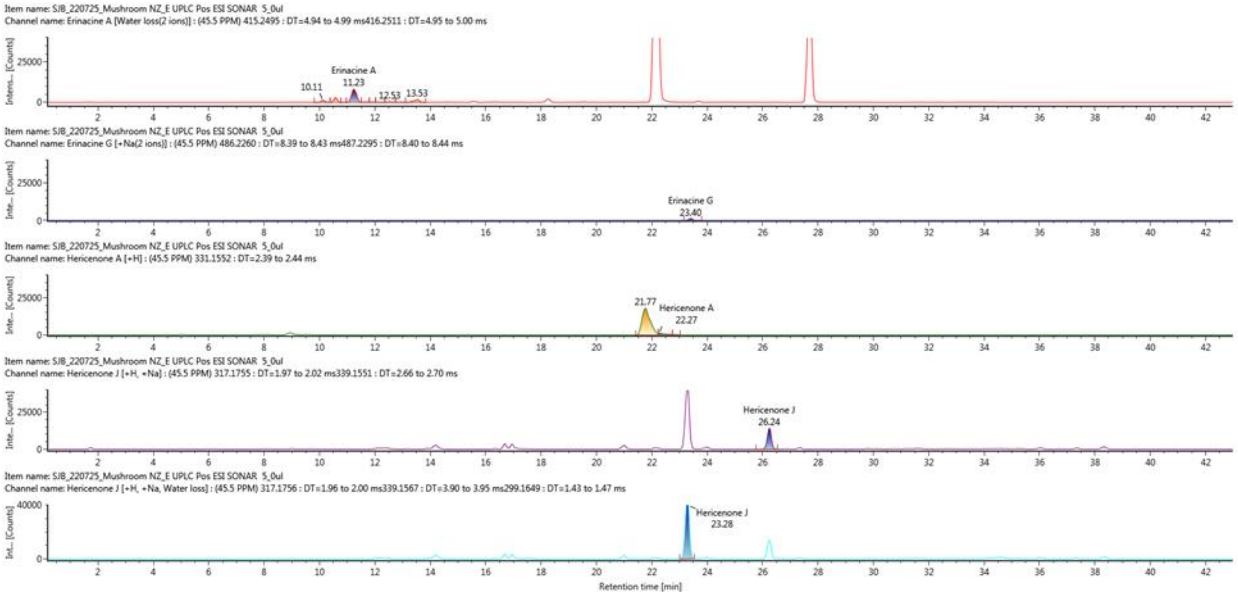

Figure 2. Normalised chromatograms from Figure 1.

The two US samples of Lions Mane were somewhat different. The two samples were similar so we mainly looked at data for the Wilderness Poets sample.
The chromatograms for this ethanol extract showed fewer "hits". However, we do see Hericenone J (or Erinacerin B) again at the same retention time as in the NZ sample. We also see Hericene B. Figure 4 shows the normalised chromatograms.

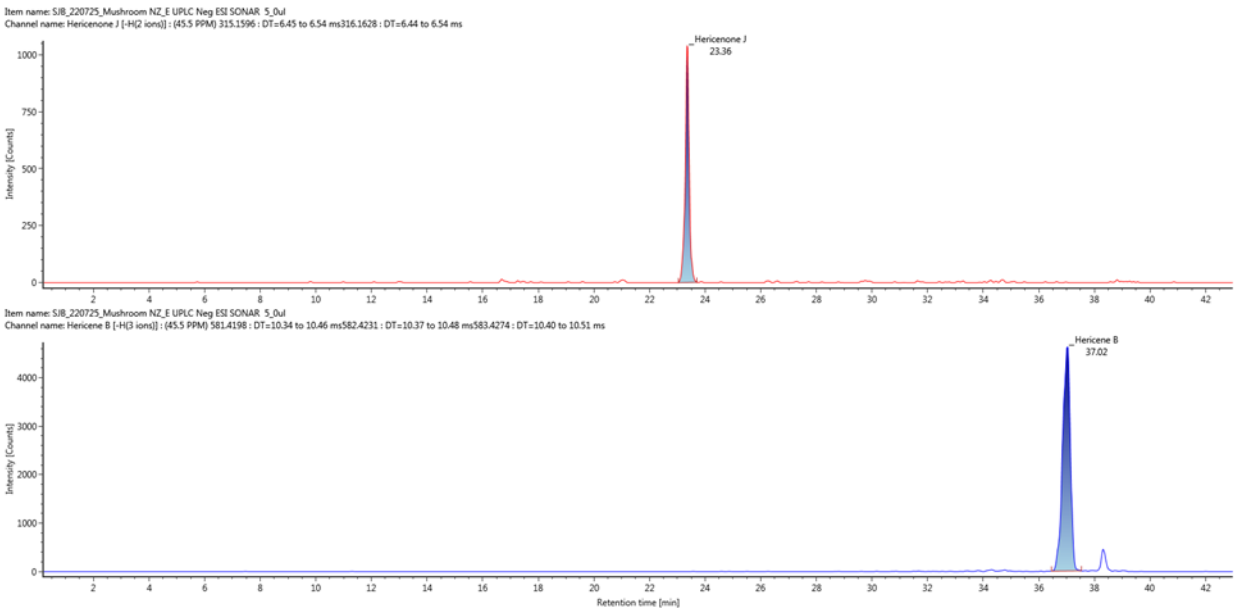

Figure 3. Metabolite peaks for identified components in the Wilderness Poets Mushroom.

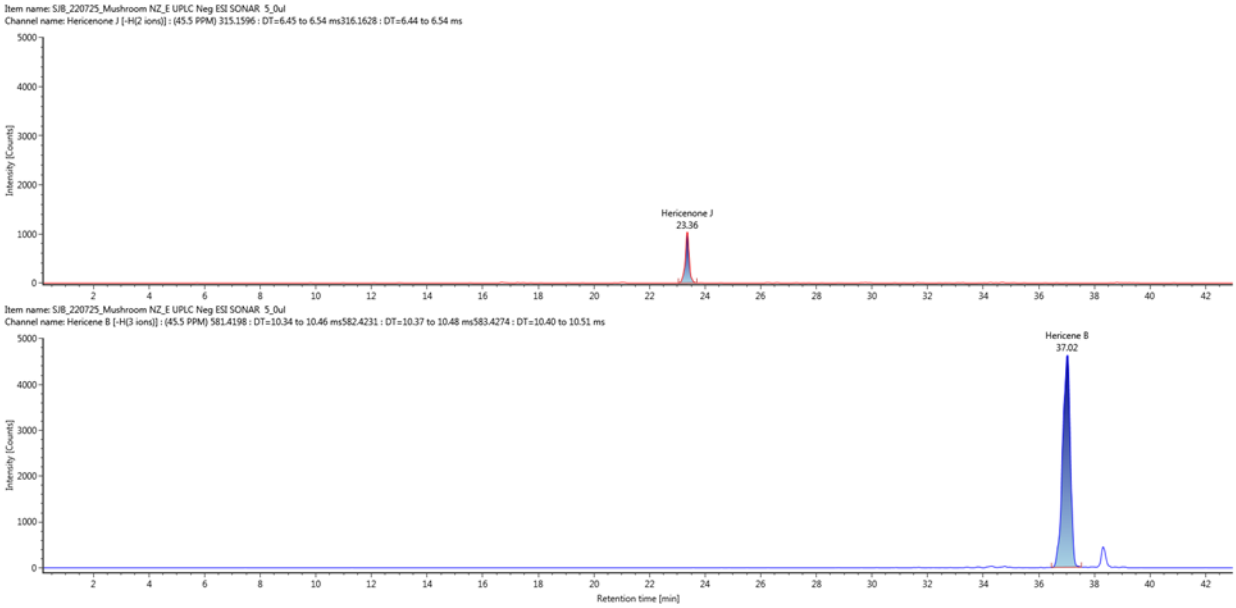

Figure 4 Normalised chromatograms from Figure 3.

Conclusions.

The *H. novae-zelandiae* powder has been compared with two US Lion's Mane mushroom powders. The glucan content in the NZ sample is around one quarter of that in the US samples. The α/β-glucan ratio in the NZ sample is typical of Heracium however there was a problem with determination of the α/β ratio in the two US samples. This may be due to interfering substances.

A high-resolution MS analysis of the ethanolic extract of the NZ sample identified 4-5 hericenone/erinacine type compounds. Only two compounds were identified in the US extracts. The metabolites could not be quantified without standards.