Extraction Experiment Plans

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Let's spend this week doing some planning.  I've attached a couple of
documents that Tom put together on his initial work on Boswellia samples.
I would like to see the solvent testing have a bit more repeats so that we
can make real calls that are not based on sample to sample variance.  Take
a good read through the attached, then pitch me an experimental design
that will help us design an efficient and effective extraction.  When you
have something put together we can sit down and do a solid go-through.
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Previous Extraction

Notes

- Chloroform is denser than sample, methanol is less dense. This makes sense.
- Consistent choice of samples: slightly translucent, yellow tinge, brown or orange discolorations avoided.
- 3 mL of solvent used.
- Sonication and vortexing treatments.
- Obtained samples of similar mass.

Block Design

Randomized Block

Treatment	*B. sacra*
MeOH w/ Sonication	
MeOH w/ Vortexing	
CHCl3 w/ Sonication	
CHCl3 w/ Vortexing	

No measurement made after extraction.

Pros and Cons

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+ Methanol and Chloroform are good starting solvents
- Low sample surface area
- Samples likely had moisture
- No quantitation
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Second Extraction

Goals

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+ More replicates
+ Good solvent choices
+ Replicates of different species
- Less sample to sample variance
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Notes

BE VERY CLEAR!!! This will probably end up in methods.

Perhaps an Erlenmeyer on a shaker platform is better suited for this extraction. More movement of the solvent. However, a volatile solvent wouldn't really work for this. Only really works for DMSO, perhaps.

Should I:

- Use one piece for each treatment? Such as, cut once piece into 4 equally sized pieces, and subject to 4 different treatments?
  This would account for measuring sample to sample variance.

Measuring mass before and after of remaining piece sounds like a good bet.

Consider Soxhlet extraction, though perhaps an idea.

- Has been done before on frankincense, find a paper on this.
- **Probably not a good idea. Deuterated solvents disappearing into the air sounds unfortunate.**

I think it would be more beneficial to crush or break the sample prior to addition to the solvent. Seems like the sample is pretty tough for the solvent to eat away at because of its composition.

- Perhaps after breakage, a visit to the desiccator may also be fruitful.

Want to test time points at 1 hour and 2 hours to coincide with previous experiment.
I do not know which solvent was tested for the NMR standard. If it is the same as is in at the lab bench, it is the CDCl3.

Probably use 30 minute intervals.

Using sonication, vortex.

- Consider using shaker instead of vortex, however
  - Solvent may evaporate.

Here is what we can strive for:

Replicate Block

Treatment	***B. sacra***	***B. sacra***	***B. sacra***
MeOH w/ Sonication			
MeOH w/ Vortexing			
CHCl3 w/ Sonication			
CHCl3 w/ Vortexing			

Measurement of the mass difference or NMR spectra quality can be used for quantitation.

Just need to figure some things out:

- What do I care about? It's not entirely clear what Tom's conclusions were.
  - Possibilities:

What do we care about?	What do we do?
Extraction Length	Using one or two extraction conditions (same solvent and different method, or vice versa). Prepare 4 samples, and extract them at once, removing samples from one at a time.
Solvent	Use different solvents for the same amount of time
Species	We may want to test methods across species.

It may be a good idea to do all of these experiments, but we don't have that many samples.

In theory, the system should take into account different starting masses of compound. For example, some samples contain pieces of wood which contribute to a change in their total mass. We cannot really minify that. Thus, should we care about the initial mass of the sample?

Here are my beginning assumptions:

- Starting mass does not matter *that much*. As long as samples are chosen within a reasonable difference in mass from one another, we are doing our best.
- We are looking at extraction *length*.
  - It is important to note that all samples could have different compound compositions. However, this is good and contributes to our purpose.
- We assume we can apply the same method to all sample species.
- Each bag of sap *is a sample* and each sap piece *is a pseudoreplicate*.

    - I only say this because we cannot guarantee that the sap is from a different tree or the same tree.
    - I don't think this really matters too much, but it is important to note.
- Vortexing seems like a bad way to do it, but the most concurrent. Vortexing is going to have to be serial, but it has the best efficiency.
    - Assuming 90 samples, and 2 hours per sample extraction, it will take 180 hours, or 7.5 days worth of work. Is this what Tom did? Is there a better way to use the sonicator?
- Extraction will be completed using normal solvent, and dried down to be resuspended in deuterated solvent.
    - This may introduce some error due to oxidation of the dissolved products.
    - Basically, do we extract in normal or deuterated solvent?