

Optimizing the phytochemical specific extraction process of alkylamides present in *Echinacea purpurea* root (EPR) for further research

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Abstract

Alkylamides are naturally occurring chemical-compounds classified as phytochemicals, which are of interest for their bioactive abilities giving many plants their medicinal properties. The purpose of researching the phytochemical specific extraction process is to deduce the most efficient method of alkylamide extraction that yields the highest abundance of this specific chemical compound. Once this process is thoroughly researched, conclusions will allow future researchers to be able to extract alkylamides efficiently to use for experimentation and as standards for future experiments. *Echinacea purpurea* root which is known for being high in alkylamide levels was dried, ground up and combined first with a solvent (95% ethanol) for the primary mass extraction. Distillation and freeze drying then yielded an extracted product of phytochemicals that will be used in the second wave of extractions. Following the primary extraction process, an array of different solvents will be combined with equal amounts of extracted material from the primary step, testing to see which yields the most alkylamides while minimizing the extraction of additional phytochemicals. Different solvents used in the second wave of extraction will result in different levels of alkylamides produced, which will then allow for conclusions to be drawn about the most efficient method of extraction for this specific phytochemical.

Putting phytochemicals into context

Echinacea purpurea root (EPR) is a medicinal plant that contains bioactive phytochemicals belonging to the alkylamide family. Used by many First-Nation communities to treat upper respiratory tract infections and ailments, EPR has in recent years gained popularity for its immune-stimulatory effect by the scientific community. Among the many phytochemicals present in EPR, the alkylamides are primarily responsible for giving EPR its therapeutic potential. Motivated to facilitate further research on alkylamides present in EPR, this project looks to conclude which method of extraction and fractionation optimizes the yield of specific phytochemicals. The methodology that was chosen encompassed first grounding up the root material followed by three separate stages of combining it with a solvent and finally using a roto-vap and freeze dryer to end up with isolated phytochemical samples. By future isolation of alkylamide samples, future research on this specific phytochemical can easily be done and will allow researchers to draw more conclusions about its bioactivity.

Methodology

Preparation:
Step 1

- EPR was broken down by hand, then processed in a laboratory grade mill, which reduced it to a medium grade powder.
- This increased the surface area of the root, allowing more solvent to come into contact with the material in the following step

Extraction: Step 2

- The ground up material (1078.3 g) was then combined with 10.8 L of solvent (95% Ethanol) and was left mixing over night. The following day the mixture was filtered to remove any sediment and placed in containers. The same process was repeated two more times with the left over material, however only 5.4 L of solvent was used.

Extraction: Step 3

- To remove the solvent a roto-vap was used which used kinetic energy, thermodynamics and negative pressure. The extracted sample fluid was reduced down to a sludgy consistency. Further freeze drying was needed to end up with a final pure phytochemical product.



Observations and results

The extraction process selected for this project worked quite well in extracting phytochemicals from the original raw root sample. The following list is a compilation of observations and results that were recorded during the processing, extraction and distillation process. Further results will be recorded after the final step, freeze drying, that will allow for a dry powder sample to be obtained.

- ❖ Combining the ground up EPR material with a solvent resulted in the release of an array of phytochemicals observed through the alteration in pigmentation of the solvent.
- ❖ Processing the phytochemical containing solvent through the roto-vap machine resulted in the distillation and removal of the ethanol and left behind some fluid (water) that would need to be freeze dried.
- ❖ The first extraction of EPR resulted in the darkest pigment afterwards and therefore the highest yield of phytochemicals compared to the following two extractions on the same material.



Conclusion

- ❖ 95 % ethanol is capable of extracting phytochemicals from an organic sample such as EPR and is relatively easy to remove through distillation.
- ❖ The first solvent extraction of phytochemicals yielded more than the consecutive two extractions. This allows us to conclude that the majority of phytochemicals are extracted during the first extraction process.

Future steps and speculative conclusion:

- ❖ The next step in concluding which solvent optimizes alkylamide extraction will include separating the extracted phytochemicals into groups and using different solvents to fraction out alkylamides specifically.
- ❖ It is expected that a lipophilic solvent such as hexane or ethyl acetate will yield the most alkylamide while minimizing the extractions of unwanted phytochemicals present in the sample.

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