Small-Scale Isolation and Mass Spectrometry-Based Analysis of Bioactive Metabolites from Rare Medicinal Plants

Jasenka Piljac Žegarac, Daniel Cuthbertson and Bernd Markus Lange*
Institute of Biological Chemistry, Washington State University, Pullman, WA 99164

Introduction

The World Health Organization estimates that between 60-80% of the world's population relies on traditional medicine for primary healthcare needs. People worldwide use between 50,000 to 80,000 flowering plants for medicinal purposes. The vast majority of these species grow wild, while only approximately 4% are cultivated. There has been a pronounced interest in medicinal plants research in the scientific community over the past decade.

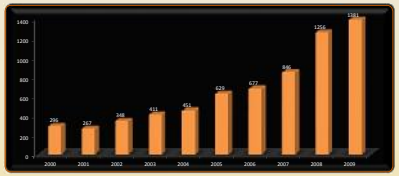


Figure 1: Number of journal articles published in the past ten years containing the phrase "medicinal plants" in the topic search. Source: Web of Science, SCI-Expanded.

The clubmoss *Huperzia squarrosa* synthesizes a wide range of alkaloids, one of which, huperzine A, is under investigation for its cholinesterase inhibitory activity and potential use in treatment of Alzheimer's disease. *Tripterygium regelii* is a vine known for anti-inflammatory and immunosuppressive properties. One of the terpenoid constituents, triptolide, is presently in clinical trials for the treatment of rheumatoid arthritis. Both plants grow very slowly and have been extensively exploited in China.
We have developed optimized extraction protocols for huperzine A and triptolide from very small amounts of starting material (as low as 10 mg fw). Spectrometric detection of bioactive metabolites in extracts obtained from various plant organs was achieved using a Rapid Resolution HPLC-QToF-MS system.

Experimental

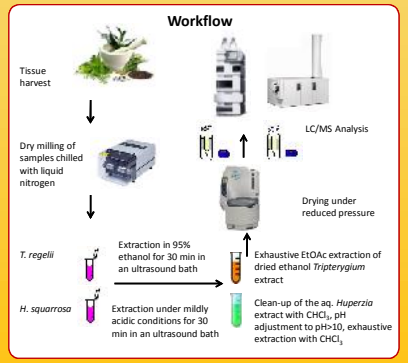
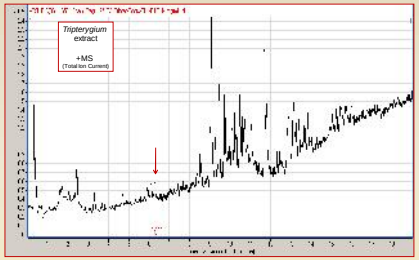


Figure 2: Major steps in extraction of huperzine A from *Huperzia squarrosa* and triptolide from *Tripterygium regelii*. Extraction protocols were optimized by comparing different solvents, pH, and vortexing vs. ultrasonic disruption to achieve maximum metabolite recovery.

Results and Discussion



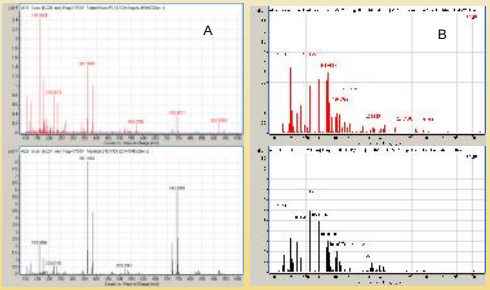


Figure 3: LC-QToF-MS chromatogram of *Tripterygium regelii* extract is shown above. The smaller images show: (A) MS spectra of the extract (above) and the triptolide standard (below) and (B) MS/MS spectra of the extract (above) and the standard (below) obtained using ESI in the positive polarity. Extraction was performed from 100 mg root tissue.

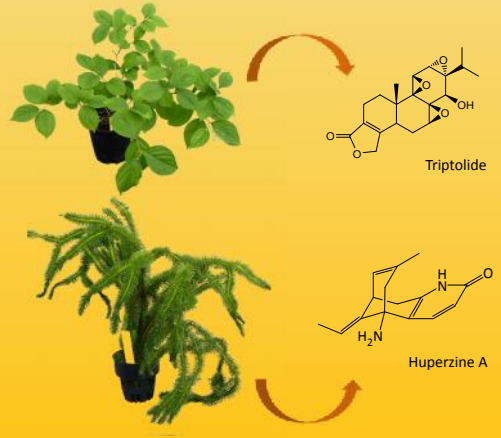
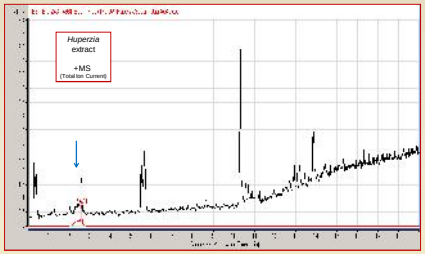


Figure 4: Molecular structures of triptolide, a diterpene isolated from the vine *Trypterigium regelii* and huperzine A, an alkaloid isolated from the clubmoss *Huperzia squarrosa*.

Results and Discussion



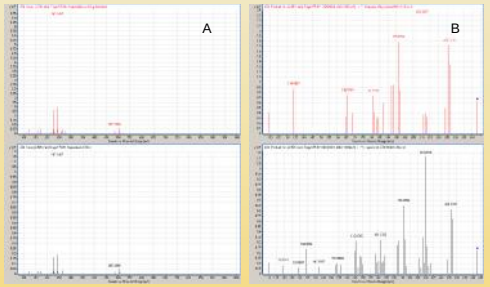


Figure 5:. LC-QToF-MS chromatogram of *Huperzia squarrosa* extract is shown above. The smaller images show: (A) MS spectra of the extract (above) and the huperzine A standard (below) and (B) MS/MS spectra of the extract (above) and the standard (below) obtained using ESI in the positive polarity. Extraction was performed from 10 mg sporophyte tissue.

Conclusions

- We optimized extraction conditions of active metabolites huperzine A and triptolide to very small amounts of starting materials.
- Ultrasonication, as opposed to prolonged vortexing, proved to yield higher concentrations of metabolites in the final extract
- The presence of huperzine A and triptolide in tissue extracts was confirmed using mass spectrometry and comparison with the MS and MS/MS spectra of authentic standards obtained using ESI in the (+) polarity.
- Total RNA extractions were performed from tissues actively synthesizing the metabolites as a step towards studying their biosynthetic pathway.

Acknowledgements

This research was conducted as a part of the project *Transcriptome Characterization of Plants Relevant to Human Health*, funded by the National Institutes of Health