Quick annotation of flavone glycosides from the *Sideritis hyssopifolia* using *in silico* metabolization

Axeille Aimond1,2, Coralie Audoin1, Yann Beauxis1, Kevin Calabro3, Olivier P. Thomas3, and Grégory Genta-Jouve1

1 C-TAC, UMR CNRS 8638 COMETE, Université Paris Descartes, 4 Avenue de l'Observatoire, 75006 Paris, France
2 Laboratoire Clarins, 5 Rue Ampère, 95300 Pontoise, France
3 Marine Biodiscovery, School of Chemistry and Ryan Institute, National University of Ireland Galway, H91 TK33 Galway, Ireland

Annotation of small molecules remains a challenge in untargeted metabolomics. During the last decade, the development of molecular networking, available through the freely accessible platform GNPS enables the annotation of known compounds using spectra libraries. The low number of spectra present in these databases is a limit towards the identification of unknowns, even when slight structural changes occur (oxidation, reduction, hydroxylation, etc.). In the present study, we applied the newly developed in silico metabolization tool MetWork to explore the chemical space of isocutellarein glycosides. A phytochemical study of the plant will complete and confirm the results by isolation of the compounds.

**Workflow used for the study**

**Plant material and Extraction process**

300 g of dried *Sideritis hyssopifolia* (cultivated at 1300 meters of altitude, in CLARINS domain in french Alps) were extracted with MeOH/DCM (1:1). The subsequent extract was then fractionated by C18-RP Vacuum Liquid Chromatography (VLC) and yielded 7 fractions of decreasing polarity.

**Why *Sideritis hyssopifolia*?**

For this study, we were interested by some polar compounds especially the flavonones described in Rodriguez-Lyon (2000). Indeed, these compounds are good model compounds as they can be easily ionized and their fragmentation leads to specific fragmentation patterns. An other advantage is that small modifications such as glycosylation or methylation make the diversity of these flavones which allowed us to show the efficiency of the new MetWork tool.

**Molecular networking and MetWork processing**

With the .mzXML data of DDA analysis, a molecular network of 7 fractions of *Sideritis hyssopifolia* was created on GNPS and then visualized in Cytoscape.

Based on MS2, the principle was to create clusters between ions with similar fragmentation patterns. Cosine parameter: 0.7

Thanks to the first steps, a single ion was selected to be metabolized in silico on MetWork.

**Phytochemical investigation**

After fractionation, the purification of the fractions obtained was performed on a preparative reverse phase HPLC JASCO (phenyl hexyl column) and allowed the isolation of several compounds. In fraction 4, the 4 flavones glycosides and 3 other not yet described for this species were purified. 2 flavone glycosides were isolated too. The structures of the molecules were elucidated by NMR and MS analyses.

**Results and Discussion**

After a comparison of the 50 structures obtained by MetWork and the 7 compounds isolated in the plant, a filter was applied on MetWork to finally obtained 26 structures for a 11 nodes cluster. One structure was found by MetWork but not isolated in the plant. 3 other nodes had several structures proposed. To make possible the selection of the right structure for each m/z, some filters have to be created in MetWork especially to determine the glycosylation site for the second sugar.

**Conclusion**

The use of MetWork was useful to find 10 structures of the nodes around the single ion chosen. 7 of these structures were confirmed by a phytochemical work and NMR and MS analyses, so the comparison of the structures with the two approaches was successful. The MetWork tool was confirmed as a good tool to help annotation of the nodes in a molecular network. The optimisation of the filtering with contrasts is ongoing and will help to refine the future annotations.

**Acknowledgments**

The authors thank the ANRT to support us with this project.

References:


**Appendix**

A molecular network was built and 7 of the isolated compounds appeared in the same cluster.

**Detected compounds**

Metabolization network: 50 structures obtained

**Annotation**

The use of MetWork was useful to find 10 structures of the nodes around the single ion chosen. 7 of these structures were confirmed by a phytochemical work and NMR and MS analyses, so the comparison of the structures with the two approaches was successful. The MetWork tool was confirmed as a good tool to help annotation of the nodes in a molecular network. The optimisation of the filtering with contrasts is ongoing and will help to refine the future annotations.