Phytochemical and biological research on *Herniaria hirsuta*

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1. Introduction

Urinary stone disease is considered as an important healthcare problem which affects 10–15% of the population. In the absence of prophylactic therapies, recurrence rates are as high as 50% within 10 years. An aqueous extract of the aerial parts of *Herniaria hirsuta* (Caryophyllaceae) is a widely used herbal medicine. Despite its proven activity against urolithiasis, little is known about the active compounds and the mechanism of action.[1,2] Previous phytochemical research on *Herniaria* species revealed the presence of saponins, flavonoids and coumarins.[2] It is suggested that gastrointestinal (GI) and/or hepatic metabolites of phytochemicals present in *H. hirsuta*, most likely saponin metabolites, are responsible for the beneficial effects.

2. Approach

As a first step in the quest for the active constituents, elaborate phytochemical research on the aerial parts of the dried herb was performed, using a comprehensive extraction.[3]

To avoid extensive *in vivo* studies, biotransformation of the lyophilized infusion of *H. hirsuta* was simulated using an *in vitro* GI model. A negative control sample, lacking enzymes and fecal suspension, and a blank sample, without infusion, were included. Samples were taken before and after the stomach phase, after the intestinal phase and after 2, 4, 6 and 24 hours of colon phase and analyzed with ultra-high performance liquid chromatography-photodiode array-high resolution mass spectrometry (UHPLC-PDA-HRMS), to monitor the relative abundances of the compounds over time.

A data-analysis workflow was optimized to render as much information as possible from the longitudinal LC-MS data. XCMS was used to convert the raw data into features via peak-picking followed by grouping. EDGE was used for extraction of significant differential profiles.[4,5] These time profiles were evaluated for the difference between sample, blank and negative control, expressed as q-values (false discovery rate (FDR) corrected p-values). To reduce the number of false positives among the significant results, a machine learning model was trained on the significant positive results. An app was built, called Tinderesting, to manually rate the importance of significant profiles. This model was used to reduce the amount of non-interesting profiles remaining in the negative mode data.

3. Results

Multiple flavonoids and saponins were (tentatively) identified based on analytical standards or spectral and chromatographic data. Besides the known saponins, 15 new saponins were tentatively identified as glycosides of (acetylated) medicagenic acid and zanhic acid.

Q-values indicating differences between time profiles obtained during *in vitro* simulation of GI biotransformation between sample, blank and negative control. The number of profiles with a q-value below an arbitrary 0.4 cut-off value was 16.4% of 10001 profiles and 27.7% of 12351 profiles in positive and negative mode respectively. LC-MS data showed a decrease in relative abundance of several compounds over time, especially in the colon phase. Additionally, the relative abundance of the aglycones of the saponins increased.

4. Discussion

Biotransformed phytochemical LC-MS data are complex and difficult to interpret. An automated data analysis workflow was developed for unbiased screening for metabolites and revealed the biotransformation of possible saponin prodrugs to their respective aglycones. Future perspectives will include *in vitro* microsomal S9 investigation on the additional effect of liver biotransformations. A well-characterized biotransformed extract will be evaluated *in vitro* for its activity against urinary stones.

References

1. Atmani F, Slimani Y, et al. Effect of aqueous extract from Herniaria hirsuta L. on experimentally nephrolithiasic rats. J Ethnopharmacol. 2004;95(1):87-93.
2. van Dooren I, Foubert K, et al. Saponins and Flavonoids from an Infusion of Herniaria hirsuta. Planta Med. 2016;82(18):1576-83.
3. Bijttebier S, Van der Auwera A, et al. Bridging the gap between comprehensive extraction protocols in plant metabolomics studies and method validation. Analytica chimica acta. 2016;935:136-50.
4. Storey JD, Xiao W, et al. Significance analysis of time course microarray experiments. PNAS 2005;102(36):12837-12842
5. Leek JT, Monsen E, et al. EDGE: extraction and analysis of differential gene expression. Bioinformatics 2005;22(4):507-508