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Identification of Fungal H\textsuperscript{+}-ATPase Inhibitors by Microfractionation and HPLC-HRMS-SPE-NMR

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Background - Fungal Fight

A large number of fungal proteins have been proposed as potential targets for novel antifungal agents.\textsuperscript{1} However, current available antifungal agents are primarily targeting the intracellular membrane biosynthesis\textsuperscript{2} and thus need to enter the fungus to act. In our search for novel and more efficient antifungal compounds, we are focusing on the plasma membrane (PM) H\textsuperscript{+}-ATPase enzyme as target.

Plants are exposed to a wide array of patho-pathogenic fungi in their natural habitat, and have been forced to develop antifungal metabolites in order to survive.\textsuperscript{3} Hence, as previously suggested by Monk and coworkers,\textsuperscript{4} it is reasonable to assume that some plants have the PM H\textsuperscript{+}-ATPase enzyme as target for the antifungal metabolites. However, plant extracts are very complex mixtures, and the traditional bioassay-guided fractionation used for identification of individual bioactive compounds are very time-consuming and suffer from inherent low resolution during the fractionation process. To circumvent this, we have developed a bioassay-guided screening for high-resolution bioassay profiles using a high-performance liquid chromatography (HPLC), high-resolution mass spectrometry (HRMS), solid-phase extraction (SPE) and nuclear magnetic resonance (NMR) system. In the present work, we report crude extract screening of 48 plant extracts - fungi PM H\textsuperscript{+}-ATPase inhibitors - followed by high-resolution bioassay and HPLC-SPE-NMR analysis for identification of individual bioactive constituents.

Results - Crude extract screening

\textbf{From 48 plants to 20 plants}

Extracts were tested in three different concentrations and those showing inhibition higher than 95% for all concentrations or a concentration-dependent activity profile were selected for semi-high-resolution screening.

Results - Semi-HR-screening

\textbf{From 20 plants to 2 compounds}

The 20 samples selected for semi-high-resolution screening (assay resolution: 2.66 data points per min) were assayed for their ability to inhibit the PM H\textsuperscript{+}-ATPase.

Results - HPLC-HRMS-SPE-NMR

Detailed analysis of HRMS and NMR data acquired via HPLC-HRMS-SPE-NMR analysis allowed optimized workflow.

Concluding remarks

- Thorough investigation of 48 plant extracts for fungal PM H\textsuperscript{+}-ATPase inhibitors led to identification of two active metabolites, i.e., Chebulagic acid (1) and Tellimagrandin II (2).
- Systematic combination of crude extract screening, high-resolution screening and HPLC-HRMS-SPE-NMR analysis allowed optimized workflow.
- High-resolution PM H\textsuperscript{+}-ATPase inhibition assay allows subsequent HPLC-SPE-NMR analysis to target bioactive constituents only.
- Cryogenic probe detection (1.7 mm) allowed characterization of metabolites (high PM H\textsuperscript{+}-ATPase inhibition) from analytical-scale HPLC of crude extract.

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References