Correlation of *Mt*SK Inhibitory Activity with Chemical Constituents in *Alpinia galanga* Identified by LC-MS

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Alpinia galanga, a plant in the Zingiberaceae family, has been reported to be active against *Mycobacterium tuberculosis*. Galangal has been used traditionally to treat many bacterial infections and a variety of other ailments by boiling the rhizome to make a tea or grinding it into a paste.

Among the compounds reported in galangal, 1´-s-1´acetoxychavicol acetate (ACA) is a known antitubercular compound. Based on the literature reports, hexane and dichloromethane (DCM) extracts of galangal were prepared and tested in this study for inhibition of the *Mycobacterium tuberculosis* shikimate kinase (*Mt*SK). *Mt*SK catalyzes the fifth reaction of the *Mt*SK pathway to produce shikimate-3-phosphate (S3P). S3P production was measured using Liquid Chromatography-Mass Spectrometry Quadruple-TOF (LC-MS Q-TOF). These extracts, along with ACA standard compound and rottlerin, a known SK inhibitor, were tested for their inhibitory activity against *Mt*SK at concentrations of 50 µg/mL and 50 µM, respectively. The hexane extract displayed the highest *Mt*SK inhibition of 47% whereas DCM extract, rottlerin and ACA were all categorized as inactive with *Mt*SK inhibition rates less than hexane extract. The screening results suggest that ACA may not work through this mechanism of action. The focus of the research was then transitioned to identifying putative, previously undocumented compounds in *A. galanga* that work within ACA's molecular network. This information will assist future researchers assessing ACA's antitubercular properties.

The documentation of these compounds was performed through a variety of software and experimentation. A combination of Global Natural Products Social Molecular Networking and Mass Professional Profiler (MPP) software was used in the chemical profiling of the potential compounds, otherwise known as bioactives. The MPP analysis was able to produce a Venn Diagram displaying the number of unique compounds in each extract and which were present in both extracts. This is shown in Figure 1. Twelve known compounds have been potentially identified in the extract. Among them, six compounds (genistein, pinocembrin, kaempferide, naringenin, 4-hydroxybenzaldehyde and 3-O-acetylepinobaskin) occurred in *A. galanga* and *A. katsumadai*. The remaining compounds have not been previously documented as being present in *A. galanga* or its family, and three compounds (acacetin, naringenin, and alantolactone) have been selected for confirmation of presence in the extract. LC-MS-based chemical fingerprinting and profiling of the bioactives is in progress for comparison against standard compound spectra to confirm the presence of these chemical constituents in *A. galanga*. The major impact of this work in natural products drug discovery is that it serves as an example of the combined application of mass spectrometry and chemoinformatic tools to identify bioactives, with the broad goal being the identification of new antitubercular compounds that could be used to treat multi and extensively drug resistant strains of *Mycobacterium tuberculosis*.

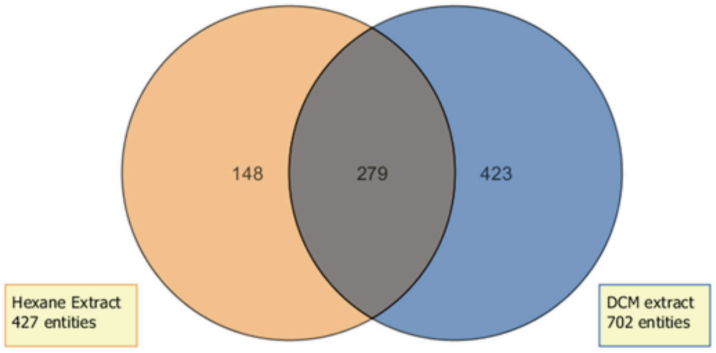

Figure 1: Identification of unique bioactive compounds in the hexane extract of *A. galanga* using Mass Professional Profiler software.

Statement of Research Advisor

Madison had to carry out extensive literature searches to design the extraction procedure of *A. galanga* to obtain extracts rich in the antitubercular constituents, perform *Mt*SK inhibitory activity screening, and identify bioactives using mass spectrometry and chemoinformatic tools. She has established a platform for more detailed studies on natural products biochemometrics of *A. galanga*.

– Angela Caldéron, Drug Discovery and Development