Integration of Bioactivity – Global Natural Product Social Molecular Network (GNPS): Proof of Conception Açaí

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Euterpe oleracea Mart, commonly known as açaí, is a fruit present in botanical dietary supplements (BDS) frequently taken by cancer patients. The açaí extracts have cancer-related properties: antioxidant, antiproliferative, cytoprotective, and anti-inflammatory. The goal of my research is to prove that Global Natural Product Social Molecular Network (GNPS) can be integrated with a bioactivity tool and produce a visual molecular network for açaí and identify known and unknown compounds related to these anti-cancer properties. GNPS is a cutting-edge software program which has been integrated with bioactivity in our laboratory.

This method compares two different commercially available açaí supplements prepared using acidic methanol. The extraction procedure consisted of maceration of the capsule powder, followed by sonication and evaporation of the solvent. Liquid chromatography-mass spectrometry (LC-MS) was used to identify the mass of compounds from the two different acidic methanol extracts of açaí formula 1 (F1) and formula 2 (F2). Parallel artificial membrane permeability assay (PAMPA) mimics a human gastric intestinal track and shows how much of the extract is passively diffused, on the acceptor side and non-passively diffused, on the donor. PAMPA was done at a range of concentrations (1,000-1.95 µg/mL), which accounts for human relevant doses (4.494-6.557 µg/mL) and potential overdose. CYP3A4 is a major liver enzyme that metabolizes many anticancer drugs; the donor and acceptor site compounds have been tested for inhibition of hepatic CYP3A4. The CYP3A4 inhibition assay was analyzed through LC-MS of each extract formulation by measuring the amount of metabolite made in the presence of açaí extract. The last step is to compline all the data collected from LC-MS and MS/MS and upload it to GNPS. GNPS is the core computer program used, but other software programs also aid in the process (MZmine, Rstuido, and Cystoscope) of creating a visual bioactive molecular network.

The LC-MS identified which compounds were present in each extract formulation and compared to a chemical fingerprinting library of all açaí compounds previously identified. The two formulations, even though both aqueous extracts of açaí, had very different chemical compositions. BDS are commonly not standardized or strictly regulated. CYP3A4 inhibition was anticipated for F1, since we had also observed CYP3A4 inhibition previously with the methanol extracts. Inhibition by F2 was not as well observed; the acceptor side had no inhibition and the donor side had very slight inhibition. Figure 1 shows how all the data collected can be integrated to generate a bioactive visual molecular network for açaí acidic methanol extracts. This figure identifies the mass of each compound, along with if it is known or unknown, and the ones that have high bioactivity and low bioactivity.

This research shows that Integrated Bioactivity – Global Natural Product Social Molecular Network (GNPS) can

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differentiate known and unknown natural products from a botanical extract and aid in the creation of a visual molecular network of bioactive açaí constituents. In the future, the. GNPS-Bioactivity tool can be used for other botanical dietary supplements and the isolation of high bioactive compounds.

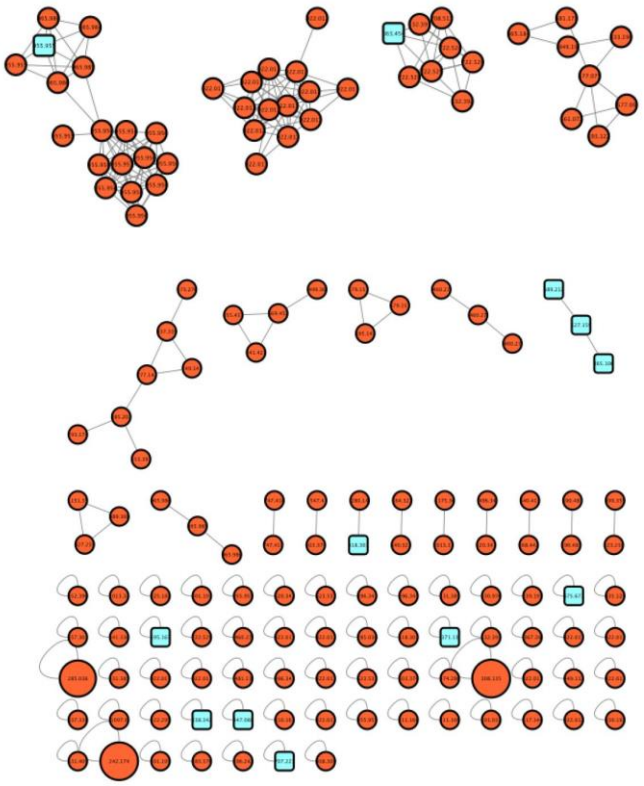

Fig. 1. Visual molecular network produced with GNPS, blue squares are known & orange circles are unknown

Statement of Research Advisor

Madeline has learned to perform LC-MS analysis of botanical dietary supplements and analyze data using Integrated Bioactivity – Global Natural Product Social Molecular Network (GNPS) for identification of CYP3A4 inducers in açaí. Madeline developed strong natural products chemistry literature reading and problem-solving abilities. Her contribution was critical to providing an example of a shorter and less laborious process to identify bioactive compounds in complex botanical extracts.

- Dr. Angela I. Calderón, Harrison College of Pharmacy

Authors Biography

Madeline Kunze is a junior-year student pursuing a B.S. degree in Chemical Engineering at Auburn University. She has played key research roles in integration of GNPS with the bioactivity tool and in writing a review paper about the benefits of traditional medicine for anticancer treatment.

Kabre L. Heck is a Graduate Teaching Assistant from the Harrison College of Pharmacy in the Department of Drug Discovery and Development. Kabre received her undergraduate degree in Medical Studies from Lenoir-Rhyne University in 2019 and is currently pursuing a PhD in Pharmaceutical Sciences with an emphasis in Medicinal Chemistry. In the Natural Products Laboratory of Dr. Calderón, Kabre investigates potential interactions between pharmaceuticals and botanical dietary supplements and utilizes liquid chromatography-mass spectrometry for quantitation of metabolites and performing structural elucidation.

Angela I. Calderón, Ph.D. is an Associate Professor in the Department of Drug Discovery and Development, Harrison School of Pharmacy, Auburn University. She received her degrees in Pharmacy and Pharmacognosy from the University of Panama, Panama, the University of Illinois at Chicago, and the University of Lausanne, Switzerland. Dr. Calderón specializes in natural drug products chemistry. She works to apply mass spectrometry to natural products drug discovery, and quality and safety assessment of botanical dietary supplements. Dr. Calderón enjoys educating graduate and undergraduate students as the next generation of natural products researchers. with unique skills in mass spectrometry.