Antioxidant activity and Chemical Composition of Acai Berries from Different Locations

Dr. Nadja Cech (Mentor)

Ashleigh Musso (Undergraduate Student)

This statement certifies that both the student (Ashleigh Musso) and faculty mentor (Dr. Nadja Cech) were involved in the development of this OUR URCA award application.

A. Project Goals

Many of the deadliest human diseases, including heart disease, cancer, Alzheimer's disease, and even aging itself [1] are linked to oxidative stress. Research suggests that the consumption of various antioxidant-rich foods, particular darkly colored fruits and vegetables, can help overcome oxidative stress and aid in the treatment or prevention of disease. One food with purported antioxidant properties is the Amazonian super fruit "acai" (*Euterpe oleracea*). Several studies suggest health benefits associated with the consumption of acai [2-4]. However, the specific cellular mechanisms by which these effects occur, and the chemical components responsible, are not known. This lack of knowledge is a problem because it prevents acai from being fully utilized to improve human health.

Recently, Dr. Gregory Raner (Principal Investigator) and Dr. Nadja Cech (Co-Investigator) in the UNCG Department of Chemistry and Biochemistry were awarded a grant from the National Institutes of Health to study the antioxidant properties of acai berry. In the early stages of conducting this work, we realized that there is considerable variation in the composition of acai berries depending on their geographic source and method of preparation. We have identified one source of acai berries with particularly favorable biological activity, and the goal of this URA proposal is to determine what chemical characteristics of acai berries from that source make it more effective. This is a side-project to our main grant proposal, and makes an excellent undergraduate research project because it is interesting and important but also tractable.

B. Methods the student will utilize to achieve goals

Acai (*Euterpe oleracea*) berries have already been obtained from two different sources. Genetic analysis and morphological evaluation of voucher specimens have confirmed correct identification of genus and species for these samples. An additional set of 5 different *E. oleracea* samples will be obtained from commercial sources, and methanolic extracts will be prepared from these samples and from freeze dried berries from the other two sources (which are currently maintained at -80 ºC awaiting further analysis).

High performance liquid chromatography (HPLC) coupled to electrospray ionization mass spectrometry (ESI-MS) will be employed to characterize the extracts from the 7 different batches of *E. oleracea* berries. These analyses will be conducted in the Cech laboratory. The data will be analyzed both in a targeted fashion (focusing on known anthocyanins, thought to be active constituents) and an untargeted fashion (using principal component analysis) to identify compounds that are similar or different among the extracts. These results will then be compared with biological assay data to identify chemical signatures of the most biologically active extracts. Our ultimate goal is to determine whether biological activity correlates with the compounds that have been reported to have antioxidant activity, and to identify additional biologically active compounds.

Biological assays will be conducted in the Raner laboratory to evaluate the antioxidant activity of each extract and to provide data that can be compared with the analytical data, as described above. These experiments will employ a HepG2 cell line transfected with an Nrf2-ARE (antioxidant response element) reporter. This cell line is used regularly in the Raner laboratory for the purpose of screening extracts and fractions for antioxidant activity.

C. Role of faculty mentor

Dr. Cech will oversee the project and will meet weekly with Ashleigh to discuss progress and plan future experiments. When problems arise, she will give suggestions for how experimental design can be modified, and help with troubleshooting. Dr. Cech will arrange meetings with the Raner group as necessary and will review reports from Ashleigh prior to submission to the Raner group. She will draft an outline of the manuscript that is expected to be an outcome of this project, will edit Ashleigh's contributions, and will coach her through the submission process. Dr. Cech has been involved with submission of manuscripts with undergraduate coauthors on numerous previous occasions. Ashleigh has strong writing skills and has previously written excellent laboratory reports about her other work in the Cech laboratory.

D. Role of the Student

Ashleigh Musso has been working in the Cech laboratory for 1 year. So far she has collaborated with another student to complete data acquisition for a paper. This project will be her first independent project. Ashleigh has proven herself to be productive and independent in the laboratory, and has generated a nice publication-quality dataset. She is a very responsible student (GPA 3.93) and an active member of the Cech laboratory.

Ashleigh will be responsible for conducting the experiments described under methods. She will perform all chemical analysis and interpret the resulting data (with help from Dr. Cech and other members of the Cech laboratory). Ashleigh will attend weekly laboratory meetings with other members of the Cech group and present her findings. She will assist a graduate student (PhD level) in the Raner laboratory with the biological assays, and will be involved in interpreting the data from these assays. This cross-disciplinary research experience (working in an analytical laboratory and a biological laboratory) will be excellent training for Ashleigh. At the end of the summer, a report of all observations, calculations, and data will be prepared by Ashleigh and shared with the Raner group. Ashleigh will be a coauthor on any publications that use these data. Ashleigh will present the results of her research at the UNCG undergraduate research symposium in spring of 2015.

E. Benefits to the faculty mentor

This research is directly related to (although not part of the original aims of) an NIH grant that was recently funded to support ongoing research in the Cech and Raner groups. Dr.

Cech and Dr. Raner have worked together extensively in the past, and have previously co-supervised a number of graduate and undergraduate students and coauthored several publications [5, 6]. This collaboration is beneficial to both the Cech and Raner groups, as it enables free exchange of data and ideas that become future publications and grant proposals. Ultimately, the project proposed for Ashleigh will benefit the mentor (Cech) by enabling further collaborations with the Raner group and by providing data to support a publication that will demonstrate progress on funded research. Currently, there are not sufficient funds in the NIH funded project budget to support a summer student from the Cech group. Thus, funding from the URA will enable experiments that would not otherwise be possible and will facilitate an expanded role for the Cech group in this interdisciplinary project.

F. Benefits to the student

Ashleigh Musso is a junior undergraduate in the UNCG Department of Chemistry and biochemistry who will be graduating in May of 2015. Ashleigh plans to enroll in medical school and pursue an MD degree. Thus far, the research Ashleigh has conducted in the Cech group has been analytically focused but has not had a biological or biomedical component. By engaging in the proposed URA project, Ashleigh will gain an understanding of methods involved with medically related research. The critical thinking and research skills that she learns by taking part in research will also be beneficial in medical school and beyond.

G. Justification of the award amount

We request $3,000 total in funds to support a stipend for Ashleigh Musso during summer of 2014. Ashleigh is a 1st generation college student and works to pay for her living expenses and tuition. The requested stipend will enable her to spend her summer working in the research laboratory rather than in a job unrelated to her academic goals. The proposed project is very demanding in time for sample preparation, data collection, and data analysis. Finishing this project will require Ashleigh to work full time during summer 2014. Additional funds needed to pay for project expenses will be supported by an NIH grant "Antioxidant activities of freeze dried acai berry (*Euterpe oleracea*) extracts" (Gregory Raner, Principal Investigator, Nadja Cech, Co-Investigator).

References

1. Valko, M., D. Leibfritz, J. Moncol, M. Cronin, M. Mazur, and J. Telser, Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*, 2007. **39**(1): 44-84.

2. Schauss, A.G., X. Wu, R.L. Prior, B. Ou, D. Huang, J. Owens, A. Agarwal, G.S. Jensen, A.N. Hart, and E. Shanbrom, Antioxidant Capacity and Other Bioactivities of the Freeze-Dried Amazonian Palm Berry, Euterpe oleraceae Mart. (Acai). *54*, 2006. **22**: 8604–8610.

3. Jensen, G.S., X. Wu, K.M. Patterson, J. Barnes, S.G. Carter, L. Scherwitz, R. Beaman, J.R. Endres, and A.G. Schauss, In Vitro and in Vivo Antioxidant and Anti-inflammatory Capacities of an Antioxidant-Rich Fruit and Berry Juice Blend. Results of a Pilot and Randomized, Double-Blinded, Placebo-Controlled, Crossover Study. *J. Agric. Food Chem.*, 2008. **46**(18): 8326–8333.

4. Mertens-Talcott, S.U., J. Rios, P. Jilma-Stohlawetz, L.A. Pacheco-Palencia, B. Meibohm, S.T. Talcott, and H. Derendorf, Pharmacokinetics of Anthocyanins and Antioxidant Effects after the Consumption of Anthocyanin-Rich Açai Juice and Pulp (Euterpe oleracea Mart.) in Human Healthy Volunteers. *J. Agric. Food Chem.*, 2008. **56**(17): 7796-7802.

5. Cech, N.B., K. Tutor, B.A. Doty, K. Spelman, M. Sasagawa, G.M. Raner, and C.A. Wenner, Liver enzyme-mediated oxidation of Echinacea purpurea alkylamides: Production of novel metabolites and changes in immunomodulatory activity. *Planta Med.*, 2006. **75**(15): 1372-1377.

6. Raner, G.M., S. Cornelious, K. Moulick, Y. Wang, A. Mortenson, J. Hill, C. Cunningham, and N.B. Cech, Effects of Herbal Products and their constituents on Human Cytochrome P4502E1 Activity. *Food Chem. Toxicol.*, 2007. **45**: 2359-2365.