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1-1-2017

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Setzer, William N., "Cherokee Traditional Medicine: Natural Products from *Nyssa sylvatica*" (2017). *RCEU Project Proposals*. 259.

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Cherokee Traditional Medicine: Natural Products from *Nyssa sylvatica*

A Proposal for the Research or Creative Experience for Undergraduates (RCEU) Program Summer 2017

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Project Summary: The bark infusion from *Nyssa sylvatica*, “blackgum tree”, was used by the Cherokee Native Americans to treat fevers, cancer, gonorrhea, wounds, urinary tract infections, and intestinal worms.^{1,2} We hypothesize that there are biologically active compounds in the bark that can be used to treat infectious diseases. The overall goal of this research is to isolate and identify biologically active natural products from the bark extract of *N. sylvatica*. There have apparently been no previous studies on this tree in regard to biological activity screening or natural products characterization. The research will involve collecting and extracting *N. sylvatica* bark with chloroform and with methanol, screening the crude bark extracts for biological activity (antibacterial, antifungal, cytotoxic, antiparasitic activities), carrying out activity-directed preparative chromatography, and isolating and identifying bioactive compounds. ***We are very experienced in bioactivity screening,³ natural product isolation, purification, and structure elucidation.⁴***

The student will be supervised by Dr. Setzer every day during the conduct of this research. Dr. Setzer’s office (MSB 315) is directly across the hall from the laboratory (MSB 312) and next door to the departmental computer facility (MSB 317), so he will be available at all times during the day and evenings for consultation. The student’s work will not only fit directly into our overall research in natural products drug discovery, but also is a stand-alone project with the scope suitable for undergraduate summer research; the plant, extraction, isolation, activity screening, and structure determination will belong to the student. Dr. Setzer has a good track record in working with undergraduate researchers (more than 170 individuals at UAH) and publishing their results (56 peer-reviewed publications based on undergraduate research have appeared since 2010).

Student Prerequisites: There are no coursework or academic standing prerequisites. We will instruct and oversee all the student needs to carry out the project. This project does organic solvents and chemicals; some understanding of chemical handling and safety would be beneficial.

Student Duties:

Collection and Extraction of *Nyssa sylvatica*. *N. sylvatica* is a ubiquitous tree in our area; collection will not be problematic. The student will collect, chop, and dry the bark. The student will carry out extraction of dried bark using solvent extraction (Soxhlet extraction using chloroform and then methanol). The collection and extraction of plant materials will be carried out under the direct supervision of Dr. Setzer.

Isolation of Bioactive Compounds. The student will carry out chromatographic separation using flash chromatography.⁴ Bioactivity screening on the crude extract and chromatographic fractions will be carried out in our laboratories in the Shelby Center (SST 324).³ Purification of bioactive compounds will be carried out using various purification techniques (usually recrystallization or chromatography).

Structure Elucidation of Compounds. The RCEU participant will collect and be involved in interpretation of spectroscopic data. We will utilize mass spectrometry (MS), infrared spectrophotometry (IR), and nuclear magnetic resonance (NMR) spectroscopy.⁴ The student will work very closely with Dr. Setzer collecting spectral data as well as interpretation to determine structures. In addition to hands-on experiences with the spectroscopic instruments (IR, NMR, MS), the student will become familiar with computer modeling of structure and spectra utilizing several software packages that we have available in our department.

Manuscript Preparation. Dr. Setzer encourages all undergraduate student researchers to write up their results in the form of a manuscript for publication. The RCEU participant, under the supervision of Dr. Setzer, will help prepare the manuscript(s), which may include data from other undergraduate or graduate students.

Expected Results and Deliverables. The *N. sylvatica* bark will be collected at the beginning of the summer, the bark chopped and air-dried. Initial preparative flash chromatographic separation of the extract will take approximately one week. Additional chromatographic separation of crude fractions is very variable and depends on the number of components in the mixture, the solvents needed for the separation, and whether the components are crystalline or not. Spectroscopic data for purified compounds are collected as the materials are purified.

Mentor Supervision and Interaction: Collection and extraction of plant materials, bioactivity screening, spectroscopic measurements and spectral interpretation, and manuscript preparation will be supervised by Dr. Setzer. We will hold regular group meetings each week. Note: In addition to the bioactivity screening we do here at UAH, we also collaborate with several other groups for additional screening (antileishmanial, antitrypanosomal). This project is particularly suitable for an undergraduate summer researcher and will provide hands-on experience with several laboratory techniques not typically encountered as well as experience with modern analytical instrumentation.

References

1. Cozzo DN. Ethnobotanical classification system and medical ethnobotany of the Eastern Band of the Cherokee Indians. Ph.D. dissertation, University of Georgia, 2004.
2. Hamel PB, Chiltoskey MU. *Cherokee Plants and Their Uses – a 400 Year History*. Stephens Press, Asheville, NC, 1975.
3. Setzer MC, Newby JS, Moriarity DM, Setzer WN. A phytopharmaceutical survey of Abaco Island, Bahamas. *American Journal of Essential Oils and Natural Products*, **2015**, 2(5), 10-17.
4. Dosoky NS, Moriarity DM, Setzer WN. Phytochemical and biological investigations of *Conradina canescens* A. Gray. *Natural Product Communications*, **2016**, 11, 25-28.