Apr 1st, 11:00 AM - 2:00 PM

Evaluating Antioxidant Activity of Selected Plant Species Native to Cedarville, Ohio

Daniel A. Benson
Cedarville University, danielbenson127@cedarville.edu

Alexander P. Treide
Cedarville University, atreide@cedarville.edu

David Woodfield
Cedarville University, dwoodfield@cedarville.edu

Joshua A. Sitler
Cedarville University, joshuasitler@cedarville.edu

Denise S. Simpson
Cedarville University, dsimpson@cedarville.edu

See next page for additional authors

Presenters
Daniel A. Benson, Alexander P. Treide, David Woodfield, Joshua A. Sitler, Denise S. Simpson, and Robert L. Paris

Evaluating the Antioxidant Activity of Selected Plant Species Native to Cedarville, Ohio

Daniel Benson[1], Alex Treide[1], David Woodfield[1], Joshua Sitler[1], Denise S. Simpson[2], and Robert L. Paris[1]

[1] Department of Science and Mathematics, [2] Department of Pharmaceutical Sciences, Cedarville University

INTRODUCTION

Over the past several decades, there has been an increase in the number of synthetic drug molecules developed and utilized to treat various conditions. Although these synthetic drugs have proven useful, there has been growing public concern regarding the potentially negative long-term effects of synthetic agents on the body. As a result, there has been an increased interest in identifying and utilizing plant extracts and purified compounds since they are perceived to be a more natural alternative to synthetic drugs.

The goal of this study was to evaluate the specific antioxidant properties of alsike clover, *Trifolum hybridum,* when grown under differing environmental conditions. The alsike clover was collected from the campus of Cedarville University, Cedarville, Ohio for testing. Alsike clover was removed from the field in January 2013, and transplanted indoors under grow lights for 14 days. These plants were then subjected to three separate 60-day treatments: control treatment - watering to field capacity with no fertilizer; positive treatment - watering to field capacity with fertilizer; and negative treatment - half of the water given to the field capacity treatment with no fertilizer.

The rationale for choosing these different treatments was to evaluate the effects of specific growing conditions on bioactive secondary metabolite production in alsike clover. The biological evaluation was accomplished by conducting diphenylpicrylhyrazyl (DPPH) free-radical scavenging and Folin Ciocalteu assays to assess the concentration of polyphenolic compounds. Results from these experiments indicate that the biological and chemical profiles of alsike clover can be influenced by the environmental conditions under which the plants are grown.

METHODS

DPPH Assay (assessing antioxidant activity)

Preparation of DPPH solution

- A 0.25% (w/v) solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared in methanol and stored protected from light.

Preparation of gallic acid solutions

Gallic acid was used as a standard.

- A gallic acid stock solution of concentration 1 mg/mL was prepared in methanol.
- Gallic acid calibration standards were prepared by diluting the stock solution to give the required concentrations.

Preparation of ascorbic acid solutions

Ascorbic acid was used as a reference.

- An ascorbic acid stock solution of concentration 1 mg/mL was prepared in water.
- Ascorbic acid reference solutions were prepared by diluting the stock solution to give the desired concentrations.

Preparation of plant extracts

- Plant extract stock solutions of concentrations 1 mg/mL were prepared in methanol.

DPPH assay procedure

a) 200 µL of the gallic acid solutions of concentrations 10, 20, 40, 80, and 100 µg/mL and the test extracts were added to a 96-well plate followed by 5 µL of the freshly made DPPH solution.
b) Blank experiments were carried out using 200 µL of the test solutions and 5 µL of MeOH to the 96-well plate.
c) The experiments were done in triplicate.
d) Plates were incubated in the dark for 30 minutes at 25 °C and then read at 520 nm on a Promega Glomax®-Multi Detection System.

DPPH calculation

I% inhibition of free radical = [Abs(control)- Abs (sample)/Abs control] x 100

Folin-Ciocalteu Assay

Preparation of 7.5% w/v sodium carbonate solution

- A 7.5% w/v solution of sodium carbonate was prepared in deionized water

Preparation of diluted Folin-Ciocalteu reagent solution

- A 10% Folin-Ciocalteu solution was prepared by diluting the commercially available reagent in deionized water

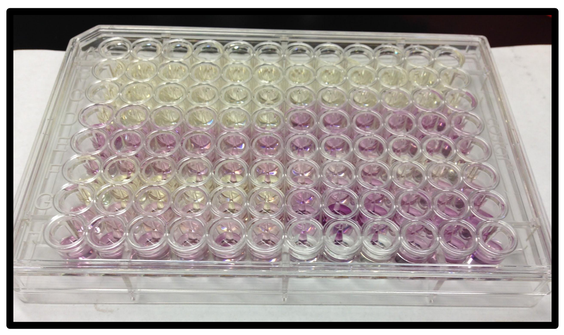
Preparation of gallic acid calibration standards

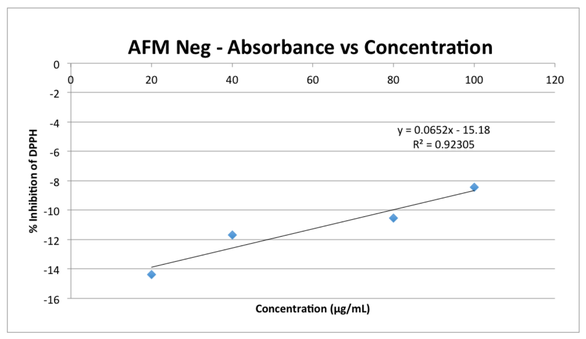
- Gallic acid stock and standard solutions were prepared as described above.

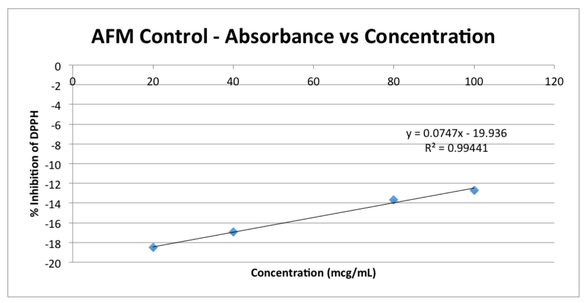
1. Gallic acid solutions (20 uL) of concentration 100, 200, 400, and 500 ug/mL were added to individual wells in triplicate
2. 20 uL of extract at concentrations of 20, 40, 80, and 100 ug/mL were plated in triplicate
3. Control samples were prepared with sample solution (20 uL) and deionized water (200 uL) only
4. Blank samples were prepared with Folin-Ciocalteu reagent (100 uL), sodium carbonate (100 uL) and water (20 uL) only
5. 100 uL of Folin-Ciocalteu reagent was added to each well. The plate was swirled to mix the reagents and then allowed to stand for minutes at room temperature
6. 100 uL of 7.5% w/v sodium carbonate solution was then added to each well
7. The plate was incubated at room temperature in the dark for 30 minutes
8. Absorbance was read at 750 nm

RESULTS

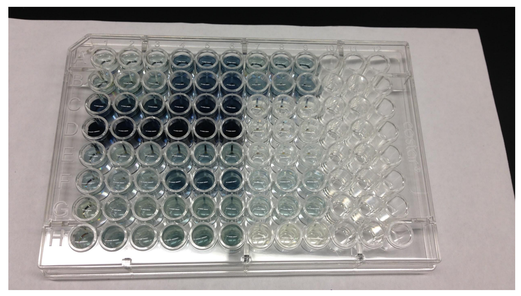
DPPH Data

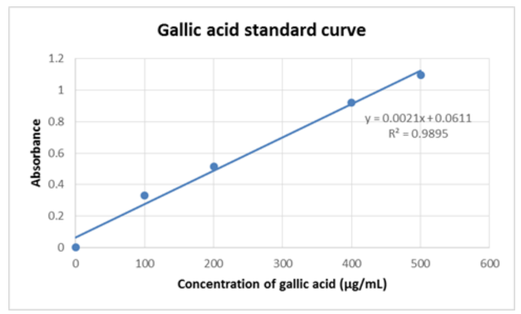


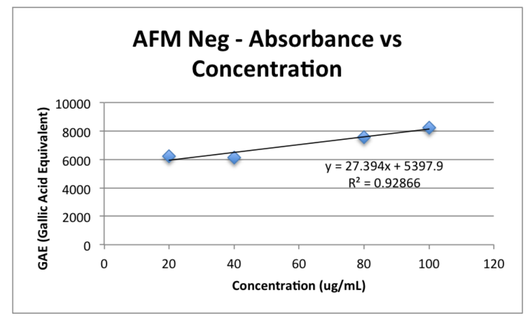


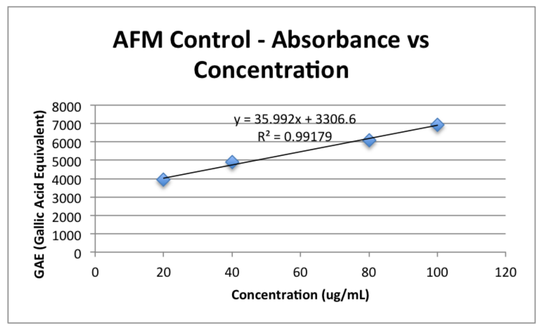


Folin-Ciocalteu Data









DISCUSSION

Antioxidants are important for countering oxidative stress that has been linked to pathological processes such as cancer, injury to cells and Alzheimer's Disease. Both the total phenolic content and DPPH free radical scavenging assays are indicators of antioxidant activity.

The results of this study indicate that the biological activity of plants can be optimized by altering the environment in which the plants are grown. The DPPH assays suggest that the percent inhibition varies with the growing conditions of the plants. The Folin assays also indicate an increase in polyphenolic compounds that was proportional to the concentration of plant extract used. However, we were not able to establish a clear correlation between growing conditions, the level of polyphenolic compounds, and antioxidant ability of the extracts.

Future Direction

This research project has allowed us to examine the biological activity of a select number of species under varying environmental conditions. Additional collected samples, such as honeysuckle, alsike clover, white clover, and red clover, will be investigated over the coming semesters using DPPH, Folin-Ciocalteu, and FRAP assays.

ACKNOWLEDGEMENTS

Financial support from the Department of Science and Mathematics and the School of Pharmacy, Cedarville University is gratefully acknowledged.

REFERENCES

1. McLaughlin, J., Lingling, R., & Anderson, J. (1998). The use of biological assays to evaluate botanicals. *Drug Information Journal*, *32*, 513-524.
2. Herald, T. J., Gadqil, P., & Tilley, M. (2012). High-throughput microplate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *J. Sci. Food Agric., 92,* 2326-2331..