“*Nyssa Sylvatica* (Black Gum) Bark: Bioactivity and Extraction”

Kyle Albert

Research Advisor: Dr. Diep Ca

Department of Chemistry

Shenandoah University

Winchester, Virginia

Abstract

The *Nyssa Sylvatica* (known as Black Gum) tree bark is rumored to be used by Native American tribes to treat dysentery and parasitic infections. However, no scientific reports have been found on the bark.

The goal of this project was to develop a new method of extraction, perform an extraction of the bark using solvents of different polarities, determine the total polyphenols, 331.2 mg Gallic-acid/g crude extract, and flavonoids, 16.2mg Rutin/g of crude extract, present in the bark of the Black Gum tree, and assess if the bark extract possess any biological activity.

Introduction

With the Black Gum bark rumored to treat dysentery and parasitic infections, a bioassay must be done to assess the accuracy of this statement. Due to the lack of access to a microplate reader a brine shrimp based bioassay will be used in substitute. The bark must first be extracted however, and with the time constraints of one summer and the goal of extracting with three different solvents, a new method has been developed. The new method utilizes sonication during extraction to make the process quicker without sacrificing quantity or quality of the plant extract. Analyzing the bark extracts using HPLC allows for Chlorogenic-Acid Derivatives (CGA's) to be identified. CGA's contribute towards compounds having anti-carcinogenic and antioxidant properties. Similarly, polyphenols and flavonoids also contribute towards anti-carcinogenic and antioxidant properties. If the bark possesses high concentrations of CGA's or polyphenols and flavonoids it should be biologically active.

Experimental

New Method of Extraction

The conventional method of extraction is three rounds of 72 hours of solvent exposed to the ground bark, followed by filtration and vacuum-evaporation. The crude extract is then fractioned with solvents with different polarities.

We developed a new method, in which the ground bark was extracted directly with three different solvents, each with different polarities. The ground bark went through three rounds of two hours of solvent exposed to the bark in a sonicating bath, separating bark from plant extract, and evaporation of solvent from plant extract utilizing vacuum evaporation. To test the effectiveness of this new method, the same procedure is repeated but with three hours of bark exposed to the solvent.

Extraction

The extraction was performed with n-hexane, Ethyl Acetate, and Methanol, as was the protocol by Julinton Sianturia and Hideo Hayashic (1). The dried and ground up bark was exposed to three rounds of n-hexane while in a sonicating bath, filtering the hexane extract from the bark between each exposure. The hexane extract underwent vacuum evaporation until dry and was suspended in hexane after recording the mass.

This process was repeated with Ethyl Acetate and then Methanol.

The entire extraction process is repeated with three hour extraction times instead of two hour.

Determination of Polyphenols and Flavonoids

Methods modified from "Total Phenolics and Flavonoids…" (2) and "Development and validation…" (3) to obtain Gallic-Acid and Rutin equivalents; to evaluate the Polyphenol and Flavonoid content of the bark extracts respectively.

Brine Shrimp Bioassay

A brine shrimp bioassay was performed to assess the biological activity of the plant extracts. One gram of *Artemia Salinia* were hatched in salt water, and were exposed to three different concentrations of plant extracts for 24 hours. The lethality percent, percentage of shrimp that died after 24 hours of exposure, was then calcualted.

HPLC Analysis

The HPLC analysis of the extract was performed with acetonitirile and DI water, both of which contained 1% formic acid.

Results and Discussion

Bark Extraction

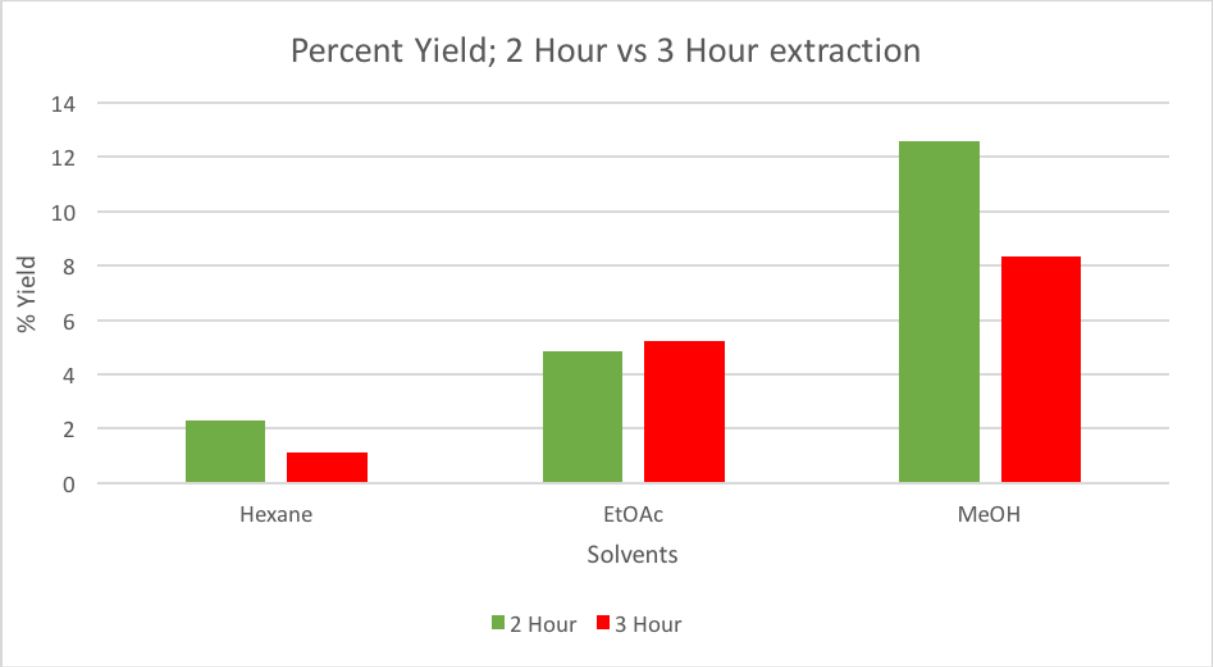

Figure 1. Percent yields of the 2 Hour or 3 Hour extraction time with each solvent

The two hour extraction had a higher percent yield of crude extract for Hexane and MeOH but not for EtOAc, however during the three hour extraction the amount of bark and solvent used was doubled which may have impacted the results.

Polyphenols

	2 Hour	3 Hour
Solvent	**Polyphenols (mg)/crude extract (g)**	**Polyphenols (mg)/crude extract (g)**
Hexane	9.203	Undetectable
EtOAc	182.170	80.231
MeOH	139.9	139.0

Table 1. Polyphenols content (mg/g crude extract) of the 2 Hour and 3 Hour extractions with each solvent.

The two hour extraction was more effective than the three hour extraction at obtaining polyphenols from the bark. The EtoAC extracted significantly more polyphenols during the two

hour extraction than the three hour extraction. Polyphenols are measured as a Gallic-acid equivalent.

Flavonoids

	2 Hour	3 Hour
Solvent	**Flavnonoids (mg/g crude extract)**	**Flavnonoids (mg/g crude extract)**
Hexane	Undetectable	Undetectable
EtOAc	8.663	12.657
MeOH	Undetectable	3.543

Table 2. Flavonoids content (mg/g crude extract) of the 2 Hour and 3 Hour extraction and each solvent

The three hour extraction was more effective than the two hour at extracting flavonoids and EtOAc is the most effective solvent for obtaining flavonoids. The flavonoids are expressed as a Rutin equivalent.

Brine Shrimp Bioassay results

Results (% Dead)				
	Blank	**500ppm**	**1000ppm**	**5000ppm**
MeOH	0	10	0	10
EtoAc	8	0	20	100
Hexane	18	0	40	100

Table 3. Percent of brine shrimp that died in each solvent at each concentration

The Brine shrimp bioassay yielded no significant results, and it's likely that 100% of the shrimp died in the 5000ppm of EtOAc and Hexane due to a hard precipitate forming over the surface of the beaker rather than from the plant extract.

HPLC Analysis

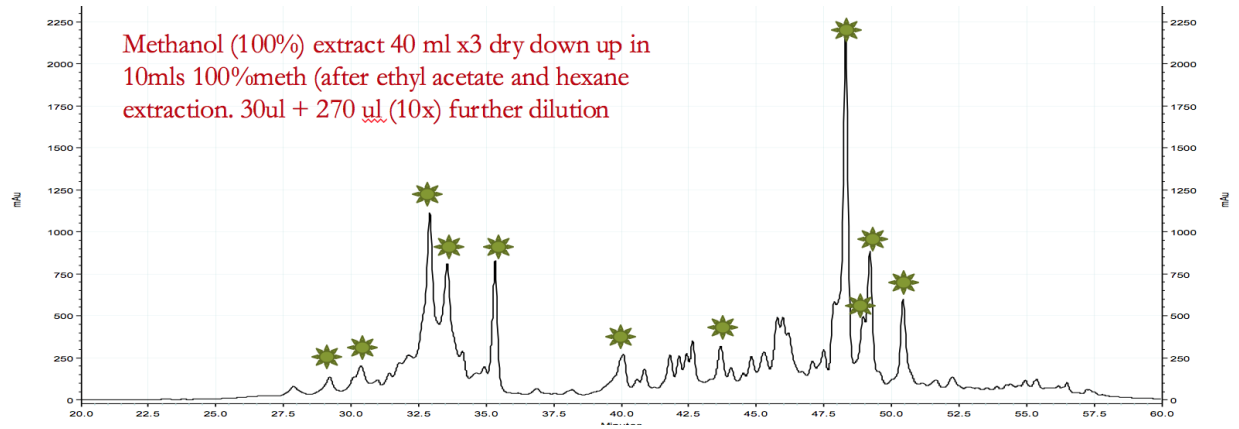

Figure 2. Chromatogram of the Methanol extraction of the bark. Each star indicates a CGA

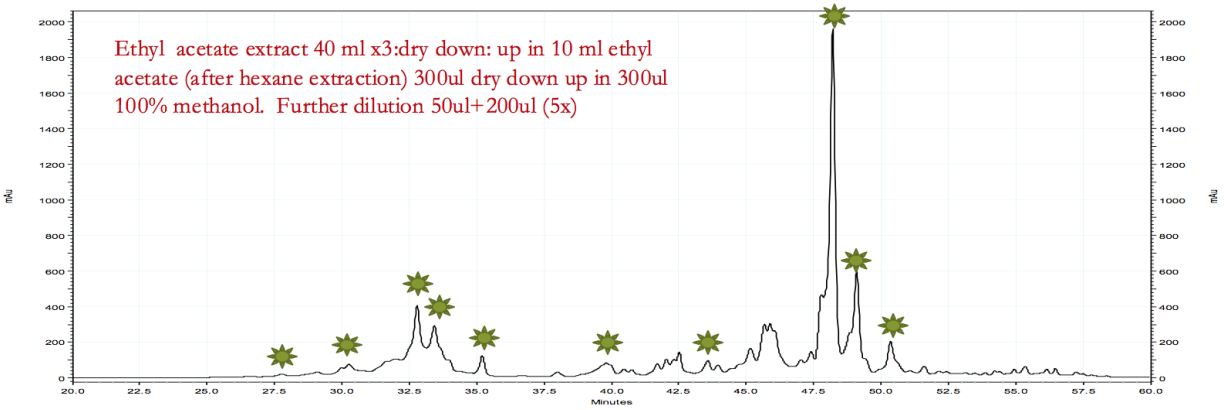

Figure 3. Chromatogram of the Ethyl Acetate extraction of the bark. Each star indicates a CGA

The Black gum bark possess many chlorogenic-acid derivatives and MeOH is the most effective of the three solvents at extracting them (Hexane extract was unable to be analyzed).

Conclusion

The Black Gum bark showed no biological activity based on the results of the brine shrimp bioassay, however, this does not necessarily mean that the bark is not biologically active. The shrimp may have been a poor indicator for the type of activity the bark has and the concentrations used to test may have been too low. The high number of CGA's in the bark suggests that the bark should show biological activity and the concentration of polyphenols and

flavonoids in the bark further support this. The new method of extraction is also a suitable substitute over the conventional method, as a sufficient amount of extract was obtained in order to conduct HPLC analysis, a bioassay, and polyphenol and flavonoid determination. Utilizing sonication helps break down the bark and allow for the solvent to extract more effectively and vacuum evaporation prevents damage of natural products that traditional heat evaporation may cause. It is likely that the bark of the Black Gum tree is biologically active and it would be worthwhile to perform a bioassay utilizing a developed cell line and a microplate reader to confirm or deny this claim.

Acknowledgment

Virginia Foundation for Independent Colleges

College of Arts and Sciences Summer Research Fund

Dr. Combest of the Bernard J. Dunn of Pharmacy

References

(1) New bisamide compounds from the bark of Aglaia eximia (Meliaceae), Phytochemistry Letters 13 (2015), Julinton Sianturia, Hideo Hayashic

(2) Total Phenolics and Flavonoids in Selected Medicinal Plants from Kerala, International Journal of Pharmacy and Pharmaceutical Sciences 6.1 (2014), Biju John, V R K Reddy

(3) Development and validation of UV spectrophotometric method for simultaneous estimation of rutin and quercetin in noisome formulation, Der Pharmacia Lettre (2014), S. P. Chaudhari, M. P. Ratnaparkhi