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Classifying the Extractive Components of the Hardwood Species Catalpa based on Polarity

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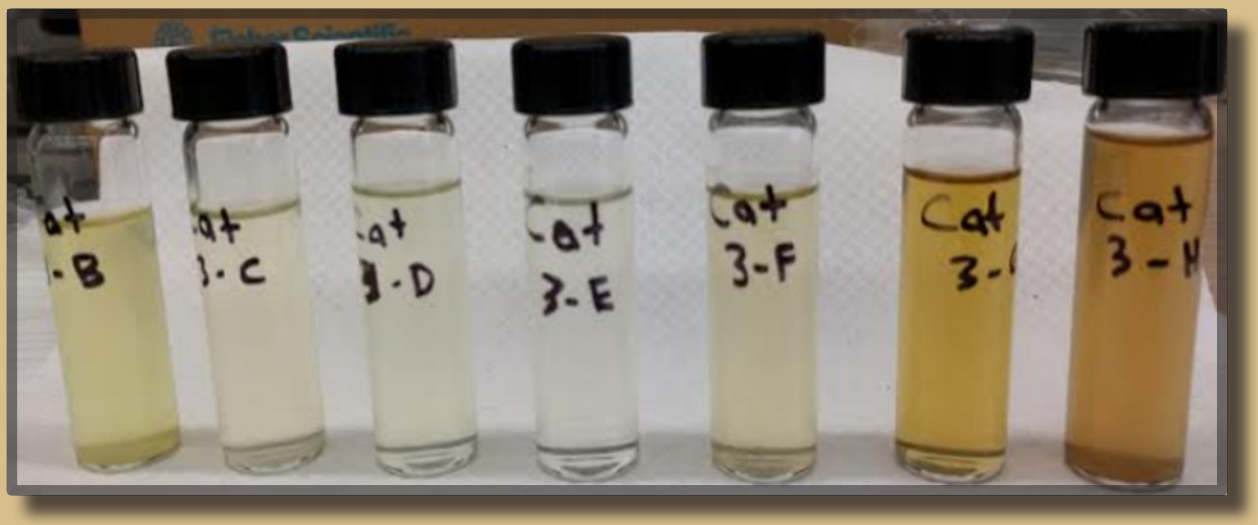

Abstract

Trees contain a number of different extractives that may have specific biological activities, many of which are capable of protecting the tree against different forms of infection. The hardwood species catalpa (*Catalpa speciosa*) is one of many types of wood that are rich with these chemicals. Researchers are often interested in extractives because knowledge of the defense mechanisms of trees against xenobiotics could potentially be applied to pharmaceutical development. For instance, literature indicates that catalpa wood often contains high concentrations of sterolic and phenolic compounds, some of which have been shown to exhibit antinociceptive properties (decreased pain sensitivity) in humans. Investigating these compounds and their effects helps to build an understanding of how different chemicals can be manipulated to benefit people. In this study, we used liquid–liquid extraction as an efficient and easy protocol to separate the different extractive components into varied concentrations of polar and nonpolar fractions. This protocol could help expand the knowledge base of extractive compounds from wood and could be used in pharmaceutical development. Isolated compounds were then further characterized by gas chromatography–mass spectrometry (GC–MS). Resulting data from the GC–MS procedure indicated that many of the extractives identified from catalpa were sterolic derivatives and precursors, including octahydro-phenanthrene and naphthalenol-like compounds as well as various assorted sterols and saponin fragments.

Keywords: Extractives; sterolics; phenolics; saponins; liquid–liquid extraction; gas chromatography–mass spectrometry; polar–nonpolar compounds; fractionation; analyte; supernatant; solvents; percentages

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Classifying the Extractive Components of the Hardwood Species Catalpa Based on Polarity

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Introduction

Wood extractives have been shown to exhibit antixenobiotic properties (Edwards and Ericsson 1999, Sjöström 1993). Literature has shown that catalpa (*Catalpa speciosa*) extractives are often rich with small phenolic compounds, in addition to larger molecules such as saponins and sterols. Both of these larger molecular compound groups have shown a wide range of biological activity, collectively exhibiting anti-inflammatory, antinociceptive (decreased pain sensitivity), anticarcinogenic, and many other properties (Muñoz-Mingarro and others 2003, Castillo and Rossini 2010, Okuda and others 1975). Structurally, sterols contain a four-ringed hydrocarbon nucleus and are often identified as common signaling molecules found within different organisms, notably plants. Saponins, a similar phytochemical, are glycosides. They are larger structures that contain a sterol-like compound bound to a sugar molecule and, in many cases, have similar effects as sterols (Arabski and others 2012).

Through the methods of separation by liquid–liquid extraction (LLE) and analysis by gas chromatography–mass spectrometry (GC–MS), researchers have been able to isolate certain compounds from extractives and characterize them based on respective data (Gutierrez and others 1998). The objective of this study was to develop an efficient and easy protocol for separating different extractive compounds by taking advantage of their varying polarities. Different tree species, depending on their classification as a hardwood or a softwood, contain varying levels of polar and nonpolar extractive content. For example, because catalpa is a hardwood, its extractives probably contain higher amounts of polar compounds than do most softwoods (Moore and others 2015). This polarity protocol could help expand the knowledge base of extractive compounds contained within wood and could potentially be used in pharmaceutical development. The ability to manipulate chemical compounds and test their efficacy in living organisms is the cornerstone of pharmaceutical and clinical research. By observing extractives and their biological activities, researchers can learn various ways in which organisms combat infection and could use this information to develop new pharmaceutical products.

Experimental Methods

Sample Preparation

Catalpa wood was ground in a Wiley mill (Thomas Scientific, Swedesboro, New Jersey, USA) into 1-mm particles using a size 40 mesh. The particles were then soxhlet-extracted using 9:1 acetone/H_2O for 8 to 12 h. Solvent was then evaporated off, leaving viscous samples behind, which were stored in the freezer.

Liquid–Liquid Extraction

Three preweighed vials (one for each replicate), with approximately 90 mg of extractive sample in each, were successively washed with 9 mL of each solvent ratio (solvents were hexane and acetone; Table 1). As the trials progressed, the solvents changed from nonpolar (solvent

Table 1—Solvent ratios used for each successive washing of catalpa samples in the liquid–liquid extraction process

	Ratio of solvents used for each solvent wash						
Solvents in ratio	Vial B	Vial C	Vial D	Vial E	Vial F	Vial G	Vial H
Hexane/acetone (v/v)	10:90	20:80	30:70	45:55	60:40	80:20	100:0

ratios B-D) to mostly polar (solvent ratios F-H). Vials were sonicated and vortexed and then centrifuged for 1 min at 1,000 rpm. Supernatant was then decanted into 7-mL vials, which were dried with nitrogen. The analyte was recovered by allowing the solvent to evaporate from the 7-mL vials overnight.

Gas Chromatography–Mass Spectrometry

Analysis of the extractives was performed using a Hewlett Packard (Palo Alto, California, USA) HP 5890 Series II Plus gas chromatography–mass spectroscopy instrument equipped with a Hewlett Packard HP-5 capillary column (30 m, 0.25-mm i.d., 0.25-µm film thickness). Column temperature was initially 60 °C for 1 min, then was gradually increased to 300 °C at 10 °C/min and kept there for 3 min. Extractives were dissolved in 500 to 1,000 µL of 1:1 (v/v) acetone/methanol, depending on weights, and then pipetted into 1,000-µL gas chromatography vials. Dissolved samples were then injected automatically into the instrument.

Results and Discussion

Visual Observations

Figure 1 shows the colors of the supernatants from the LLE. Polar compounds in wood extracts tend to have a more intense color than nonpolar compounds. Starting with a majority hexane (nonpolar) solvent on the left and moving toward a majority acetone (polar) solvent on the right, the colors of the vial contents generally became more intense in color. However, the supernatant in vial B shows an intense pale-yellow color, despite it containing mostly nonpolar components. This led to the conclusion that supernatant colors are reliant on factors of both dissolved analyte and polarity of soluble compounds.

Polarity Fractionation with Liquid–Liquid Extraction

Data from Figure 2 show that Catalpa has a higher percentage of polar (~60%) than nonpolar (~40%) content (Moore and others 2015). As shown in Figure 3, the first solvent ratio B dissolved most of the nonpolar compounds immediately but the largest portion of analyte (more than half) was dissolved between the polar solvent ratios F and H. This shows that Catalpa's majority polar composition has a very high affinity for acetone. As referenced in the literature, polar compounds that dissolved in vial G were mostly phenolic compounds (Muñoz-Mingarro and others 2003). Figure 3 also shows very minimal variance among replicates, indicating that experiment results were consistent throughout each trial. The largest difference occurred for vial C, which contained mostly nonpolar compounds (80%).

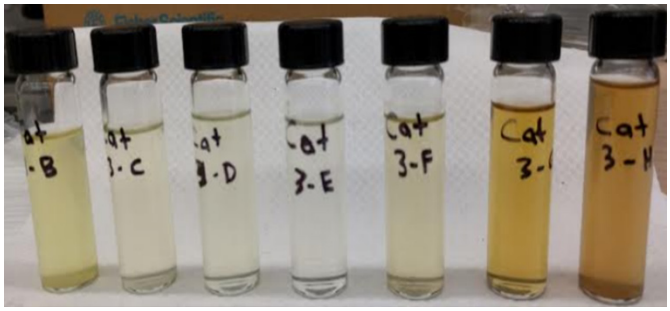

Figure 1—Vials of each supernatant resulting from washing catalpa samples with solvent ratios B through H. Supernatants from nonpolar solvent ratios (B–D) are on the left, the supernatant from the median solvent ratio (E) is in the center, and those from polar solvent ratios (F–H) are on the right.

Despite both phenolic and sterolic compounds having hydroxyl groups, phenols with one aromatic ring are more polar than sterols or naphthalenes. Because of this difference, most of the phenolic components were identified in vials from the more polar solvents (F–H). These compounds also had significantly shorter retention times. More nonpolar vials (B–E) contained many compounds with a backbone similar to naphthalene and sterols, all of which had much longer retention times than polar components.

Gas Chromatography–Mass Spectrometry

GC–MS data showed that, as literature suggests, catalpa extractives were made up of multiple phenolic and sterolic compounds. There appeared to be three primary compounds

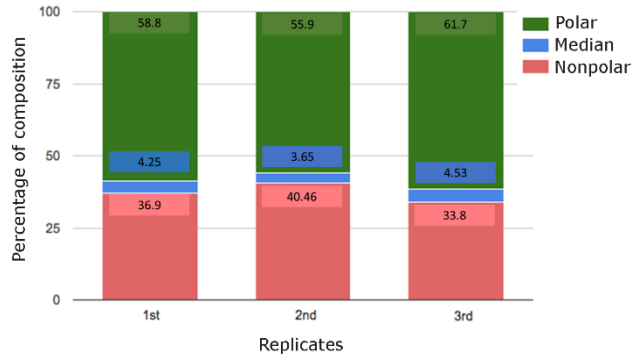

Figure 2—Relative compositions of each replicate and their respective polarity. This chart shows that the polar solvents consistently dissolved slightly higher percentages than both the median and nonpolar solvents combined.

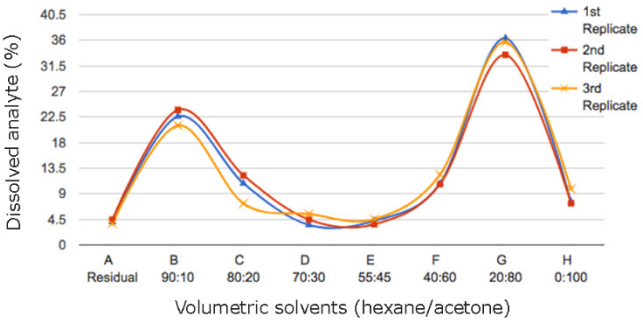

Figure 3—Progression of analyte amounts that solvent ratios successively dissolved for each replicate. The most effective solvent ratios at dissolving analyte were B and G, both of which are represented by the peaks.

on which many derivatives were based. The first was naphthalene, a bicyclic benzene compound, which according to Rokade and Sayyed (2009) is able to adopt many different chemical moieties. For example, 1-naphthalenemethanamine, identified in the GC–MS spectra in vial B, is a molecule with a naphthalene base in addition to a methylamine functional group. The second common structural base was a phenol, from which various compounds found in catalpa, such as 2-methoxy-4-vinylphenol, are believed to be derived. The third was gonane, a tetracyclic hydrocarbon compound that constitutes the backbone of all sterolic nucleus structures. Chemical structures of the common backbones are shown in Figure 4, and chromatograms for identified compounds are shown in Figure 5.

In addition to those that were easy to identify, a large array of compounds found by GC–MS were not as easily classifiable. Many assorted terpenoid compounds were found that did not align with literature sources. These could have been either derivatives of more common identified compounds or fragments of larger molecules. When phenanthrene, or any type of bi- or tricyclic compound, is encountered, the assumption is that these structures are

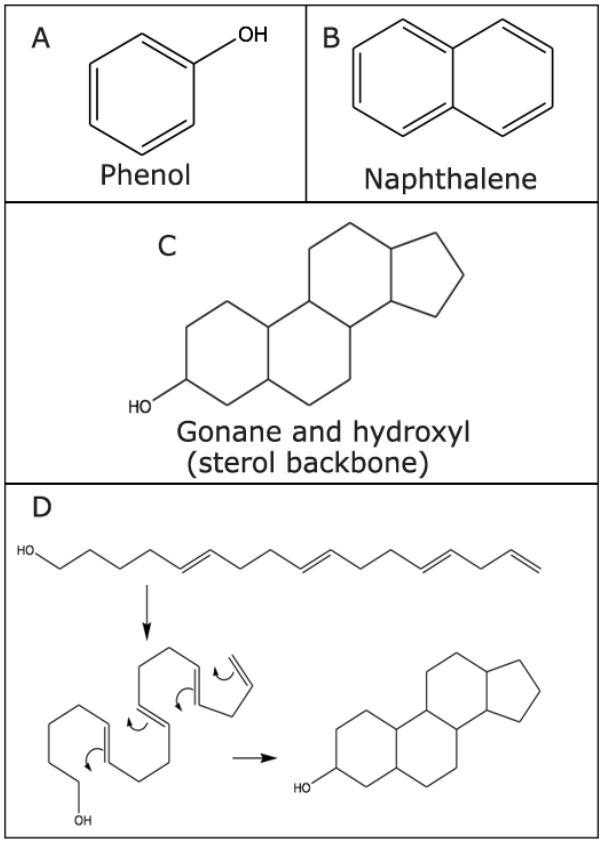

Figure 4—Chemical structures of (A) phenol, (B) naphthalene, and (C) gonane hydrocarbon. All three structures are speculated to be backbone structures off which many extractive derivatives are based. (D) Biosynthesis for gonane, the sterol nucleus (Nes 2011).

some sort of precursor to or fraction of a larger sterol-based molecule. The precursors to sterol biosynthesis are long-branched alkenes that self-assemble into multiringed structures. Premature or early cyclization can occur as well, yielding products that resemble both aromatic rings and linear polyfurcated (branched) alkene structures. Sterolic precursors such as these were found in high concentrations, reinforcing the knowledge that sterols are common species of catalpa extractives.

Despite such a prominence in the literature, sterols and saponins were only identified in very small quantities from the GC–MS spectra. This could have been due to a number of different factors. Compared with other extractives (e.g., phenols), sterols and sterolic compounds (e.g., saponins) probably had a difficult time volatilizing in the GC–MS. Smaller phenolic compounds volatilize because of their low molecular weight and polar state. In the case of larger molecules, their extensive hydrocarbon nuclei, increased hydrophobicity, and high molecular weight prevent them from being able to volatilize completely. This could cause them to either not elute from the column whatsoever or to fractionate in the process of volatilization, becoming smaller derivative-like compounds (two- or three-ringed compounds). This would also explain the high incidence of multiple sterolic fragments.

Conclusions

The colors of the supernatants were primarily related to the polarity of the soluble compounds in them but also partially reflected how much analyte they dissolved. Intense supernatant color reflected compounds with greater polarity, whereas the supernatant with less polar compounds had less intense color. Average percentage weights of the samples in the vials were consistent among the three replicates.

It was determined that separation of extractive compounds into polar and nonpolar portions was beneficial for classifying compounds into the major groups. GC–MS results showed that most phenolics dissolved best in polar solvents and had a higher volatility whereas sterolic and naphthalene derivatives dissolved best in earlier nonpolar vials and had lower volatility, if any.

To further identify and classify compounds from these extractives, future work will include gas chromatography × gas chromatography time of flight mass spectrometry (GC×GC TOFMS) analysis. GC×GC is known for its separation power to deconvolute complex compounds with similar volatility and polarity. It will also separate larger molecules (greater than 20 carbons) to be identified as whole molecules, not just fragments. NMR spectroscopy is another efficient method that will be considered for characterizing larger molecules once they are separated.

The chemicals from catalpa extractives will be investigated further to identify which fractions have biological activity. Sterols, along with their precursors, are known to be relevant to hormonal signaling in a wide variety of species,

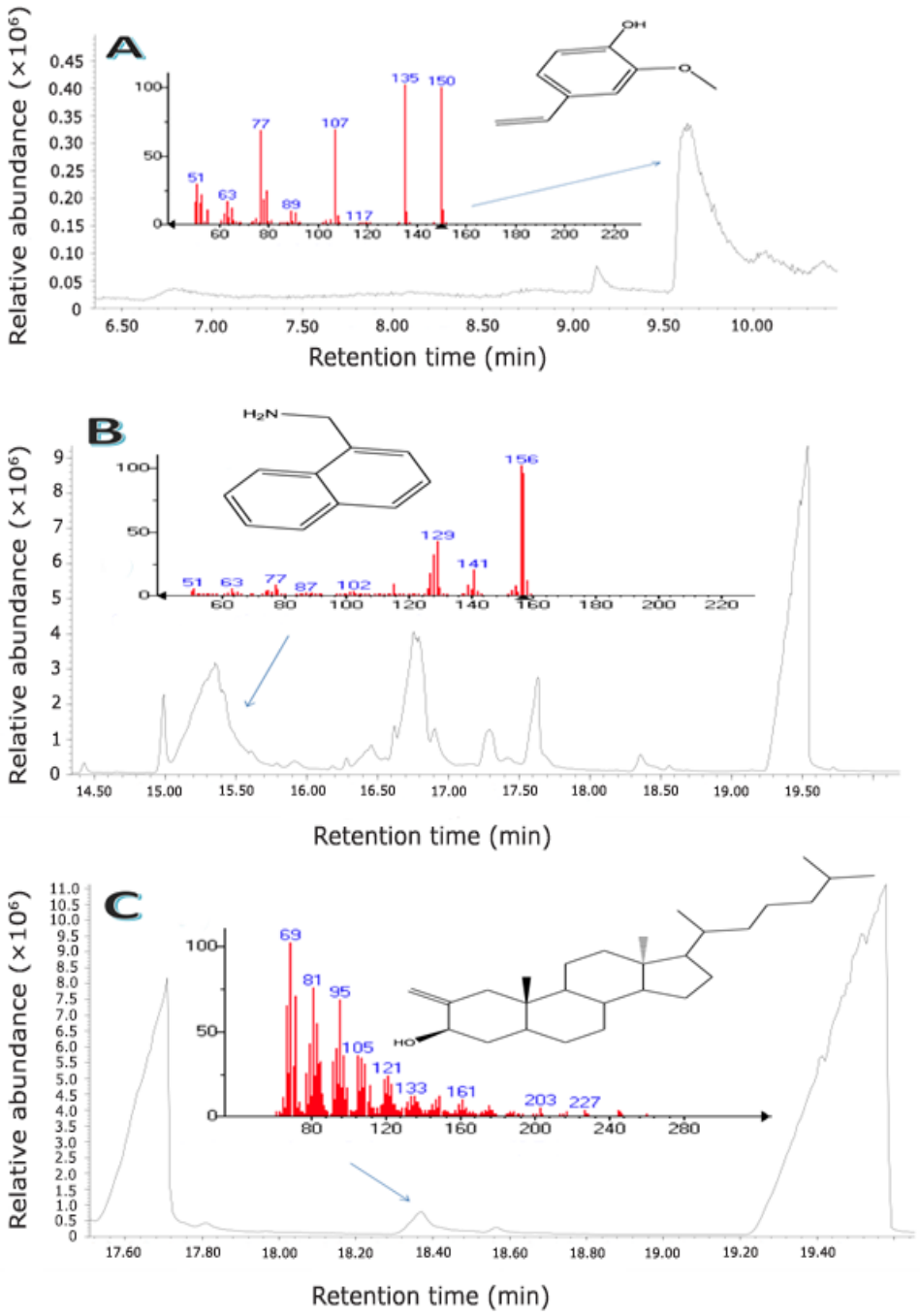

Figure 5—Gas chromatography–mass spectrometry data including chromatograms, spectra, and chemical structures for (A) 2-methoxy-4-vinylphenol, (B) 1-naphthalenemethanamine, and (C) cholestanol. Each of the three given structures is a derivative compound whose chemical type is featured prominently in both literature and experimental data. Arrows indicate which of the chromatogram peaks the spectra were drawn from.

both plants and animals (Edwards and Ericsson 1999, Sjöström 1993). Phenolic compounds, present in both pharmaceutical supplements and diets, have been effective in decreasing the occurrences of cardiovascular disease and cancer and also reducing the plasma levels of cholesterol in patients with those diseases (Stütz and Petranyi 1984). Saponins are commonly used in soap and known for their foaming characteristic. They also rapidly increase calcium uptake and aid in the body's digestive tract (Cornell University 2019).

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