

Characterization of extractives in durable and non-durable hardwoods: Black locust, Catalpa, and Honey mesquite

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Abstract

Hardwoods are a class of tree that holds multiple types of extractives, some of which have their own specialized biological activity. Characterizing these compounds in greater depth can help virologists understand how natural chemicals are able to resist infection, leading to pharmaceutical uses. Hardwoods are known to contain high concentrations of different phenolic compounds which exhibit anti-microbial properties. Liquid-liquid fractionation was used to separate extracts with the same polar characteristics in different solvents. GC/MS spectra showed Black Locust, Catalpa, & Honey Mesquite, had a number of potential biological-active phenolic compounds despite differences in durability.

Keywords: extractives, liquid-liquid fractionation (LLF), Gas Chromatography / Mass Spectrometry (GC/MS), polar / non-polar compounds, separation

Introduction

Hardwoods are a class of tree species that contain compounds exhibiting a wide range of anti-tumor & anti-fungal properties.¹ These compounds are extractives that act as deterrents against various forms of biological attacks.² *Bignoniaceae* (Catalpa - CAT), *Prosopis glandulosa* (Honey Mesquite - HM), & *Robinia pseudoacacia* (Black Locust - BL) are all Hardwoods, which suggests that their compositions are rich with polar compounds.³ Literature also suggests that Hardwoods are usually more durable than Softwoods.⁴ HM and BL are considered to be highly durable woods while CAT is far less durable by comparison.⁵ It is also noteworthy that CAT has a very small concentration of fatty acids compared to the two other hardwood species.⁶

Literature has shown that both CAT and BL contain large amounts of phenolic compounds. In CAT, these phenolics are saponins & sterols, both of which exhibit anti-inflammatory properties⁵ while in BL, these compounds are mostly flavonoids which exhibit antifungal properties.^{8,1} Most of the major components in these Hardwood phenolics are complex as well. For instance, BL is rich with resorcinol, commonly used to synthesize a wide range of products, including pharmaceuticals.⁹ BL also contains resveratrol and piceatannol, stilbenes that have been used in antibiotic treatment and stress reduction.¹⁰ HM has been shown to possess a number of indolizidines which have shown promise for exhibiting anticarcinogenic and antimalarial properties.¹¹

Given that BL is extremely durable, it has been prominent in the research industry and often used in products like medicines, ointments, lotions, etc.^{5,8,12} Since CAT is notoriously non-durable, it has been neglected by many researchers. Similar to CAT, HM has also been moderately disregarded since information about its extractives is very rare. More emphasis needs to be put on identifying the extractive components of HM and CAT, and their possible biological activity.

This information could potentially be used for pharmaceutical development. By observing extractives and their biological activities, researchers can learn various ways in which organisms combat infection. Through methods of separation by Liquid-Liquid Fractionation (LLF) and analysis by Gas Chromatography/Mass Spectrometry (GC/MS), researchers are able to isolate certain compounds from extractives and characterize them. This research project aims to identify and compare the chemical compositions of different hardwood species.

Experimental

Sample Preparation

Wood samples of BL, HM, and CAT were collected and Wiley milled to 40 mesh. Soxhlet extraction was used to wash wood samples in 9:1 acetone: water for 10 hours. The extractives were dried with a rotary evaporator and placed in the freezer.

Liquid-Liquid Fractionation

Solvent solutions of acetone and hexane were prepared in the following ratios (acetone: hexane): 10:90, 20:80, 30:70, 45:55, 60:40, 80:20, 100:0. Approximately 100 mg of each wood extractives sample was placed in a separate 3 mL vial and washed with the following (acetone: hexane) solutions. The samples were washed with 9 mL of each solution, decanted into a 7 mL vial and dried with nitrogen. Each extract was washed, sonicated, vortex mixed, and allowed time for the solids to settle. There were three replicates for each sample.

Gas Chromatography/Mass Spectroscopy

Analysis of the extractives was performed using an HP 5890 Series II Plus Gas Chromatography/Mass Spectroscopy instrument equipped with an HP-5 capillary column (30 m, 0.25 mm i.d., 0.25 μ m film thickness). Column temperature was initially 60 °C for 1 min, then gradually increased to 300 °C at 10 °C/min, and kept there for 3 min. Extractives were dissolved in 500-1000 μ L of 1:1 (v/v) acetone: methanol, depending on weights, then pipetted into 1000 μ L GC vials. Vials of dissolved sample were then injected automatically into the instrument.

Results and Discussion

Figure 1, shows that the progressions of BL and HM are similar, with two increases in concentration towards the more polar solvents. Phenols are known to contribute to the durability of the tree⁴ and, based on observations, fall near the middle of the polarity spectrum. The spike in the graph at the 55:45 solution for BL and HM is most likely due to the presence of phenols in both species. CAT has a large peak in the most non-polar solution and then again at the 20:80 solution. The majority of compounds from CAT have an affinity for the extremes of the polarity spectrum while the majority of BL and HM fall near the middle of the polarity spectrum. The vials that were mostly non-polar (B-D, in Figure 1) have a lighter color than the vials that were mostly polar (F-H). This suggests that polar compounds from Hardwood extractives tend to have a deeper color than the non-polar.

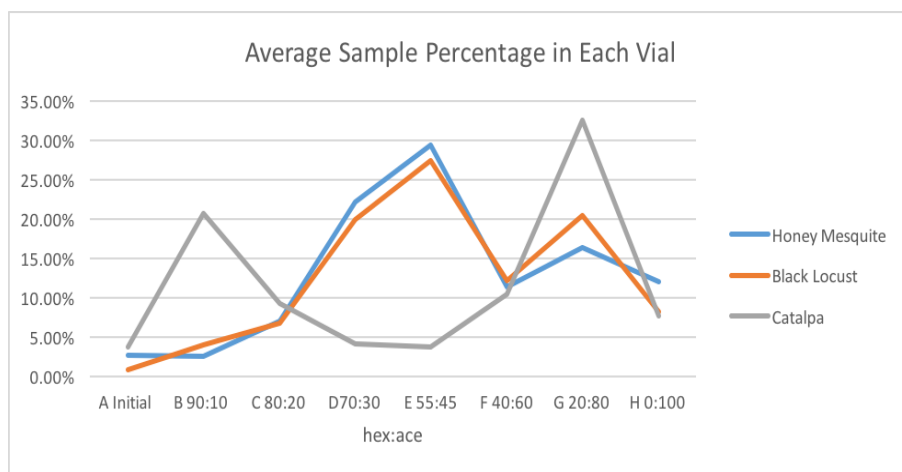


Figure 1. Weight percentages of extractives collected by solvent fractions for Honey Mesquite, Black Locust and Catalpa.

When comparing results from GC-MS analysis, BL and HM share many of the same compounds. N-Hexadecanoic acid, 9,12-Octadecanoic acid (Z,Z), 1,2-Benzenediol, and Oleic acid were all found in vials of both BL and HM. N-Hexadecanoic acid has been shown to exhibit anti-inflammatory properties in humans.⁹ 1,2-Benzenediol may contribute to the durability of HM and BL as it exhibits both antimicrobial and antiparasitic properties.¹³ In humans, 1,2-Benzenediol has been shown to exhibit antioxidant properties and has been used in anticancer agents with promising results.¹³ Fatty acids identified from the hardwoods, in addition to having their own biological activity, are also known to be precursors to larger compounds none of which were identified in this study.⁸ Figure 2 shows the spectra from the analyses on the hardwood. Literature suggested a number of complex phenols in BL and CAT while GC-MS only identified simple phenols. Both of these observations suggest that in future trials it may be necessary to use other analytical methods, such as Liquid Chromatography, to identify possible complex phenols.

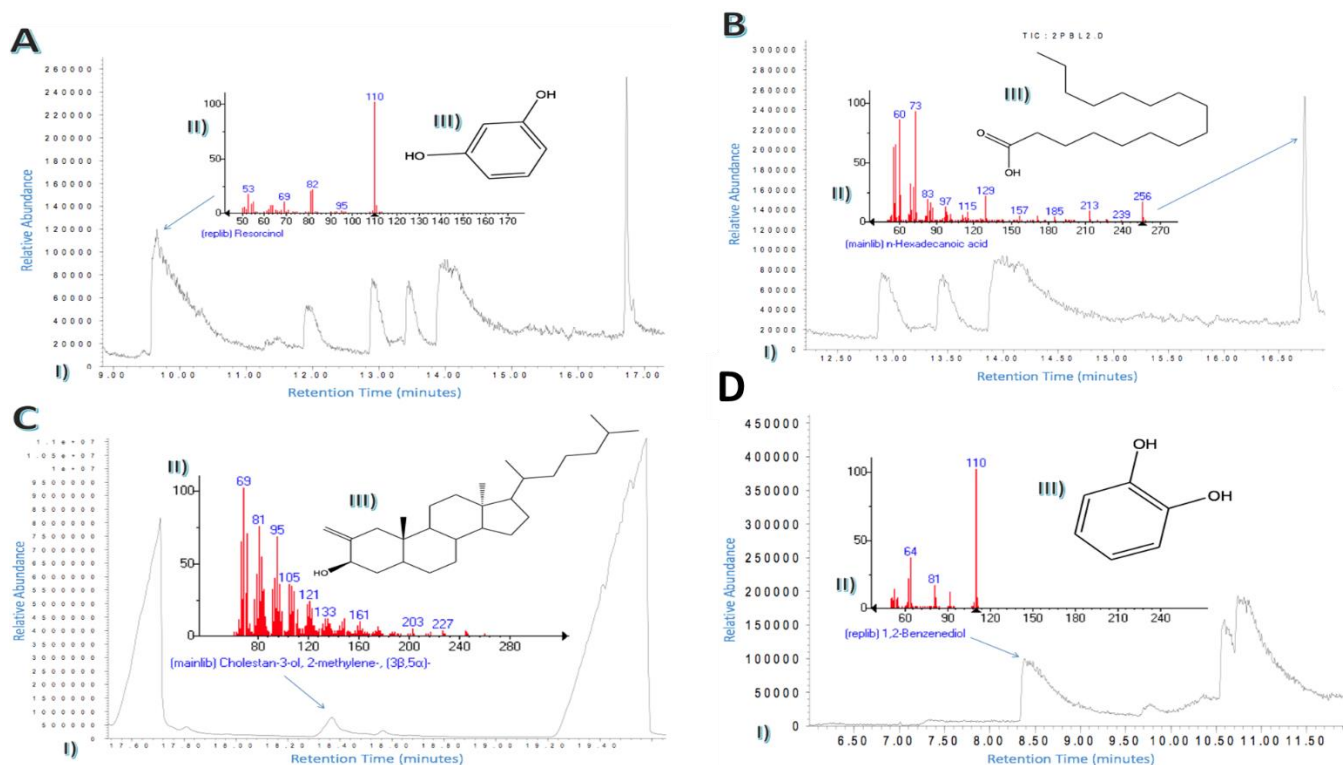


Figure 2. GC/MS analyses of A. Resorcinol (BL) B. Hexadecanoic acid (BL) C. Cholesterol (CAT) D. Benzenediol (HM) (I) Chromatogram (II) Spectra (III) Structure

While all three woods have similar percentages in their nonpolar vials (shown in table 1), CAT actually has the highest percentage of polar compounds. This is unexpected as CAT is the least durable wood of the three.⁴ This observation brings more attention to individual chemicals or to composition of certain chemicals that could be the reason for durability. Both HM and BL have similar percentages of compounds in each phase. As BL and HM are much more durable than CAT, these compounds may be responsible for their durability.

Table 1. Summation of weight percentages for polar, non-polar and median of HM, BL and CAT wood extractives.

<u>Woods</u>	<u>Non-Polar (B-D)</u>	<u>Polar (F-H)</u>	<u>Median (E)</u>
HM	31.89%	39.84%	29.31%
BL	30.76%	40.94%	27.40%
CAT	37.06%	58.79%	4.14%

Conclusions

Honey Mesquite, Black Locust, and Catalpa are all considered to be hardwoods, but their durabilities vary greatly. Both HM and BL appeared to be the most similar in terms of trends from extractives whereas CAT deviated the most. HM and BL have a number of compounds in common (N-Hexadecanoic acid, 9,12-Octadecanoic acid

(Z,Z), 1,2-Benzenediol, and Oleic acid) which may contribute to their durability. Each of the tree species contained high levels of phenolic compounds, consistent with hardwoods and their significant polar content^{6,7,1}. No complex phenols were identified but were seen in literature, suggesting that a different analytic method needs to be used.

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